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PRÉTRAITEMENTS PHYSICO-CHIMIQUES ET BIOTRANSFORMATION DES EAUX USÉES ET DES BOUES D'ÉPURATION: DEVENIR DES PERTURBATEURS ENDOCRINIENS (EDCs) ET DES COMPOSES PHARMACEUTIQUEMENT ACTIFS (PhACS)

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DEDICATED

To my teachers, parents and my wife Nilima who have lend me a moral support during this travail

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RÉSUMÉ

La croissance de la population et l'urbanisation ont conduit à la libération de composés perturbateurs endocriniens (EDCs) et des composés pharmaceutiquement actifs (PhACs) dans l'environnement. Ces composés sont détectés dans les eaux usées (WW) et dans des boues d'épuration (WWS) à travers le Canada.

Le problème majeur avec ces composés est celui de leur détection (ng ou pg) principalement dans les milieux ayant des matrices complexes tels que les WW et les WWS. De plus, l'élimination des WW et des WWS constitue un problème global inévitable à cause de la présence des composés organiques tels que les EDCs et les PhACs. En outre, l'axe de recherche actuel met l'accent sur une approche de valeur ajoutée (VAP) plutôt que sur une approche d'élimination. En effet, au cours du traitement des WW et des WWS, une possibilité de dégradation concomitante de ces composés organiques serait envisageable et nécessite de ce fait une investigation. Ainsi, la présence des composés organiques toxiques et leur dégradation par des traitements physico-chimiques et biologiques devraient faire l'objet d'une attention particulière. Cependant, ces traitements peuvent parfois conduire à la formation des métabolites plus toxiques que les composés parents. Les problématiques soulevées par la présence des composés organiques toxiques dans les WW et les WWS, à savoir leur détection, leur mécanisme de dégradation et la formation des sous produits, nécessite une uchuche.

Le bisphénol A (BPA) est un perturbateur endocrinien largement utilisé dans les industries de plastique et des pates à papier. À côté des autres sources de production, le BPA est relargué dans les systèmes aquatiques par l'intermédiaire des stations d'épuration (WWTP). Plus encore, d'autres composés pharmaceutiquement actifs, tel que la carbamazepine (CBZ) y sont détectés. La CBZ est le composé pharmaceutique le plus persistant et qui est détecté à une fréquence élevée dans les différents milieux environnementaux. Afin d'évaluer la toxicité des effluents traités et rejetés dans l'environnement, l'identification et la quantification du BPA et du CBZ dans les WW et les WWS seraient d'un intérêt majeur.

Le BPA a été mesuré, dans les échantillons provenant des stations d'épuration de la communauté urbaine de la ville de Québec (Canada), avec la méthode du LC-MS/MS. Les résultats montrent que le BPA est présent à des quantités significatives (0.07 µg L^{-1} à 1.68 µg L^{-1} dans les WW et 0.104 µg g^{-1} à 0.312 µg g^{-1} dans les WWS) dans les effluents de ces stations d'épuration. L'efficacité d'élimination du BPA dans le circuit a été de 76%. Cependant, des concentrations environementalement significatives de 0.41 µg L^{-1} demeurent présentes dans les

effluents traités. En ce qui concerne l'analyse des CBZ dans les WW et les WWS, une méthode rapide et fiable permettant d'analyser des échantillons à haut débit pour générer de manière plus rapide les données, les détecter et les surveiller a été mise au point et valide. Le procédé ultrarapide (15s/échantillon) est basé sur l'utilisation de la désorption thermique induite par diode laser avec ionisation chimique à pression atmosphérique (LDTD-APCI) couplée à la spectrométrie de masse en tandem (MS/MS). La limite de détection de la méthode a été de 12 ng L^{-1} dans les WW et de 3.4 ng g^{-1} dans les WWS. Par ailleurs, trois méthodes d'extraction à savoir l'extraction par ultrasons (USE), l'extraction par micro ondes (MAE) et l'extraction accélérée par solvant (ASE) avec trois conditions différentes de solvant tel que le méthanol, l'acétone, et l'acétonitrile: éthyle acétate (5 :1, v/v) ont été comparées sur la base des procédures de blanc et de la méthode de récupération. En général, et comparativement à la USE et la MAE, l'ASE a montré la meilleure efficacité d'extraction avec du méthanol. En outre, la quantification de la CBZ dans les échantillons de WW et WWS a révélé la présence des contaminants dans toutes les étapes d'épuration.

Plusieurs technologies de traitement incluant diverses méthodes de prétraitement, telles que l'hydrolyse, l'oxydation Fenton, l'ultra-sonication et la ferro-sonication ont été développées dans le but de dégrader le BPA et la CBZ dans les WW et WWS et pour la production de produits à valeur ajouté (VAPs) a'basé WWS. Les effets de différentes méthodes de prétraitements, comme par exemple l'hydrolyse alcaline (AH), l'hydrolyse thermique (TH), l'hydrolyse alcaline thermique (TAH), l'oxydation thermique (TO) et l'oxydation alcaline thermique (TAO), sur la solubilisation des WWS et la dégradation simultanée du BPA ont été étudiés. Les résultats indiquent que parmi les différentes méthodes de prétraitements AH, TH et TAH, cette dernière a amélioré de façon significative la solubilisation des WWS (41.6% de solide en suspension (SS), 70.7% de solides en suspension volatile (VSS) et de 48.5% de demande chimique en oxygène (COD)) avec une dégradation élevée de 38.4% de BPA. La solubilisation des SS, des VSS et des COD s'est avérée basse dans les prétraitements TO et TAO, comparativement au prétraitement TAH. Cependant, en raison des processus d'oxydation, une dégradation plus élevée de BPA (75.0% et 78.9%) a été observe respectivement dans les procédés de prétraitements TO et TAO. Parmi les autres méthodes de prétraitement, à savoir l'ultra-sonication (US), l'oxydation Fenton (FO) et la ferro-sonication (FS), une solubilisation élevée (39.7%, 51.2%, 64.5% et 17.6%), respectivement pour les SS, VSS, COD et du carbone organique soluble (SOC), a été observe pendant les processus de prétraitement de ferro-sonication durant 180 minutes résultant ainsi à une forte dégradation de 82.7% de BPA. Pour ce qui est de la CBZ, parmi les diverses méthodes de prétraitement utilisées (US, FO et FS), une hausse de la demande chimique en oxygène soluble (SCOD) et du carbone organique soluble (SOC) correspondant respectivement à une augmentation de 63 à 86% et 21 à 34% a été observe pendant les processus de prétraitement au FO. Ce processus a permis d'obtenir un fort taux de rabattement du CBZ dans les WW (84 à 100%). Par ailleurs, ce travail a conduit à une investigation de la dégradation photocatalytique de la CBZ présent dans les WW avec de nanoparticules de TiO_2 et ZnO. L'utilisation des nanoparticules de TiO_2 et ZnO, a permis respectivement une dégradation de 100% et 92% de la CBZ dans les eaux usées pendant une période d'irradiation de 120 min. Ceci pourrait être attribué à la dégradation oxydative de la CBZ par le TiO_2 et le ZnO en tant qu'une bande directe d'excitation des semi-conduits résultant en une séparation des trous électroniques.

De plus, l'identification des différents sous-produits formés durant les prétraitements de US, FO et FS pour la dégradation du BPA des WWS et de la CBZ des eaux usées a été analysée. Des sous-produits similaires ont été observés dans tous les trois processus d'oxydation avancés (AOPs). La formation des sous produits à savoir le 3-hydroxybisphenol A, l'hydroquinone et la 4-hydroxyacetophenone, a été rendue possible par les réactions des radicaux d'$OH^{\bullet}$ avec le BPA, l'époxycarbamazepine et l'hydroxycarbamazepine pendant la dégradation de la CBZ. En outre, l'activité œstrogénique des échantillons prétraités aux US, FO et FS avec la CBZ et ses sous produits a été mesurée avec la méthode du Yeast Estrogen Screen (YES). Les résultats issus du YES test indiquent qu'aucun échantillon prétraité ne montre une activité œstrogénique. De plus, la dégradation du BPA par des enzymes laccases produits par *Sinorhizobium meliloti* dans les boues brutes et prétraitées a aussi été déterminée. Le résultat indique une forte activité des laccases (230.9 U L^{-1}) dans les boues prétraitées au TAH résultant de la dégradation du BPA (21.9%). Ceci suggère une dégradation biologique concomitante du BPA. Parmi les méthodes de prétraitement du WWS aux US, FO et FS, une forte activité de la laccase (207.9 U L^{-1}) a été observée dans les boues ayant subit un prétraitement à la ferro-sonication pendant 180 minutes résultant à une hausse du taux de rabattement du BPA de 16.2%.

Dans une autre mesure, l'application du modèle de surface de réponse dans la prédiction et l'optimisation de la ferro-sonication et l'ozonation partielle de prétraitement de la dégradation du BPA à partir du WWS a été analysée. Ce processus d'optimisation a permis de conclure qu'une dégradation maximum de BPA correspondant à 88% pourrait être obtenue avec 163 minutes de temps d'ultrasonication, une concentration de 2.71 mg L^{-1} $FeSO_4$, et un pH de 2.81 avec 22 g L^{-1} de concentration de solides en suspension (SS). Pour ce qui est du processus d'ozonation, les conditions optimales pour une dégradation de 100% du BPA ont été de : 24 g L^{-1} SS, pour un pH de 6.23 avec une dose d'ozone de 26.1 mg g^{-1} SS pour un temps d'ozonation de 16.5 minutes. De plus, l'effet de la ferro-sonication (FS) et de l'ozonation partielle (OZ) dans les conditions optimales de la rhéologie du WWS a été étudié. De tous les modèles rhéologiques étudiés, la loi de puissance a été la plus marquée avec une confiance d'ajustement de 90% à 97% en décrivant la rhéologie des boues brutes et prétraitées. Les résultats issus de cette expérimentation montrent aussi une réduction de la viscosité et de la taille de particules en combinaison avec une hausse de la dégradation du BPA de WWS.

Ainsi, d'une part, le prétraitement des WW et des WWS suivi d'une valeur ajoutée (prétraitement physico-chimique combiné avec un traitement biologique) indique une diminution significative de la concentration du BPA et de la CBZ. D'autre part, la méthode développée pour l'analyse des CBZ dans les WW et dans les WWS par la technique du LDTD/APCI-MS/MS pourrait être appliquée pour l'analyse à haut débit d'une grande variété de composés organiques dans les WW et dans les WWS ceci sans faire appel aux solvants (c.à.d. pas de phase mobile) pendant la quantification, respectant les principes de la chimie verte.

ABSTRACT

Growing population and urbanization have led to the release of endocrine disruptor compounds (EDCs) and pharmaceutically active compounds (PhACs) to environment- detected in wastewater (WW) and wastewater sludge (WWS) across Canada. The biggest problem with these compounds is their detection (ng or pg) mainly in media with complex matrices, such as WW and WWS. Moreover, WW and WWS disposal is an inevitable global problem due to presence of these organic compounds, including EDCs and PhACs. In addition, currently research thrust is being laid on the value-addition (VA) approach rather than disposal. In fact, during the course of VA of WWS, there is a possibility of concomitant degradation of these organic compounds, which needs to be investigated. Thus, the presence of toxic organic compounds and their degradation using physico-chemical and biological treatments needs to be addressed. However, these treatments can sometimes result in production of more toxic metabolites than the parent compound. Thus, the big lacuna in detection of these contaminants, their degradation mechanism and formation of by-products in WW and WWS needs to be addressed.

Bisphenol A (BPA), an EDC, largely used in plastic and paper industry, ends up in aquatic systems via wastewater treatment plants (WWTPs) among other sources. Further, among PhACs, carbamazepine (CBZ) is the most persistent pharmaceutical compound that detected at the highest frequency in different environmental media. The identification and quantification of BPA and CBZ in WW and WWS is of major interest to assess the toxicity of treated effluent discharged into the environment. BPA was measured in samples from Urban Community of Quebec wastewater treatment plant located in Quebec (Canada) using LC-MS/MS method. The results showed that BPA was present in significant quantities (0.07 µg L^{-1} to 1.68 µg L^{-1} in WW and 0.104 µg g^{-1} to 0.312 µg g^{-1} in WWS) in the WWTP. The treatment plant was efficient (76 %) in removal of BPA from process stream, however, environmentally significant concentrations of 0.41 µg L^{-1} were still present in the treated effluent. Further, for analysis of CBZ in WW and WWS, a rapid and reliable method enabling high-throughput sample analysis for quicker data generation, detection, and monitoring was developed and validated. The ultrafast method (15 s per sample) is based on the laser diode thermal desorption-atmospheric pressure chemical ionization (LDTD-APCI) coupled to tandem mass spectrometry (MS/MS). The method detection limits was 12 ng L^{-1} in WW and 3.4 ng g^{-1} in WWS. Furthermore, three extraction methods, ultrasonic extraction (USE), microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) with three different solvent condition such as methanol, acetone and

acetonitrile:ethyle acetate (5:1, v/v) were compared on the basis of procedural blank and method recovery. Overall, ASE showed the best extraction efficiency with methanol as compared to USE and MAE. Furthermore, the quantification of CBZ in WW and WWS samples showed the presence of contaminant in all stages of the treatment plant.

Many treatment technologies, including various pre-treatment methods, such as hydrolysis, Fenton oxidation, ultrasonication and ferro-sonication were developed in order to degrade BPA and CBZ in WW and WWS and for the production of WWS based value-added products (VAPs). The effect of various pre-treatment methods, including alkaline hydrolysis (AH), thermal hydrolysis (TH), thermal alkaline hydrolysis (TAH), thermal oxidation (TO) and thermal alkaline oxidation (TAO), on solubilization of WWS and simultaneous degradation of BPA was investigated. The results showed that among AH, TH and TAH pre-treatments, TAH significantly improved the solubilization of WWS (41.6% suspended solid (SS), 70.7% volatile suspended solid (VSS) and 48.5% chemical oxygen demand (COD)) with higher degradation of BPA (38.4%). SS, VSS and COD solubilization were observed to be lower in TO and TAO pre-treatment as compared to TAH pre-treatment. However, higher degradation of BPA (75.0% and 78.9%) was observed in TO and TAO pre-treatment due to the presence of oxidation process. Among ultrasonication (US), Fenton's oxidation (FO) and ferro-sonication (FS) pre-treatment, higher SS, VSS, COD and soluble organic carbon (SOC) solubilization (39.7%, 51.2%, 64.5% and 17.6%, respectively) was observed during ferro-sonication pre-treatment process carried out for 180 min resulting in higher degradation of BPA (82.7%). In case of CBZ, among the various US, FO and FS pre-treatments carried out, higher soluble chemical oxygen demand (SCOD) and soluble organic carbon (SOC) increment (63 to 86% and 21 to 34%, respectively) was observed during FO pre-treatment process, resulting in higher removal of CBZ (84 to 100%) from WW. Further, this work also investigates the photocatalytic degradation of CBZ from WW using TiO_2 and ZnO nanoparticles. By using TiO_2 and ZnO nanoparticles, 100% and 92% degradation of CBZ, respectively from wastewater was achieved during 120 min of irradiation time. This can be attributed as oxidative degradation of CBZ by TiO_2 and ZnO as direct bandgap excitation of the semiconductor results in electron-hole separation.

Further, identification of different by-products formed during US, FO and FS pre-treatment for degradation of BPA from WWS and CBZ from WW was addressed. Similar by-products were observed in all the three advanced oxidation processes (AOPs), 3-hydroxybisphenol A, hydroquinone and 4-hydroxyacetophenone due to the reaction of $OH^{\cdot}$ radicals with BPA and epoxycarbamazepine and hydroxycarbamazepine during CBZ degradation. In addition, the estrogenic activity of US, FO and FS pre-treated sample with CBZ and its by-products was carried out by Yeast Estrogen Screen (YES) assay method. Based upon the YES test results, none of the pre-treated samples showed estrogenic activity.

Further, BPA degradation by laccases produced by *Sinorhizobium meliloti* in raw and pre-treated sludge was also determined. Higher activity of laccases (230.9 U L^{-1}) was observed in TAH pre-treated sludge resulting in degradation of BPA (21.9%) suggesting concomitant biological degradation of BPA. In between, US, FO and FS pre-treated WWS, higher activity of laccases (207.9 U L^{-1}) was observed in ferro-sonicated pre-treated sludge (180 min ultrasonic time) resulting in higher removal of BPA (16.2%).

Further, the application of response surface model in predicting and optimizing the ferro-sonication and partial ozonation pre-treatment for degradation of BPA from WWS was investigated. Through this optimization process, it was found that maximum BPA degradation of 88% could be obtained with 163 min ultrasonication time, 2.71 mg L^{-1} $FeSO_4$ concentration, pH 2.81 with 22 g L^{-1} suspended solids (SS) concentration. For ozonation process, the optimum condition for 100% degradation of BPA was: 24 g L^{-1} SS, 6.23 pH with ozone dose of 26.1 mg g^{-1} SS for 16.5 min ozonation time. Further, the effect of ferro-sonication (FS) and partial ozonation (OZ) under optimum condition on the rheology of WWS was investigated. Among all the rheological models studied, the power law was more prominent (confidence of fit 90% to 97%) in describing the rheology of the raw and pre-treated sludge. The results from this experiment showed a decrease in viscosity and particle size together with a higher degradation of BPA from WWS.

Thus, pre-treatment of WW and WWS followed by value-addition (physico-chemical pretreatment combined with biological treatment) resulted in decrease in significant concentration of BPA and CBZ. Further, the method developed for analysis of CBZ in WW and WWS by LDTD/APCI-MS/MS can be applied for the high-throughput analysis of wide variety of organic compounds in WW and WWS without using the solvents (no mobile phase) during quantification respecting the green chemistry principles.

ABRÉVIATIONS

AH: Alkaline hydrolysis

AOP: Advanced oxidation process

ASE: Accelerated solvent extraction

APCI: Atmospheric pressure chemical reactions

BPA: Bisphenol-A

CBZ: Carbamazepine

EDC: Endocrine disrupting compounds

FO: Fenton's oxidation

FS: Ferro-sonication

GPE: Greatest potential for human exposure

LC-MS/MS: Liquid chromatography-mass spectrometry/mass spectrometry

LDTD: Laser diode thermal desorption

LOD: Limit of detection

MAE: Microwave-assisted extraction

MEC: Measured environmental concentration

OZ: Ozonation

PhACs: Pharmaceutically active compounds

PNEC: Predicted no-effect environmental concentration

SPE: Solid phase extraction

TH: Thermal hydrolysis

TAH: Thermal alkaline hydrolysis

TO: Thermal oxidation

TAO: Thermal alkaline oxidation

US: Ultrasonication

VAP: Value added products

WWTP: Wastewater treatment plant

WW: Wastewater

WWS: Wastewater sludge

Units

kDa: Kilo Dalton

mPa.s: milli pascale. Seconde

mV: millivolt

p/p: poids / poids

rpm: rotation par minute

UFC: unité formant colonie

v/p: volume/ poids

v/v: volume/ volume

U: unité d'activté enzyamatique

VVM: Volume per Volume per Minute

gds: Gram dry substrate;

U/gds: Units per gram dry substrate;

gdw: Gram dry weight

U/ml: Units per mL of medium

INTRODUCTION

La population croissante et l'urbanisation ont mené à la libération des Perturbateurs Endocriniens (EDCs) et des Composés Pharmaceutiques Actifs (PhACs) dans l'environnement. Les EDCs sont définis comme des substances ou mélanges exogènes qui altèrent les fonctions du système endocrinien et causent ainsi des effets adverses sur la santé d'un organisme intact, ou sur sa progéniture, ou sur des (sous) populations (IPCS, 2005). Les EDCs et PhACs sont une préoccupation environnementale émergente, dû à leur présence dans l'environnement aquatique et les impacts potentiels sur la faune, incluant les écosystèmes aquatiques et les êtres humains (Kolpin et al., 2002). L'intérêt pour les EDCs et les PhACs s'est développé au cours des dernières années, en parallèle de nouvelles technologies analytiques extrêmement sensibles développées pour la détection de ces composés à des niveaux très bas (aux niveaux du nano et du pico) dans l'environnement.

Selon la Gazette du Canada (2007), le bisphenol-A (BPA) est un composé organique à haute priorité dans l'évaluation du risque de santé humaine puisqu'il est considéré comme présentant un fort potentiel à l'exposition humaine (GPE) et a été classifié par d'autres agences sur la base de la toxicité pour la reproduction. Le BPA a été identifié comme un EDC par l'Agence de Protection Environnementale des États-Unis (EPA) et par le Fonds mondial pour la nature (WWF) et devient un problème social d'intérêt croissant (Mohapatra et al., 2010a). Selon l'EPA, "le BPA est un agent exogène qui agit sur la synthèse, la sécrétion, le transport, la liaison, l'action ou l'élimination des hormones naturelles du corps responsable de la maintenance de l'homéostasie, de la reproduction ou du comportement". De plus, parmi les PhACs, la carbamazépine (CBZ) est le composé pharmaceutique le plus persistent, détecté en hautes concentrations dans différents environnements (Zhang et al., 2008). La CBZ est utilisée seule ou en combinaison avec d'autres médicaments pour contrôler certains types de crises. La CBZ est parfois aussi utilisée pour traiter les maladies mentales, la dépression, le syndrome de stress post-traumatique, l'arrêt de drogue et alcool, le syndrome des jambes sans repos, les diabètes insipides, certain syndromes de douleur et une maladie infantile appelée chorée (Miao et Metcalfe, 2003). **De telles préoccupations ont accru le besoin de techniques originales et nouvelles d'assainissement pour supprimer efficacement le BPA et la CBZ de plusieurs milieux environnementaux contaminés incluant l'eau, les eaux usées (WW), les boues d'épuration (WWS), les sédiments et les sols.**

Les eaux d'égout entrant dans les stations de traitement des eaux usées (WWTPs) augmentent en complexité jour après jour en raison de l'addition de nouveaux contaminants, qui forment la charge principale des affluents. Le BPA et CBZ peuvent se retrouver dans les systèmes industriels et municipaues de traitement des eaux usées, soit à travers une décharge directe dans les égouts, soit après ruissellement des eaux pluviales. De plus, le prétraitement des WW et des WWS est adopté pour détruire les composés organiques et augmenter la solubilisation de la boue. Plusieurs méthodes sont employées pour le prétraitement de la boue, incluant le traitement mécanique (Tiehm et al., 2001), le traitement thermo-alcalin (Mohapatra et al., 2010b), le traitement chimique (Mohapatra et al., 2011a), le traitement oxydatif (Mohapatra et al., 2011 b, c) et le traitement par radiation (Lafitte-Trouqué and Forster, 2002). Le choix d'une de ces méthodes dépend fondamentalement du coût du processus ainsi que d'autres facteurs, tels que la concentration et le volume des affluents à traiter.

Récemment, les WWS ont été sujettes à une réutilisation pour la production de produits à valeur ajoutée (VAPs) au travers de la voie de bioconversion. La bioconversion des WWS en VAPs (bio-pesticides ou autres agents de bio-contrôle, des inoculateurs microbiens, des enzymes industrielles, des bioplastiques et autres bio-polymères) a été envisagée avec des résultats fructueux et encourageants (Brar et al., 2008). En effet, le prétraitement des WWS et la bioconversion subséquente en VAPs permet une réduction du volume et une amélioration de la qualité de la boue et des gains potentiels à partir des ventes de produits microbiens. Afin de protéger l'écosystème et les consommateurs ou pour sensibiliser le public à l'usage bénéfique des WWS, ces dernières doivent être exemptes de tout type d'EDCs et de PhACs, comprenant le BPA et la CBZ. Ainsi, il est nécessaire d'étudier le devenir du BPA et de la CBZ pendant ou après le prétraitement des WW et WWS et de garantir une pratique innovante de production de VAPs basée sur les WWS, sans aucun de ces contaminants.

CHAPITRE I

SYNTHÈSE

PARTIE 1: REVUE DE LITTÉRATURE

1.1. BISPHENOL A (BPA)

1.1.1. Sources de BPA

Le BPA a été détecté dans des affluents, effluents, et WWS des WWTPs (Mohapatra et al., 2011d; Huang et al., 2012) et a été relâché dans l'environnement par les effluents de traitement des eaux usées, par les lixiviats de décharge (via l'hydrolyse du BPA des plastiques), ou par la dégradation naturelle des plastiques polycarbonés dû à une solubilité aqueuse modérée et une pression de vapeur faible. Le BPA est aussi utilisé comme agent de réaction dans la production de papier sensible à la température avec des couches colorées. Par conséquent, les effluents de papeterie (que ce soit la fabrication ou le recyclage de papier) et les produits de papier recyclés, comme le papier toilette (qui contribue significativement aux eaux usées) doivent aussi être considérés comme des sources importantes de BPA dans les WW et WWS (Gehring, 2004). La libération du BPA dans les courants d'eau est minimisée par l'augmentation des installations de WWTPs dépendant de conditions de traitement spécifiques (Cespedes et al., 2008).

1.1.2. Effets endocriniens du BPA

Plusieurs études ont montré que le BPA est un agent chimique avec un potentiel *œstrogénique* beaucoup plus fort que précédemment annoncé par les municipalités et les agences de régulation (vom Sall et al., 2012). Récemment, des effets significatifs de faibles doses de BPA ont été rapportés dans des expériences sur les animaux, incluant des effets adverses sur les niveaux sanguins des animaux et sur les niveaux moyens du sang humain (vom Sall et al., 2012). De très faibles doses de BPA ont été rapportées causer la prolifération des cellules cancéreuses de la prostate humaine, des maladies cardiovasculaires, des diabètes de type 2 et des anomalies enzymatiques du foie dans un échantillon représentatif de la population des É.-U. (vom Sall et al., 2012). La valeur *œstrogénique* calculée en utilisant la recombinaison de dosage de levure fournit une valeur qui transforme la « concentration » en « *œstrogénicité* » et permet ainsi de présenter un niveau de risque (Tiwari et al., 2012).

Le BPA a aussi démontré une action paradoxale en bloquant les effets bénéfiques de l'estradiol sur la formation des synapses neuronales et en perturbant l'action des hormones thyroïdiennes. Les risques potentiels du BPA incluent des effets sur la reproduction et le développement et des effets neurochimiques et comportementaux (vom Sall et al., 2012). Bien qu'il n'y ait pas beaucoup d'études publiées rapportant les effets directs sur la santé humaine dus à l'exposition au BPA, son effet sur les animaux a suggéré que les humains peuvent aussi être affectés par le BPA en consommant de l'eau et des nourritures contaminées. Les études de suppression/dégradation présentent des méthodes analytiques élaborées pour estimer précisément la concentration du BPA dans ces environnements.

1.1.3. Méthodes pour l'analyse de BPA

En raison de ses diverses caractéristiques, la détermination de la concentration de BPA doit être effectuée dans plusieurs compartiments des processus de traitement et des eaux réceptrices. La détermination du BPA dans les WW et les WWS comprend différentes étapes, comme l'échantillonage, l'extraction, le nettoyage et la quantification. La Fig. 1 présente un schéma des différentes étapes nécessaires à l'analyse du BPA dans ces environnements, en utilisant différentes méthodes analytiques.

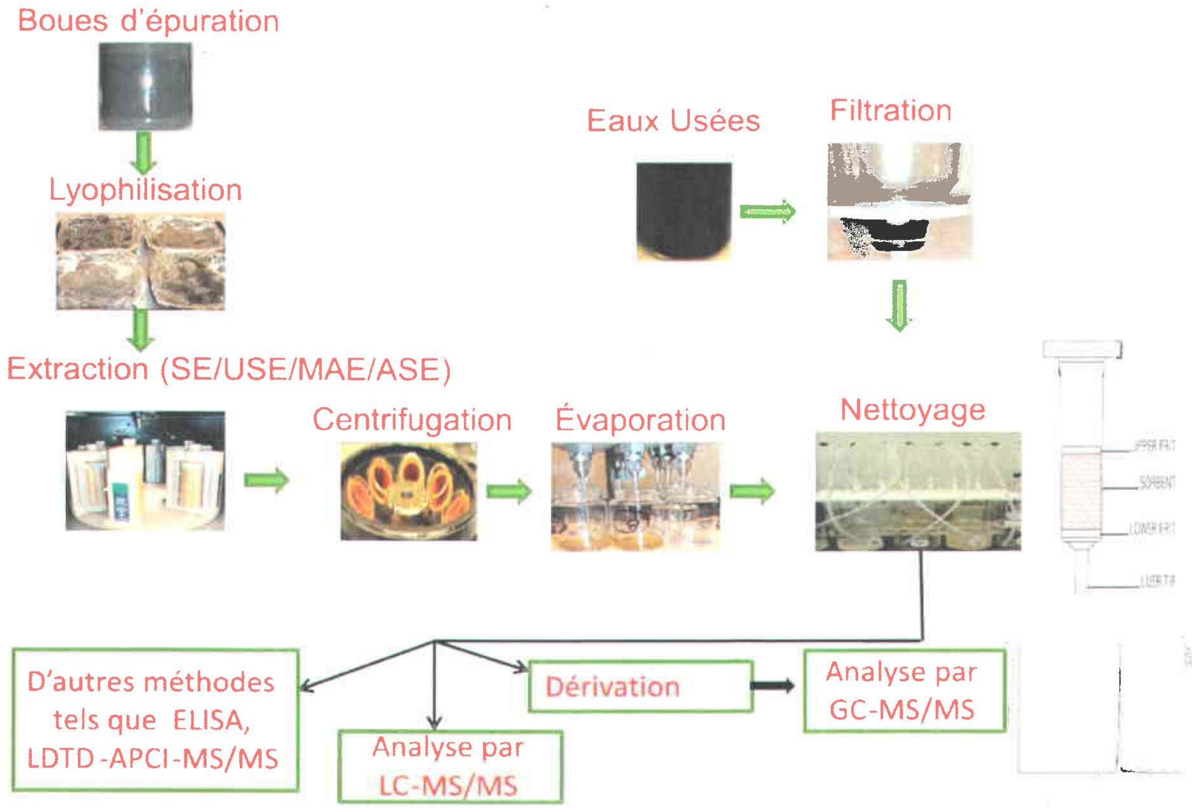


Figure 1. Diagramme schématique de la procédure analytique pour l'analyse du BPA dans les WW et les WWS

Le BPA a été majoritairement analysé par des méthodes chromatographiques, et peu de méthodes analytiques sont disponibles pour la détermination du BPA dans les échantillons environnementaux. Les méthodes d'analyses par chromatographie phase gazeuse-spectrométrie de masse (GC-MS) (Santhi et al., 2012) et par chromatographie phase liquide-spectrométrie de masse (LC-MS) (Dorival-Garcia et al., 2012) sont utilisées pour la détermination du BPA dans les WW et WWS. Les systèmes LC-MS/MS sont hautement sensibles et spécifiques (Mohapatra et al., 2011 a,b,c) et constituent un outil puissant et populaire pour quantifier les composés polaires, comme le BPA, à des niveaux du nanogramme (ng) ou du pictogramme (pg). La comparaison de la sensibilité entre les différentes techniques analytiques pourrait être faite dans l'ordre : LC-MS/MS > GC-MS/MS > LC-MS.

Généralement, l'extraction doit être effectuée avant la détermination du BPA par ces méthodes analytiques. De plus, après une revue des différents articles basés sur des méthodes d'extraction pour l'analyse du BPA dans les WWS, il ressort que les extractions assistées par ultrasons et par microondes sont les plus adaptées sur une base temporelle et de concentrations de solvants utilisées. La méthode de l'extraction de la phase solide (SPE) est habituellement utilisée pour l'extraction du BPA dans les WW. Le choix d'une technique analytique est ainsi une étape importante pour déterminer la concentration du BPA avant la sélection de la méthode de prétraitement ou de traitement pour l'éliminer.

1.1.4. Prétraitement et la dégradation du BPA

1.1.4.1. Hydrolyse alcaline, alcaline thermique et acide

L'hydrolyse de la boue est une façon de rompre la majeure partie de la fraction solide de la boue en molécules solubles et moins complexes, et a ainsi des effets sur la viscosité et la filtrabilité de la boue (Mohapatra et al., 2010b, 2011d), ce qui contribue significativement à la destruction des agents pathogènes et des composés organiques. Les études sur les prétraitements par hydrolyse suggèrent que la méthode peut faciliter la biodégradation des EDCs par traitement biologique subséquent comme la digestion mésophile anaérobie, les réacteurs biologiques séquentiels, etc. Cependant, aucune des études menées jusqu a présent n'a rapporté le prétraitement par hydrolyse des WWS comme objectif pour la suppression ou la désintoxication du BPA.

1.1.4.2. Oxydation et peroxydation de Fenton

L'oxydation de Fenton a été exploitée en laboratoire et dans les WWTPs pour la stabilisation de la matière organique, incluant la suppression des contaminants organiques tels que les contaminants pharmaceutiques et les EDCs (Padoley et al., 2011). L'étude de Gozmen et al. (2003) a montré que la conversion du BPA par l'oxydation de Fenton augmente avec le ratio molaire Fe^{2+}/BPA et que le remplacement du système ferreux/ferrique par le couple cuivreux/cuprique résulte en une dégradation plus rapide du BPA. Le couple ferreux/ferrique a cependant été plus efficace en termes de conversion et de minéralisation totale contre la charge utilisée. Bien que le réactif de Fenton soit fortement actif dans les solutions acides, il ne serait pas approprié car l'utilisation de sels métalliques en tant que catalyseurs entraine une pollution additionnelle. La formation de sous-produits par l'oxydation, tels que le phénol, l'acétate et l'oxalate pendant la peroxydation de Fenton (Neamtu et Frimmel, 2006).

Tableau 1. Devenirs possibles du BPA dans les WW et les WWS via différentes méthodes de prétraitement

Méthode de Pre-treatment	Devenir du BPA
Hydrolyse alcaline	Hydrophobicité de BPA peuvent se convertir au caractère hydrophile. Il peut subir une transformation chimique et donc se partitionné en plusieurs phases en conséquence
Hydrolyse alcaline thermique	Procédé pour diviser et décomposer une majeure partie de la fraction solide des boues en molécules solubles et moins complexe, en raison du BPA se partitionnée qui peut entraîner une destruction du composé.
Hydrolyse acide	Semblable à une hydrolyse alcaline et thermique, il peut transformer le composé en moitiés simples.
Ultrasons	BPA à l'interface eau peut être décomposé thermiquement et une grande quantité de radicaux réactifs sont générés par la dissociation thermique de l'eau. Ceux-ci peuvent déclencher une série de réactions radicalaires résultant de la décomposition des composés générateurs de polluants ayant un poids moléculaire plus faible et une plus faible toxicité.
Oxydation Fenton	La substance peut oxyder, la coagulation et la minéralisation, en tant que résultat de réduire son activité oestrogénique dans la solution et éventuellement moins ou plus de composés organiques toxiques peuvent se former.

1.1.4.3. Ozonation

L'ozonation est une étape habituellement ajoutée au processus de traitement biologique pour un traitement poussé des effluents d'eaux usées dans les WWTPs. Des études ont été menées sur le comportement des micropolluants incluant le BPA, les produits pharmaceutiques et les antibiotiques durant l'ozonation (Cheng et al., 2012).

Alum et al. (2004) ont mené une étude comparative sur la dégradation du BPA dans l'eau et les WW. Ils ont surveillé la concentration de BPA dans les WW pendant 120 minutes d'ozonation dans des conditions similaires à celle de l'eau potable. Ils ont découvert que, en 2 secondes, 99% du BPA était dégradé avec une concentration d'ozone initiale de 30 µM. Divers produits dérivés du BPA sont retrouvés, obtenus normalement pendant l'ozonation complète tels que le p-tert-butylphénol, le 2-méthyle-2, la 3-dihydrobenzofuranne, l'hydroquinone et la n-butyl-acetate, dont aucun n'est classifié comme perturbateur endocrinien.

1.1.4.4. Traitement par ultrasons

La littérature sur la dégradation du BPA par ultrasons dans les WW et les WWS est limitée. Loan et al. (2007) ont observé que le processus de Fenton était beaucoup plus rapide en présence plutôt qu'en absence d'ultrasons en termes de dégradation du BPA ainsi que du taux de dégradation, qui est accru avec une concentration croissante de Fe(II) et un pH décroissant. Les effets des gaz saturants (O_2, Ar, l'air), de la concentration de BPA (0.15-460 µmol L^{-1}), de la fréquence ultrasonique (300-800 kHz) et de la puissance (20-80 W) sur la dégradation du BPA ont été étudiés par Torres et al. (2008). Ils ont observé que pour une solution de BPA de 118 µmol L^{-1}, la meilleure performance était obtenue à 300 kHz, 80 W et avec de l'oxygène comme gaz saturant. Ils ont aussi observé divers produits intermédiaires pendant la dégradation du BPA, comme le BPA monohydroxylé, le 4-isopropénylphénol, la quinine de BPA monohydroxylé, le BPA dihydroxylé, la quinine de BPA dihydroxylé, le monohydroxylé-4-isopropénylphénol et le 4-hydroxyacétophénone. Après 2 heures, ces produits intermédiaires ont été convertis en acides aliphatiques biodégradables.

Néanmoins, les études effectuées à ce jour, au nombre limité, rapportent des produits de décomposition et/ou des produits intermédiaires du BPA après prétraitement, qui pourraient être potentiellement plus toxiques. Des études systématiques sont donc requises pour explorer la dégradation des produits intermédiaires toxiques après le prétraitement des WWS et les traitements biologiques subséquents, tels que la digestion, le compostage ou la biotransformation en produits à valeur ajoutée (VAPs).

1.1.5. Biovalorisation et dégradation du BPA

Les enzymes microbiennes sécrétées par les micro-organismes commerciaux durant la mise en valeur des WW joueraient un rôle crucial dans la dégradation du BPA. Ainsi, il est intéressant de sélectionner des micro-organismes cultivables dans des WWS pour obtenir des produits à valeur ajoutée (VAPs) et produire simultanément des enzymes pour dégrader le BPA. Le Tableau 2 présente des micro-organismes ainsi que leur système enzymatique pour la production de VAPs en utilisant des WWS comme matière première. Les micro-organismes qui synthétisent les VAPs possèdent un système enzymatique potentiellement capable de désintoxiquer ou de dégrader le BPA. La capacité de générer des produits intermédiaires toxiques doit toutefois être prise en compte, puisqu'ils peuvent persister dans le produit final.

Tableau 2. VAPs et systèmes d'enzyme pour la dégradation du BPA en WW et WWS

Micro-organisme	Enzyme	Métabolisme	VAPs
Bacillus thuringiensis	Estérases, tyrosinases et laccases	Produisent des enzymes de type laccase en relation avec leur sporulation et leur mélanine, synthétisée à travers l'action des tyrosinases sur les composés phénolés.	Bio-insecticide (Brar et al., 2009; Verma et al., 2007)
Trichoderma viride	Oxidases, laccases et hydrolases	Ces enzymes sont produites comme des enzymes de dégradation puisque *T. viride* est un champignon saprophyte et il dégrade la lignocellulose. Les oxydases/ hydrolases détruisent l'anneau phénolique par clivage oxydatif.	BCA et enrichissement de terreau pour les cultures agricoles (Verma et al., 2005).
Sinorhizobium meliloti	Laccases et lipases	Les lipases peuvent aider davantage dans la destruction des liaisons ester, si elles sont formées durant des réactions mixtes.	BCA (Ben Rebah et al., 2001)

Les procédés de production de VAPs basés sur les WWS utilisent différentes souches bactériennes et fongiques comme agents de valorisation. Des cellules de *Rhizobium sp.* et de *Sinorhizobium meliloti* sont utilisées comme inoculant dans les cultures de légumineuses et comme agent de bio-contrôle (BCA) en agriculture. Les laccases produites par *S. meliloti* (Rosconi et al., 2005) ont le potentiel de dégrader les composés organiques, pendant la bio-conversation des WWS en VAPs.

1.2. CARBAMAZEPINE (CBZ)

1.2.1. Sources de carbamazepine et ses metabolites

Chaque année, 1014 tonnes de carbamazepine (CBZ) sont consommées dans le monde (Intercontinental Marketing Services (IMS) Health data, 2007) et plus de 30 tonnes doivent être traitées dans des effluents. Au Canada, environ 28 tonnes ont étés vendues en 2001 (Zhang et al., 2008). Une fois ingérée, l'adsorption de la CBZ dans l'organisme est lente et incontrôlable, et environ 2 à 3% de la dose appliquée sera rejetée dans l'urine (Elmquist et al. 1991). La CBZ est métabolisée par le cytochrome P450 dans le foie produisant de nombreux métabolites (Guneysel et al., 2008). Ces métabolites peuvent inhiber la CBZ active pharmaceutiquement.

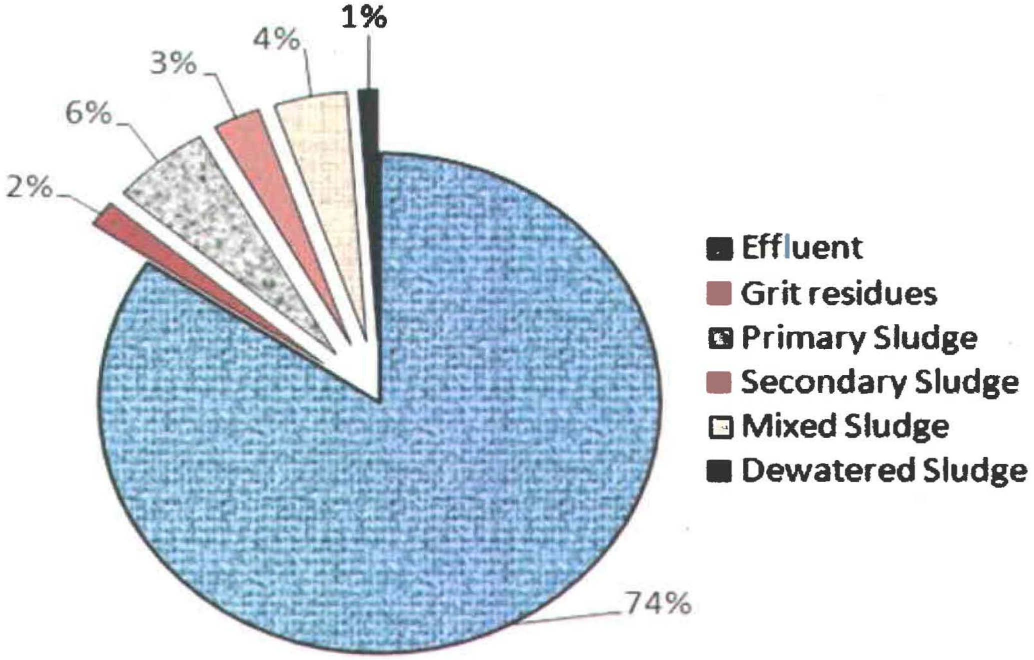


Figure 2. Flux massique journalier de CBZ dans les différent effluents d'une usine de traitement des eaux usées. Les quantités sont ici exprimées de façon relative par rapport à la somme des effluents (%).

Du fait de l'augmentation de la consommation induisant une augmentation de la production et de la pharma-cinétique de la CBZ lors d'un traitement thérapeutique normal (ex: demi-vie, excrétions urinaires et fécales, et métabolisme) ses composés parents ainsi que les métabolites sont rejetés dans les eaux usées. La Fig. 2 présente le bilan massique de la CBZ retrouvée dans les divers effluents d'une station d'épuration des eaux usées.

1.2.2. Effets sur la faune et la flore

Les impacts sur la santé humaine et les risques potentiels pour les écosystèmes des composés pharmaceutiques résiduels (PhACs), tel que la CBZ, même à de faibles concentrations est aujourd'hui un intérêt majeur de la recherche scientifique (Ferrari et al., 2003). De façon globale, des essais biologiques sont utilisés afin d'évaluer la toxicité de la CBZ. Des organismes sont exposés à des concentrations spécifiques afin de mesurer l'effet du produit sur ceux-ci. Les résultats sont ensuite utilisés afin de prédire les concentrations limites (CL) et ils sont ensuite comparés pour mesurer le risque environnemental (RE) (Younghee et al., 2007).

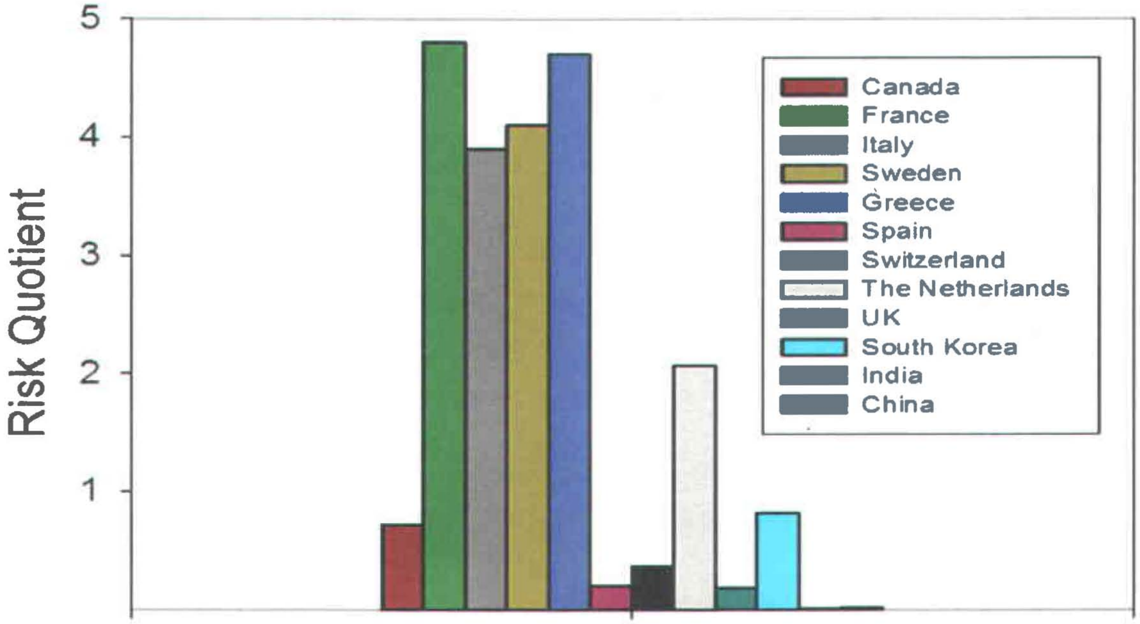


Figure 3. Ratio RE/CL de la carbamazepine relevé dans la littérature. Le risque aquatique est considéré lorsque le ratio≥ 1.

Données de: Canada (Dussault et al. 2008), France; Italy;Sweden;Greece (Ferrari et al. 2003), Spain (Martin et al. 2012), Switzerland (Lienert et al. 2007), The Netherlands (Brandhof and Montforts, 2010), UK (Jones et al. 2002), South Korea (Kim et al. 2007), India (Malarvizhi et al. 2012), and China (Zhao et al. 2010).

La caractérisation du risque est une estimation de l'incidence des effets concurrents se produisant dans un écosystème permettant la prédiction de l'effet d'une substance. Les calculs de risques (RE/CL) pour la CBZ dans différents pays sont présentés en Fig. 3. Malarvizhi et al. (2012) ont étudié les concentrations de CBZ mesurées dans le foie et les muscles d'une carpe commune, *Cyprinus carpio*. Ces derniers concluent que la CBZ induit des altérations au niveau de l'activité de la glutamate oxaloacetate transaminase (GOT), la glutamate pyruvate transaminase (GPT) et lactate dehydrogenase (LDH) dans différents organes des poissons. Une étude de Cunningham et al. (2010) sur les risques pour la santé humaine liés à la présence de CBZ dans les eaux de surface en Amérique du Nord et en Europe, conclue que la CBZ et ses métabolites représentent un risque faible pour des expositions environnementales. Cependant, des études supplémentaires seraient nécessaires afin de mesurer la toxicité de ces contaminants sur la santé humaine, ainsi que sur la dégradation/l'enlèvement de la CBZ contenue dans l'environnement incluant l'eau de surface, les eaux usées et les boues d'épuration, afin d'élaborer des méthodes analytiques afin d'estimer précisément l'effet de la concentration de la CBZ.

1.2.3. Méthodes analytiques pour la CBZ dans les WW et WWS

Le suivi des PhACs incluant la CBZ dans les écosystèmes aquatiques est devenu une priorité pour les agences gouvernementales et de régulation. Une des limitations majeures est le manque de méthodes analytiques pour les faibles concentrations. Jusqu'à maintenant, les méthodes utilisées pour mesurer la CBZ dans les WW et les WWS, incluent la chromatographie (phase gazeuse ou phase liquide), la spectrométrie de masse (MS) ou couplée (MS/MS). Les techniques de séparation utilisent la chromatographie phase liquide ou phase gazeuse, tandis que les mesures sont effectuées par MS. Cependant, du fait de la faible volatilité de la CBZ, l'analyse GC-MS nécessite une étape de dérivation, ce qui allonge le temps de préparation des échantillons, induisant un plus grand risque d'erreur et de contamination. De plus, la CBZ est thermolabile et se décompose lors de l'extraction et forme de l'iminostilben (Ternes, 2001).

Afin d'atteindre les limites de quantification de l'ordre du ng.L^{-1} pour l'analyse de la CBZ dans les matrices environnementales, des techniques avancées comme LC-MS/MS ou LC-ESI-MS/MS sont nécessaires (Ternes, 2001). Les LC-MS/MS sont ultra sensibles et permettent de quantifier les composés pharmaceutiques acides, comme la CBZ contenue dans les WW, à des niveaux de l'ordre du nanogramme ou du picogramme (Bahlmann et al., 2012). D'autre part, l'utilisation des LC-ESI-MS/MS pour la mesure de la CBZ dans le WWS et les WW du fait de la polarité, de la thermolabilité, et de la nature non volatile du contaminant offre un outil sélectif et précis (Lee et al., 2012). De plus, l'utilisation de LC-MS avec ESI est une technique efficace, car elle ne nécessite aucune dérivation que la quantification et l'identification se déroulent en une seule étape.

1.2.4. Procédés d'oxydation avancée (AOPs) pour la dégradation de CBZ à partir des eaux usées

1.2.4.1. Procédé de Fenton et procédé de Fenton photo-assistée

Le procédé de Fenton est un système d'oxydation attractif pour le traitement des eaux usées dû au fait que le fer est très abondant et constitue un élément non toxique et le peroxyde d'hydrogène est facile à manipuler et il est respectueux de l'environnement. Li et al. (2012) a observé une élimination complète de CBZ par le procédé de Fenton effectué en utilisant 20 mg L^{-1} Fe (II) et un rapport molaire H_2O_2/Fe de 2,5 (II). Ces différentes études ont montré que le processus d'oxydation de Fenton est un prétraitement, qui améliore l'élimination des produits pharmaceutiques, y compris la CBZ et semble être une solution efficace pour assurer la conformité avec la législation en matière de s'acquitter dans un milieu récepteur déterminé.

En outre, le procédé de photo-Fenton est une extension du processus de Fenton qui tire parti de l' irradiation lumineuse UV-VIS dont les valeurs de longueurs d'onde supérieures ou inférieures à 300 nm. Cruz et al. (2012) ont étudié la dégradation de la CBZ photo-Fenton dans ces eaux usées et ont observé une suppression de la CBZ de plus de 97% par le traitement de photo-Fenton employant UV254, 50 mg L^{-1} de H_2O_2, avec ou sans addition de fer (5 mg L^{-1} de Fe^{2+} ajoutée ou 1,48 mg L^{-1} de fer total déjà présent). L'applicabilité du traitement de photo-Fenton pour dégrader la CBZ a également été étudiée par Bernabeu et al. (2012) et ils ont observé une élimination complète des polluants après 120 min de temps d'irradiation. En outre, une comparaison des processus de Fenton et de photo-Fenton pour la dégradation de CBZ réalisée par Dai et al. (2012) a montré une augmentation d'environ 20% de l'efficacité d'élimination de la CBZ en utilisant le procédé UV/Fenton par rapport au procédé de Fenton.

1.2.4.2. Ozonation

L'ozonation est particulièrement approprié pour le traitement des eaux usées contenant des polluants organiques bio-réfractaires et / ou toxiques, y compris PhACs (Ternes et al., 2003). Une étude réalisée par Reungoat et al. (2012) sur l'élimination de la CBZ des effluents par ozonation a montré que plus de 90% d'élimination des polluants est possible par ozonation. Lee et al. (2012) ont étudié la faisabilité de l'ozone et de la biofiltration comme une alternative à l'osmose inverse (OI) pour éliminer les produits pharmaceutiques et les produits de soins personnels (PPCPs), y compris la CBZ présent dans les eaux usées. Ils ont conclu que les doses d'ozone de 4-8 mg L^{-1} ont été presque aussi efficaces pour éliminer les micropolluants.

En outre, un procédé d'oxydation combinée a généralement une plus grande efficacité qu'un processus d'oxydation particulier en raison de la génération accrue de radicaux hydroxyles. En outre, le rendement du traitement est renforcé si l'ozone est combiné avec une irradiation de lumière (O_3/UV) (Irmak et al., 2005) ou le peroxyde d'hydrogène (O_3/H_2O_2) (Huber et al., 2003). Pisarenko et al. (2012) ont étudié l'effet de l'ozone et l'ozone/peroxyde sur l'élimination de la CBZ. Ils ont démontré que l'ajout de peroxyde a accéléré la formation de radicaux hydroxyles et a donné une plus grande suppression de la CBZ par rapport à l'ozone seul.

1.2.4.3. Ultraviolet et peroxyde d'hydrogène (UV/H_2O_2)

Le procédé UV/H_2O_2 est un POA pour l'oxydation efficace et la destruction des substances organiques toxiques et réfractaires provenant de l'eau et des eaux usées. Kim et al. (2009) ont étudié la performance des processus UV et UV/H_2O_2 pour l'élimination des produits pharmaceutiques détectés dans l'effluent secondaire d'une station d'épuration. Ils ont observé que pour le processus d'UV/H_2O_2, une efficacité d'élimination de 90% pourrait être atteinte pour 39 produits pharmaceutiques, y compris la CBZ à une dose de 923 mJ UV cm^{-2}, ce qui indique qu'il sera possible de réduire la consommation énergétique UV nécessaire pour l'élimination efficace des produits pharmaceutiques par la combinaison de H_2O_2 et le processus d'UV.

Rosario-Ortiz et al. (2010) ont étudié le procédé UV/H_2O_2 pour l'oxydation de PhACs notamment dans les eaux usées contenant la CBZ. Ils ont observé une élimination de la CBZ de plus de 90% et ont également conclu que le traitement UV/H_2O_2 pour l'élimination de PhACs des eaux usées est une fonction non seulement de la concentration de la matière organiqu des effluents (EFOM), mais aussi de la réactivité inhérente aux radicaux hydroxyles. La suppression de PhACs est également corrélée avec la diminution de l'absorbance d'UV à 254 nm (UV 254). Kohler et al. (2012) ont étudié l'élimination des résidus pharmaceutiques dans les eaux usées des hôpitaux en utilisant une technologie avancée d'irradiation par UV. Ils ont observé que l'effet de l'irradiation par UV sur la dégradation des PhACs y compris de la CBZ a été renforcée avec des doses variables de H_2O_2 et que l'analyse des coûts des différentes options technologiques en tenant compte à la fois de la consommation d'énergie électrique et de l'efficacité d'élimination des produits pharmaceutiques, a clairement révélé une meilleure performance des lampes d'UV à basse pression comme AOP.

1.2.4.4. Procédés photocatalytiques hétérogènes

Les procédés photocatalytiques hétérogènes utilisent certains oxydes métalliques qui peuvent facilement générer des radicaux hydroxyles sur la surface des particules lors de l'absorption de la lumière UV. Rizzo et al. (2009) ont étudié les cinétiques de dégradation et de minéralisation d'une station d'épuration d'effluents urbains avec un mélange de PhACs, y compris la CBZ par le processus de photocatalyse par TiO_2. Ils ont conclu que le taux de minéralisation (évalué en termes de carbone organique total (TOC)) dans les eaux usées contaminée par des produits pharmaceutiques, y compris la CBZ a été jugée très lente ($t_{1/2}$ = 86,6 min) par rapport à celle du même mélange pharmaceutique dans l'eau distillée eau ($t_{1/2}$ = 46,5 min). Ils ont démontré que la différence dans les résultats est probablement due à l'interférence des piégeurs de radicaux libres, tels que les carbonates qui se produisent généralement en concentrations élevées dans les eaux usées.

En outre, un obstacle majeur à l'utilisation de TiO_2 comme photocatalyseur est son faible photo-efficacité en raison de la courte durée de vie des trous générés et des paires d'électrons et la nécessité d'une lumière riche en énergie. Laera et al. (2011) ont étudié un bioréacteur à membrane intégrée (MBR)-TiO_2 pour l'élimination de la CBZ. Ils ont démontré que l'adaptation de recyclage de 4:1 a entraîné le retrait de 95% CBZ. En outre, la réduction de la demande chimique en oxygène des effluents (COD), l'augmentation du rendement des boues et les essais de respirométrie ont suggéré que les produits d'oxydation étaient pour la plupart biodégradables et qu'ils n'ont pas inhibé l'activité microbienne. Ces études témoignent les avantages des procédés photocatalytiques hétérogènes pour la dégradation de la CBZ et des eaux usées.

1.2.4.5. Irradiations ultrasoniques

L'application de l'irradiation ultrasonique ou sonolyse pour l'assainissement de l'eau et des eaux usées est un domaine d'intérêt croissant. Naddeo et al. (2009) ont étudié le devenir de PhACs y compris de la CBZ dans les effluents contaminés sous irradiation ultrasonique. Ils ont étudié plusieurs conditions de fonctionnement, telles que la densité de puissance (25-100 W L^{-1}), les concentrations initiales du substrat (2,5-10 mg L^{-1}), le pH de la solution initiale (3-11), et l'air de barbotage pour l'évaluation du processus. Ils ont observé que la dégradation de la CBZ est renforcée par l'augmentation de la puissance appliquée, des conditions acides et en présence d'air dissous.

Des différentes études, il a été conclu que le principal mécanisme qui implique les AOPs est la génération de radicaux hydroxyles qui facilitent l'oxydation des composés organiques, y compris la CBZ. En raison de cette différence, le coût pour générer des radicaux hydroxyles varie considérablement dans les différents AOPs. Les points suivants peuvent être considérés pour une comparaison des POAs: (1) un équipement additionnel ou matériau de construction; (2) le ravage de lumière; (3) l'inhibition due à la présence ou à la production de différents sous-produits; (4) l'influence du pH; (5) les limitations de transfert de masse, et (6) une évaluation des coûts.

PARTIE 2: PROBLÉMATIQUE

De ce qui précède le chapitre sur l'analyse documentaire, les problèmes suivants ont été déduits:

2.1. Défis en matière d'élimination des eaux usées et des boues d'épuration

L'urbanisation croissante a conduit à la libération de composés organiques, y compris le BPA et la CBZ dans l'environnement, principalement détectés dans les eaux usées et les boues des eaux usées à travers le Canada. Le plus gros problème avec ces composés est leur détection (ng ou le niveau pg) dans des matrices complexes, tels que les eaux usées (Chang et al., 2009). Par conséquent, le choix des techniques d'analyse pour la détection devient un défi. En outre, l'élimination des boues et des eaux usées est un problème mondial inévitable. Au Québec, environ 200 000 T/an de boues tonnes riches d'epuration municipales sont produites, 33% sont recyclées par épandage agricole, compostage (Environnement et des Parcs, 2008). Toutefois, l'application sur le terrain est contestable en raison de la présence de composés organiques, y compris le BPA et la CBZ. Ainsi, la présence de composés organiques toxiques et leur dégradation en utilisant un traitement physico-chimique et biologique doit être abordé.

En outre, la décharge des perturbateurs endocriniens et PhACs y compris le BPA et la CBZ, respectivement dans les effluents des stations d'épuration dans les eaux de surface réceptrices (Saint-Laurent au Québec) peut provoquer des effets physiologiques de la vie aquatique. Le rapport d'évaluation du projet soumis par la Gazette du Canada à Santé Canada a conclu que le BPA peut causer des effets nocifs pour les organismes aquatiques à une exposition chronique à des niveaux inférieurs à ceux habituellement observés avec une courte exposition à faible dose, en particulier aux stades sensibles du développement des générations suivantes et en utilisant plus d' un mode d'action (Gazette du Canada, 2008). Un mode d'action oestrogénique du BPA est confirmé par des expériences in vitro, qui décrivent la perturbation de la fonction des cellules à 10^{-12} M ou 0,23 ppt. La dose de BPA nécessaire pour stimuler la prolifération des cellules (~ 10^{-7} M ou 23 ppb) était d'environ 100000 fois plus élevée par rapport à l'estradiol, qui stimule la prolifération cellulaire à environ 10^{-12} M (Welshons et al., 1999). En outre, selon les résultats de la présente législation européenne sur la classification et l'étiquetage des produits chimiques (92/32/CEE), Jos et al. (2003) ont classé le CBZ "R52/53 comme nocif pour les organismes aquatiques, peut entraîner à long terme des effets néfastes pour l'environnement aquatique. Ainsi, le BPA et la CBZ sont des perturbateurs endocriniens et PhACs respectivement, présents dans les eaux usées et les boues des eaux usées nécessitant une attention immédiate en termes de son retrait, en particulier avec les tendances de recyclage plutôt que les options d'élimination.

2.2. Pré-traitement physico-chimique des eaux usées et des boues d'épuration et formation de sous-produits toxiques

Des méthodes de pré-traitement (thermique, chimique ou mécanique) avant la dégradation biologique, ont été développées dans le but d'améliorer le recyclage des eaux usées et des boues d'épuration. Cependant, ces traitements peuvent parfois aboutir à la production de métabolites plus toxiques que la molécule mère. Il a été observé que les processus d'oxydation sont efficaces pour éliminer l'activité oestrogénique du BPA (Torres et al, 2008; Guo Feng et 2009), et la toxicité de la CBZ (Klavarioti et al, 2009). L'activité résiduelle des contaminants présents dans les milieux environnementaux persiste après le traitement et il y a une possibilité de formation des sous-produits toxiques de sorte que le composé pourrait devenir plus toxique que la molécule mère. Cette proportionne nécessité non seulement d'étudier la distribution et la transformation de composés organiques, y compris le BPA et CBZ dans l'environnement, mais aussi l'importance du suivi et de caractériser les produits de transformation. *Par conséquent, les études systématiques sont nécessaires pour détecter les sous-produits et explorer la dégradation de ces intermédiaires.*

Les prétraitements, tels que les ultrasons, l'oxydation de Fenton, la photocatalyse et l'hydrolyse, entre autres, sont connus pour apporter un changement dans la chimie de surface des eaux usées / des boues des eaux d'épuration (protéines et polysaccharides) (Stasinakis, 2008). Les changements dans la chimie de surface sont bien corrélés avec la rhéologie des boues d'épuration, qui est complexe. Les boues d'épuration, sont des fluides non newtoniens à savoir soit thixotrope (diminution de la viscosité avec le temps) ou pseudoplastique (diminution de la viscosité avec le taux de cisaillement). Les propriétés rhéologiques affectent le mélange du milieu de fermentation et les échanges de masse entre les phases solide, liquide et gazeux (Richard et Margaritis, 2003). *Ceci justifie des études systématiques pour établir une corrélation entre la rhéologie à différentes étapes de prétraitement avec la dégradation du BPA.*

2.3. Valorisation et la présence de BPA et CBZ

Actuellement, la poussée de la recherche est mis sur la bio-valorisation des boues d'épuration plutôt que leux élimination. Dans cette perspective, les boues d'épuration peuvent être transformées en des nombreux produits à valeur ajoutée (VAPs), comme les biopesticides, engrais biologiques, bioherbicides, les bioplastiques et les enzymes (Brar et al., 2006). Les VAP obtenus par la valorisation des boues d'épuration sont à faible coût et peuvent rivaliser avec des produits chimiques ou d'autres produits à forte intensité dans les marchés actuels. Toutefois, cette application est contestable en raison de la présence de ces composés organiques toxiques, y compris le BPA et la CBZ, entre autres. *Cela pourrait être résolu si les composés organiques peuvent être retirés des eaux usées (traitements physico-chimiques et biologiques).*

PARTIE 3: HYPOTHÉSES ET OBJECTIFS DE LA RECHERCHE

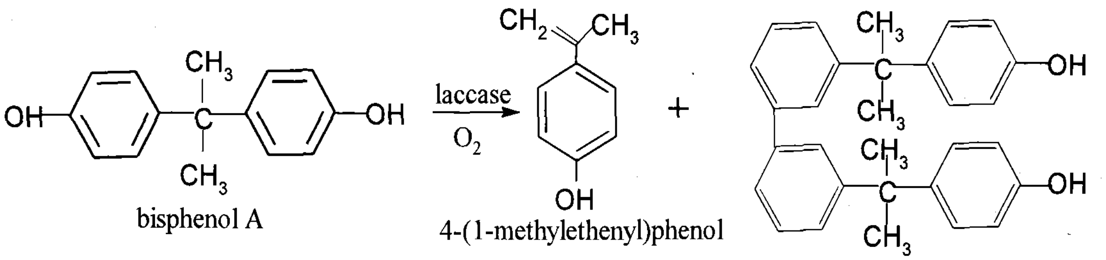
3.1. Hypothéses

Afin de quantifier la présence de BPA et de CBZ dans les eaux usées, dans les boues d'épuration, dans la dégradation simultanée de ces polluants au cours des prétraitements physico-chimiques et dans la biotransformation des boues d'épuration, les hypothèses suivantes ont été formulées:

1. Il a été rapporté que la valeur du coefficient de partition de l'eau d'octanol (log K_{ow}) a joué un rôle important dans leur élimination lors du traitement primaire (Langford and Lester, 2003). Par conséquent, il est possible que **la partition de BPA et CBZ se produise dans les eaux usées et dans les boues d'épuration des stations de traitement autant dans la phase solide que liquide des boues d'épuration ; et cela, en raison de la valeur intermédiaire du log K_{ow} (2-4 pour BPA et 2.45 pour CBZ)**. A cet effet, la quantification du BPA et du CBZ a été réalisée dans les eaux usées, dans les boues dépuration et dans les parties solides et liquides des boues d'épuration. Ainsi, ces expériences sont nécessaires avant la sélection d'un processus de prétraitement pour l'élimination du BPA et du CBZ des eaux usées et des boues d'épuration.

2. La solubilisation des boues d'épuration, la rhéologie, le potentiel zêta (en cas de dégradation du BPA) et l'accroissement de la matière organique dans les eaux usées (en cas de dégradation de CBZ) jouent un rôle essentiel dans les prétraitements physico-chimiques: le prétraitement des boues d'épuration a été adopté afin d'améliorer la solubilisation des boues et réduire les composés organiques (Weemaes et Verstrae, 1998). Cela suppose qu'avec l'amélioration de la solubilisation des boues lors des processus de prétraitement, le BPA sera transféré de la phase solide à la phase aqueuse, ce qui peut engendrer l'augmentation des protéines solubles, la concentration de carbohydrates solubles dans la phase aqueuse et la diminution des matières en suspension (SS), des solides volatils en suspension (VSS) et de la demande chimique en oxygène (COD) dans la phase solide. En outre, l'augmentation de la COD soluble et du carbone organique soluble (SOC) lors du prétraitement physico-chimique des eaux usées peut résulter de la dégradation plus élevée de CBZ. Il est possible que la rhéologie, la viscosité apparente et la taille des particules affectent l'élimination du BPA à partir des boues d'épuration en diminuant la viscosité et la taille des particules au cours de différentes étapes de traitement dans les stations d'épuration des eaux usées ; cela pourrait améliorer l'adsorption et la désorption du BPA et donc sa dégradation. La diminution de la taille des particules peut également améliorer l'accès du BPA aux enzymes microbiennes et favoriser la biodégradation des polluants au cours du traitement biologique. Cela suppose que le changement du potentiel zêta au cours des différents processus de prétraitement conduit également à modifier la taille des particules. Sur la base de la théorie de DLVO (Derjaguin, Landau, Verwey et Overbeek), une valeur élevée (négative) du potentiel zêta devrait se traduire par une répulsion électrostatique plus grande au sein des flocs, entraînant une hausse de la sensitivité des boues de cisaillement et, par conséquent, des tailles de flocs plus petites (Vogelaar et al., 2005).

3. La dégradation du BPA dans les boues d'épuration par la laccase produite par le *Sinorhizobium meliloti*: La laccase est une oxydase bleue capable d'oxyder les phénols et les amines aromatiques en réduisant l'oxygène moléculaire en eau par un système multi-cuivre (Hublik et Schinner, 2000). Par conséquent, cela suppose que la laccase est capable de dégrader le BPA à partir des boues d'épuration. Les produits possibles formés lors de la dégradation du BPA par la laccase sont présentés ci-dessous:



4. Le prétraitement physico-chimique pour la dégradation du BPA et du CBZ peut produire des sous-produits qui sont plus toxiques que les composés parents: Différents prétraitements physico-chimiques tels que les ultrasons, l'oxydation de Fenton, l'ozonation et la photocatalyse pour la dégradation du BPA et de la CBZ à partir des eaux usées et des boues d'épuration peuvent produire différents sous-produits toxiques dans le milieu. Alors, il est nécessaire de caractériser ces sous-produits et tester leur toxicité dans le milieu.

3.2. Objectifs de recherche

L'objectif global de la recherche est *la dégradation du bisphénol A et de la carbamazépine lors du prétraitement physico-chimique et de la bioconversion des boues d'épuration*. L'exécution de l'objectif global comprend des séries d'objectifs spécifiques à réaliser dans le cadre de travaux expérimentaux présentés ci-dessous:

1. Une méthode de développement pour la quantification du BPA et de la CBZ dans les eaux usées et les boues d'épuration en utilisant, respectivement, les méthodes LC-MS/MS et LDTD-APCI-MS/MS;
2. La détermination des concentrations de fond du BPA et de ls CBZ dans différentes opérations unitaires de traitement des eaux usées et de production de boues d'épuration: la détermination de la concentration et du bilan massique dans une station d'épuration des eaux usées au cours des différentes saisons;
3. L'utilisation de divers processus de prétraitement, comme l'hydrolyse alcaline (AH), l'hydrolyse thermale (TH), hydrolyse alcaline thermale (TAH), l'oxydation thermale (TO), l'oxydation alcaline thermale (TAO), l'ultrasonication (US), l'oxydation de Fenton (FO) et la ferro-sonication (FS) pour la dégradation du BPA à partir des boues d'épuration;
4. L'effet du changement de rhéologie (la viscosité et la taille des particules) et du potentiel zêta au cours des divers prétraitements des boues d'épuration sur la dégradation du BPA;
5. L'optimisation du processus de prétraitement de ferro-sonication pour améliorer la solubilisation, la biodégradabilité et la dégradation du BPA à partir des boues d'épuration en utilisant la méthode de réponse de surface;
6. L'optimisation du processus de prétraitement d'ozonation dans le but d'améliorer la solubilisation, la biodégradabilité et la dégradation du BPA à partir des boues d'épuration en utilisant la méthode de réponse de surface;

7. L'effet de la rhéologie des boues d'épuration au cours des conditions optimales de prétraitement de ferro-sonication et d'ozonation sur la dégradation du BPA;
8. La dégradation du BPA par la laccase produite par *Sinorhizobium meliloti* dans les matières premières et dans chaque boue prétraitée;
9. Différents processus de prétraitement comme l'ultrasonication (US), l'oxydation de Fenton (FO) et la ferro-sonication (FS) et la photo-catalyse pour la dégradation du CBZ à partir des eaux usées;
10. Étudier la formation des différents sous-produits dans les eaux usées et les boues d'épuration lors de la dégradation du BPA et de la CBZ avec des processus de prétraitement optimal et leur toxicité par la méthode de dosage d'œstrogénicité sur levures (YES) ;
11. Pour étudier la dégradation photo-catalytique du BPA et de la CBZ en utilisant des nanoparticules de TiO_2 et ZnO.

3.3. Originalité du travail

A partir des hypothèses précédentes, cette étude affirme son originalité en raison des points suivants:

1. La plupart des études réalisées jusqu'à ce jour ont effectué le bilan massique du BPA et de la CBZ dans les eaux usées et les boues d'épuration en se concentrant uniquement sur les échantillons totaux, négligeant ainsi le partitionnement de la fraction solide et liquide qui est une entité importante de prédiction du devenir du BPA et du CBZ dans les stations d'épuration et, particulièrement, dans les différents processus de traitement d'optimisation à venir.
2. Aucune de ces études n'a été menée sur le développement de méthode de quantification de la CBZ dans les eaux usées en utilisant la méthode LDTD-APCI-MS/MS. L'analyse de la CBZ par la méthode LDTD-APCI-MS/MS a aidé à surmonter l'utilisation traditionnelle de la chromatographie en phase liquide qui comprend l'utilisation de solvants organiques coûteux, le coût de l'entretien des pompes et des colonnes chromatographiques de remplacement. De plus, l'analyse LDTD-APCI-MS/MS a aidé à améliorer la capacité de débit élevée en MS en réduisant les séries de LC-MS/MS de 5 à 30 min de 10 à 30 s dans la série de LDTD-APCI-MS/MS.
3. La majorité des recherches sur les EDCs et les PhACs, y compris le BPA et le CBZ, respectivement jusqu'ici, a couvert leur traitement dans les solutions aqueuses

(principalement dans l'eau). En fait, aucune étude n'a été rapportée sur la dégradation du BPA dans les boues d'épuration par les différents procédés de prétraitement, comme HA, HT, HAT, OT, OAT, US, FO et la FS.

4. La rhéologie (la viscosité et la taille des particules) et le potentiel zêta peuvent affecter la dégradation du BPA à partir des boues d'épuration lors des différents processus de prétraitements. Par conséquent, l'étude de la rhéologie dans les différentes boues prétraitées pour la dégradation du BPA la rend plus originale et intéressante.
5. La biotransformation des boues d'épuration par la croissance de *Sinorhizobium meliloti* et la dégradation simultanée du BPA par la laccase produite rend la recherche proposée originale.

Dans l'ensemble, l'originalité de la recherche proposée est, « *la dégradation concomitante (avec l'évaluation des sous-produits et les tests de toxicité) d'un composé perturbateur endocrinien, le BPA et d'un composé pharmaceutiquement actif, le CBZ présents dans les eaux usées et les boues d'épuration lors du prétraitement et de la valeur ajoutée avec un suivi étroit et une corrélation avec les boues de solubilisation, ainsi que l'augmentation de la matière organique soluble et de la rhéologie.*

PARTIE 4: RESULTATS ET DISCUSSION

La partie résultats et discussion a été divisée en différents chapitres, qui sont reliés de la manière suivante: le développement de méthodes de quantification du bisphénol A (BPA) et de la carbamazépine (CBZ) dans les eaux usées (WW) et des boues d'épuration (WWS), suivie par leurs dégradation par différentes méthodes de prétraitement physico-chimiques telles que l'hydrolyse et d'oxydation avancée. La quantification des contaminants dans les différents compartiments de la station d'épuration des eaux usées permet de déterminer le type de prétraitement ainsi que la sélection des milieux (WW ou WWS) pour le traitement. En outre, l'optimisation des méthodes de prétraitement pour la dégradation du BPA et de la CBZ a été réalisée et l'effet de solubilisation des boues solides, l'augmentation de la matière organique soluble, et le changement de la rhéologie dans cette condition optimale sur la dégradation ont été étudiés. Cependant, ces traitements peuvent parfois aboutir à la production de métabolites plus toxiques que la molécule mère. Pour cela, il est nécessaire non seulement d'étudier la distribution et la transformation du BPA et de la CBZ dans l'environnement, mais aussi de suivre et de caractériser les produits de transformation ainsi que de tester la toxicité de ces produits.

4.1. Développement d'une méthode et analyse du BPA et de la CBZ dans les eaux usées et les boues d'épuration

4.1.1. Présence du bisphénol A (BPA) dans les eaux usées et dans les boues d'épuration des stations d'épuration des eaux usées de la Ville de Québec, Canada

Le BPA a été mesuré dans des échantillons provenant de la Communauté Urbaine des stations de traitement des eaux usées de Québec situé au Québec (Canada) en utilisant la méthode LC-MS/MS. Le BPA a été mesuré dans tous les flux de procédé de la station d'épuration eaux usées. La plus forte concentration de BPA a été détectée dans l'effluent et mesurée à 1,68 mg L^{-1}. Cette forte concentration de BPA dans l'effluent indique que les sources de BPA dans les eaux résiduaires urbaines étaient très importantes. Basé sur une solubilité modérée de l'eau et la faible pression de vapeur, les eaux usées et les résidus de lavage générés lors de la production et de la transformation des matériaux d'application, tels que les polycarbonates et les résines époxy sont les sources industrielles les plus probables du rejet de BPA. Parmi les quatre fractions de boues liquides (des liquides grit, des boues liquides primaires, des boues liquides secondaires et des boues liquides mixtes et épaissies), une forte concentration de BPA (0,79 µg L^{-1}) a été détectée dans le liquide grit et une plus faible concentration (0,15 µg L^{-1}) dans les boues liquides secondaires. Le BPA a été détecté dans tous les types de boues dont les boues solides. Parmi les quatre types de boues (les boues primaires, des boues secondaires et des boues mixtes et épaissies et les boues déshydratées), la plus forte concentration de BPA (0,31 µg g^{-1}) a été trouvée dans les boues primaires et la plus faible concentration (0,10 µg g^{-1}) dans les boues secondaires. La plus forte concentration de BPA détectée dans les boues primaires s'explique par une forte concentration de solides étant donné que le composé a été adsorbé sur les solides en raison de sa valeur élevée du log K_{ow} et de sa nature hydrophobe.

Après une estimation des flux quotidiens de masse de BPA, il a été observé que près de 580 g d^{-1} de BPA a été reçu par la station d'épuration. Après le traitement, environ 24% du BPA a été évacué dans l'effluent, dans un milieu récepteur des eaux de surface (fleuve Saint-Laurent). L'on peut voir que des quantités appréciables de BPA (76%) ont été enlevées par un processus de traitement physico-chimique. En ce qui concerne les résidus solides analysés, les résidus de grit, les boues solides secondaires et mixtes ne représentent pas un résultat important pour le

BPA. En revanche, 4% du BPA ont été retrouvés dans les boues déshydratées, puis incinérées. La réduction du volume de boues par l'incinération produit une pollution environnementale secondaire et elle est très coûteuse. Par conséquent, si l'incinération était arrêtée pour un usage final bénéfique des boues, tel que l'épandage agricole, la présence de BPA pourrait soulever des questions sur la réutilisation des boues avec une possibilité supplémentaire de contamination des nappes d'eaux souterraines.

4.1.2. Carbamazepine dans les eaux usées municipales et dans les boues d'épuration : quantification ultra rapide par désorption thermique par laser diode– ionisation chimique à pression atmosphérique couplée à la *spectrométrie de masse en tandem*

Dans cette étude, la distribution du médicament anti épileptique, le carbamazépine (CBZ), dans les eaux usées (WW) et les phases aqueuses et solides de boues d'épuration (WWS) de la station (Est) de traitement des eaux useés de la Ville de Québec a été etudeés. Une méthode rapide et fiable permettant l'analyse des échantillons à haut débit pour la production plus rapide de données, la détection et la surveillance du CBZ dans les WW et les WWS a été développée et validée. La méthode ultra-rapide (15 s par échantillon) est basée sur la désorption thermique par laser *diode-ionisation chimique à pression atmosphérique* (LDTD-APCI) couplée à la spectrométrie de masse en tandem (MS/MS en anglais). Les paramètres physiques de LDTD-APCI ont été optimisés pour la quantification du CBZ dans les échantillons de WW (l'effluent a été envisagé en raison de la présence d'une forte concentration de CBZ) et de WWS (les boues secondaires ont été envisagées en raison de l'effet faible de la matrice). Le dépôt de solvant, la puissance du laser, le modèle de laser et le débit du gaz vecteur sont des paramètres qui affectent la désorption thermique et l'ionisation des analytes dans de système LDTD. Ainsi, tous les paramètres mentionnés ci-dessus ont été optimisés pour l'analyse du CBZ dans les WW et dans les WWS. Les différentes conditions optimales pour les analyses du CBZ dans les WW et les WWS comprenaient: (a) un solvant optimal utilisé pour le dépôt de l'analyte à l'échantillon avec des cavités de méthanol: eau (2:1, v/v), (b) la puissance de désorption laser de 25% pour les eaux usées et 35% pour les boues d'épuration et ; (c) débit de gaz vecteur de 3 L min^{-1}. Les performances de la nouvelle méthode ont été évaluées par l'estimation de la récupération de l'extraction, de la linéarité, de la précision et de la limite de détection. Les limites de la méthode de détection étaient de 12 ng L^{-1} dans les WW et de 3,4 ng g^{-1} dans les WWS. Les précisions intra-vet inter-journée étaient, respectivement, de 8% et 11% dans les WW et 6% et 9% dans les WWS.

Par ailleurs, trois méthodes d'extraction, l'extraction par ultrasons (USE), l'extraction assistée par micro-ondes (MAE) et l'extraction accélérée par solvant (ASE) avec trois différents solvants tel que le méthanol, l'acétone et l'acétonitrile: acétate d'éthyle (5:1, v/v) ont été comparées sur la base de blanc procédural et la méthode de récupération. Les taux de récupération obtenus à partir de l'utilisation de méthanol, d'acétone et d'acétonitrile: acétate d'éthyle (5:1, v/v) sont de 85,4 à 90,1%, de 80,1 à 86,3% et de 77,6 à 84,2%, respectivement. Les taux de récupération obtenus à partir de MAE avec le méthanol, l'acétone et l'acétonitrile : acétate d'éthyle (5:1, v/v) sont de 91,1 à 95,3%, 82,1 à 87,9% et de 70,0 à 88,2% respectivement. Les taux de récupération obtenus à partir de l'ASE avec le méthanol, l'acétone et l'acétonitrile: acétate d'éthyle (5:1, v/v) sont de 96,9 à 107,0%, de 84.2 à 93.0% et 82.5 à 90.8%, respectivement. Les taux d'extraction les plus élevés de CBZ à partir de différents échantillons de WWS ont été observés avec l'ASE (P <0,01) avec le méthanol comme solvant. En outre, l'applicabilité de la méthode a été testée par la détermination du CBZ dans les WW et dans les WWS des usines de traitement des eaux usées de la ville de Québec. La quantification du CBZ dans les échantillons des WW et des WWS, a montré la présence de contaminant à toutes les étapes de l'usine de traitement.

4.2. Traitement physico-chimique et biotransformation pour la dégradation du BPA dans les eaux usées

4.2.1. Dégradation du BPA (perturbateur endocrinien) au cours du prétraitement et de la biotransformation des boues d'épuration

Diverses méthodes de prétraitement, notamment l'hydrolyse alcaline (AH), l'hydrolyse thermique (TH), l'hydrolyse alcaline thermique (TAH), l'oxydation thermique (TO) et l'oxydation alcaline thermique (TAO) ont été étudiées. Les effets de ces méthodes sur la solubilisation et la dégradation simultanée du bisphénol A (perturbateur endocrinien) dans les boues d'épuration ont été étudiés. Les résultats ont indiqué que le prétraitement des boues d'épuration par HAT a amélioré significativement leur solubilisation (41,6% de matières en suspension (SS), 70,7% de matières volatiles en suspension (VSS) et 48,5% de la demande chimique en oxygène (DCO)) et conduit a un taux de dégradation plus élevé du BPA (38,4%). Cela entraîne une augmentation des concentrations de protéines et de glucides solubles en phase aqueuse et une diminution des SS, VSS et COD en phase solide. Les performances de solubilisation et de dégradation du BPA évoluent selon ordre suivant : TAH>TAO>TO>TH>AH et TAO>TO>TAH>TH>AH. Toutefois, malgré sa grande solubilisation de matières solides et organiques, le HAT est moins efficace en termes de dégradation du BPA. Ce qui expliquerait le choix des procédés d'oxydation avancés (ultrasons, ozonation, oxydation Fenton et photo-catalyse) pour la dégradation des potentiels perturbateurs endocriniens, au détriment de l'hydrolyse.

Les procédés de prétraitement des boues d'épuration influencent généralement le comportement rhéologique des boues, notamment la structure, la force, la taille des flocs de boues et la composition des boues. La viscosité des boues brutes était de 123,8 mPa.s. Cette valeur a diminué à 56,4 mPa.s, 42,8 mPa.s, 26,3 mPa.s, 69,4 mPa.s et 58.1 mPa.s suite aux prétraitements par AH, TH, TAH, TO et TAO, respectivement. La viscosité et la dégradation de BPA ont évolué de la manière suivante : AH>TH>TAH et TAH>TH> AH, respectivement. Ces résultats ont montré que l'augmentation de la solubilisation des boues d'épuration a entraîné la diminution de la viscosité et, par conséquent, l'augmentation du taux de dégradation du BPA. La formation de fines particules a évolué dans l'ordre suivant : TAH>TH>AH. Les particules de petites tailles (26,3 µm et 42,7 µm) observées suite aux prétraitements par TAH et TH sont due à la dégradation des parois cellulaires. La température et pression élevées utilisées au cours du prétraitement par TH et TAH conduisent à la production d'espèces radicalaires. L'élévation de température, diminue l'énergie d'activation et augmente la vitesse des réactions entre le BPA et les espèces radicalaires. Cependant, l'élimination de BPA est plus élevée dans les boues prétraitées par TO et TAO (boues ayant des particules de taille plus grande) comparées aux boues prétraitées par AH, TH, TAH. Cette ambiguïté est principalement due à l'oxydation du BPA par les radicaux hydroxyles au cours du prétraitement par TO et TAO. Les boues prétraitées par TAH ont une potentiel zêta plus élevé comparé à celui des boues prétraitées par AH et TH. Le potentiel zêta élevé des boues de TAH résulte de la formation d'un plus grand nombre de flocs de boues par unité de volume de boues. Lorsque la taille des flocs diminue avec l'augmentation du potentiel zêta, la possibilité que les molécules de BPA adsorbées réagit avec des ions OH^- augmente. Ce qui conduit a un taux de dégradation de BPA élevé pendant le prétraitement par TAH.

Une plus grande activité de la laccase a été observée dans les boues prétraitées par TAH, comparées aux boues prétraitées par TH, TO et TAO. Cette tendance pourrait être due à un nombre plus élevé de cellules *Sinorhizobium meliloti* viables dans les boues prétraitées par

TAH. L'activité maximale de la laccase est observée à 24 h dans les boues prétraitées par TAH et TH, tandis qu'une période d'incubation de 12 h est requise pour les boues prétraitées par TO et TAO. La dégradation du BPA a été observée dans les boues brutes et les boues prétraitées par TH, TAH, TO et TAO, à l'exception des boues prétraitées par AH, dans lesquelles l'activité de laccase est plus faible. Ainsi, la laccase est capable de dégrader le BPA dans ces milieux.

4.2.2. Dégradation concomitante du bisphénol A au cours de l'ultrasonication et l'oxydation Fenton et production de bio-fertilisant à partir des boues d'épuration

L'efficacité du prétraitement (ultrasons 1 (US1), ultrasons 2 (US2), ultrasons 3 (US3), oxydation Fenton (FO), ferro-sonication 1 (FS1) et ferro-sonication 2 (FS2)) a été évaluée en termes de SS, VSS et de matières organiques (demande chimique en oxygène (COD)), de solubilisation et de réduction de la concentration de BPA. L'efficacité des méthodes de prétraitement pour la solubilisation et la dégradation du BPA évolue dans l'ordre suivant: FS2 > FO>FS1>US3>US2>US1 et FS2>FO>FS1>US3>US2>US1, respectivement. Ces résultats montrent que l'augmentation de la solubilisation des solides et des matières organiques au cours du prétraitement par FO et FS conduit à une meilleure dégradation du BPA dans les boues brutes. Une comparaison des six procédés de prétraitement (US1, US2, US3, FO, FS1 et FS2) a été menée afin de comprendre l'importance de la ferro-sonication (FS1 et FS2) pour le prétraitement des SS, VSS, COD et la solubilisation de SOC et la dégradation du BPA. Il a été observé que FS2 est plus efficace (39,7% SS, 51,2% SS, SCOD 64,5% et 17,6% SOC) pour la solubilisation de boue et la dégradation de BPA (82,7%). La solubilisation des solides et de la matière organique et la dégradation de BPA par FS2 était due aux conditions expérimentales, telles que: i) la diminution du pH (pH 3) conduisant à l'hydrolyse acide, ii) l'augmentation de la température dans le milieu (de 20° C à 74° C) pendant l'ultrasonication résultant de l'hydrolyse thermique acide , iii) la formation de H_2O_2 (temps élevé : 180 min) due l'éclatement des bulles et l'effet de mélange, et iv) la grande capacité de production de radicaux $OH^{\bullet}$ due à l'ajout de $FeSO_4$. L'ordre des performances en termes de solubilisation de solides et de la matière organique et de dégradation du BPA était : US3>US2>US1 et US3>US2>US1 respectivement. Des solubilisations de solides et de matière organique (30,7% SS, 41,6% VSS, COD 45,2% et 11,7% SOC) ont été observées au cours du prétraitement par US3 (intensité ultrasons = 15 W cm^{-2}, temps de réaction= 180 min). Cela a conduit à un taux élevé de dégradation de BPA (66,3%).

La viscosité, la taille des particules et la dégradation de BPA ont évolué dans l'ordre suivant US1>US2>US3; US1>US2>US3 et US3>US2>US1, respectivement. Les plus faibles viscosités et tailles des particules ont été observées après le prétraitement par FS2. FS2 permet un taux d'élimination élevé de BPA. La diminution de la viscosité améliore le transfert de masse. Ce qui conduit à une vitesse de réaction plus élevée et un transport plus aisé du BPA, principalement sa partition, entrainant sa dégradation. La diminution de la taille des particules favorise une grande interaction (due à des particules plus fines, ce qui augmente la vitesse de réaction) du BPA avec les radicaux hydroxyles et, par conséquent, la minéralisation du BPA en CO_2 et H_2O. Le potentiel zêta observé dans le prétraitement US3 était supérieur à ceux observés en US1, US2 et US3. Cela a conduit à un taux de dégradation du BPA plus élevé dans les boues prétraitées par US3. La théorie DLVO (Derjaguin, Landau, Verwey et Overbeek) suggère qu'une valeur élevée (négative) de potentiel zêta devrait se traduire par une répulsion électrostatique et une plus grande sensibilité de cisaillement au niveau de la boue, et par conséquent des flocs de petites tailles. Un potentiel zêta élevé conduit à un taux de dégradation de BPA élevé. Le potentiel zêta dans les boues prétraitées par FS2 était plus élevé que les valeurs observées dans les boues prétraitées par FO et FS.

Une plus grande activité de la laccase (207,9 U L^{-1}) a été observée dans les boues prétraitées par FS2, en comparaison aux boues prétraitées par US1, US2, US3, FO, FS1. Cette tendance pourrait être due à un nombre élevé de cellules *S. meliloti* viables dans les boues prétraitées par FS2. Étant donné que *S. meliloti* est une bactérie aérobie, l'augmentation de la solubilisation pendant les différents procédés de prétraitement conduit à une diffusion d'oxygène plus élevée et, par conséquent, augmente le nombre de cellules. L'augmentation du nombre de cellules a entraîné une production plus élevée de laccase qui a permis d'obtenir un taux plus élevé de dégradation de BPA.

4.3. Optimisation des traitements physico-chimiques destinés pour la dégradation du BPA contenu dans les boues d'épuration

4.3.1. Optimisation des paramètres du prétraitement de ferro-sonication per la méthodologie de surface de réponse

Dans cette étude, l'application du modèle de surface de réponse pour prédire et optimiser le prétraitement de ferro-sonication destiné à la dégradation du bisphénol A (BPA) contenu dans les boues d'épuration (WWS) a été étudiée. Le prétraitement de ferro-sonication a été effectué conformément au plan composite central (CCD) à quatre variables indépendantes, telles que les matières solides des boues d'épuration, le pH, le temps de traitement aux ultrasons et la concentration de $FeSO_4$. L'effet du prétraitement de ferro-sonication a été évalué en termes de hausse des matières solides des boues (matières en suspension (SS) et de solides volatils (VS)) et de la matière organique (demande chimique en oxygène (COD) et de carbone organique soluble (SOC)) et la solubilisation et la dégradation simultanée BPA dans WWS. La réponse plus élevée de la dégradation de BPA et de la biodégradabilité a été observée après 180 min d'ultrasonication, tout en utilisant une concentration de $FeSO_4.7H_2O$ de 2,43 mg L^{-1} à pH 3,0 et une concentration en solides totaux de 25 g L^{-1}. En utilisant ces conditions, SS, VS, la matière organique (SCOD et SOC) et solubilisation observée était 43,8%, 56,01%, 61,92% et 15,71%, respectivement. Grâce à ce processus d'optimisation, il a été constaté qu'une dégradation maximale de BPA de 88% pourrait être obtenue après 163 min d'ultrasonication, une concentration de $FeSO_4$ de 2,71 mg L^{-1}, pH 2,81, une concentration de SS de 22 g L^{-1}.

En outre, l'effet du prétraitement de ferro-sonication sur la biodégradation des WWS a également été étudié solides de WWS (SS et VS) et la solubilisation de la matière organique (COD et SOC) a été adoptée comme une mesure de l'efficacité du prétraitement de ferro-sonication afin d'améliorer la biodégradabilité. Il a été observé que le temps d'ultrasonication presénte un effet significatif et la biodégradation la plus élevée (32,5%) a été observée après 180 min d'ultrasonication.

4.3.2. Ozonation partielle pour la solubilisation des boues d'épuration et la dégradation simultanée du bisphénol A: études quantitatives

L'ozonation partielle a été utilisée pour améliorer la solubilisation de la matière organique et la dégradation du bisphénol A contenu dans les boues d'épuration (WWS). La méthode ultra-rapide (15 s par exemple) utilisée pour l'analyse de BPA dans les WWS est basée sur une diode laser de désorption thermique / ionisation chimique à pression atmosphérique (LDTD / APCI) couplée à la spectrométrie de masse en tandem (MS/MS). Les méthodes statistiques utilisées pour les études d'optimisation, la méthode comprend la surface de réponse avec plans factoriels fractionnaires (FFD) et plans composites centrés (CCD). Le prétraitement d'ozonation a été réalisé avec quatre variables indépendantes, telles que la concentration en solides des boues d'épuration (15-35 g L^{-1}), le pH (5-7), la dose d'ozone (5-25 mg g^{-1} de SS) et le temps d'ozonation (10-30 min). La réponse de dégradation de BPA la plus élevée (98,5%) a été observée en utilisant une dose d'ozone de 25 mg g^{-1} de SS, un temps d'ozonation de 20 min, un pH 6 et une concentration en solides de 25 g L^{-1}. En utilisant ces conditions, la solubilisation de SS, VS et l'incrément SCOD et SOC ont été observé étaient 17,8%, 14,8%, 69,2% et 18,1%, respectivement. La dégradation élevée du BPA observée était attribuable à la hausse de la solubilisation de SS, VS, SCOD et SOC. Il a été observé que, parmi toutes les variables étudiées, la dose d'ozone avait une influence plus significative (la probabilité (p) <0,001) sur l'efficacité du prétraitement d'ozonation par l'augmentation de la solubilisation des matières solides (matières en suspension (SS) et solides volatils (VS)) et la matière organique (demande chimique en oxygène (SCOD) le carbone organique soluble (SOC)), et l'augmentation de la degradation du BPA dans WWS.

A partir des résultats obtenus avec différents essais testés, le prétraitement d'ozonation avec des paramètres choisis peuvent être considérés comme un procédé d'oxydation partielle qui a joué un rôle essentiel dans l'amélioration de la solubilisation des solides et des matières organiques dans les boues et la dégradation simultanée de BPA à partir de WWS. Pendant le processus d'optimisation, il a été constaté que plus la dégradation du BPA (100%) pourrait être obtenue en utilisant une dose d'ozone de 26,14 mg g^{-1} pendant 16,47 min et SS de 24 g L^{-1}, pH 6,23. La plus forte dose d'ozone utilisée dans cette étude a été observée pour être rentable en se basant sur la solubilisation des solides et de la matière organique et de la dégradation du BPA.

4.3.3. Ferro-sonication et ozonisation partielle et biotransformation des boues d'épuration suivie par la dégradation du bisphénol A: études de rhéologie

L'effet des propriétés physico-chimiques des prétraitements, y compris les ferro-sonication (FS) et l'ozonation partielle (OZ) sur la rhéologie des boues d'épuration (WWS) a été étudié. Les prétraitements de ferro-sonication et d'ozonisation partielle ont été effectué dans des conditions optimales: 163 min d'ultrasons, 2,71 mg L^{-1} de $FeSO_4.7H_2O$ avec un pH de 2,81 et une dose d'ozone 26,16 mg g^{-1}, une durée d'ozonation de 16,47 min à pH 6,23, respectivement. Parmi les boues prétraitées par FS et OZ avec toutes les concentrations de matières solides (15-35 g L^{-1}), la viscosité la plus faible et la contrainte de cisaillement la plus faible par rapport à un taux de cisaillement ont été observées dans des boues prétraitées par OZ, montrant un comportement rhéofluidifiant important des boues. L'effet du prétraitement d'ozonation sur le comportement rhéologique a été principalement attribuable à la désintégration des agrégats de boues et la perturbation des cellules, conduisant à la rupture de la boue microbienne où les particules en interaction constante, aboutissant ainsi à la diminution de l'influence de la viscosité. Les boues brutes et prétraitées décrivent un comportement non-newtonien et pseudoplastique avec 85% à 97% de confiance de l'ajustement en Bingham, Casson, le pouvoir et les modèles d'IPC pâte pour la concentration de matières sèches allant de 15 g L^{-1} à 35 g L^{-1}. Parmi tous les modèles rhéologiques étudiés, la loi de puissance a été plus marquée (ajustement de confiance de 90% à 97%) pour décrire la rhéologie des boues brutes et prétraitées. Le prétraitement des WWS ont conduit à diminuer en raison de la diminution en indice de consistance (*K*) et d'augmenter l'écoulement comportement de l'indice (*n*).

En outre, les effets des pré-traitement de FS et OZ des WWS sur le changement de taille des particules et sur la dégradation du bisphénol A (BPA) ont également été étudiés. Les résultats ont montré une diminution de la viscosité et de la taille des particules avec une plus grande dégradation du BPA à partir de WWS. La dégradation de BPA par laccases produites par *Sinorhizobium meliloti* dans les boues brutes et prétraitées a également été déterminée. Une plus grande activité de la laccase (366 U L^{-1}) a été observée dans les boues partiellement traitée à l'ozone, ce qui augmente l'élimination du BPA (0,16 mg g^{-1}) de WWS. Les résultats présentés dans ce manuscrit sont potentiellement intéressantes pour les chercheurs afin d'étudier le comportement des boues d'épuration au cours de l'épuration des eaux usées et l'élimination des composés traces organiques.

4.4. Étude comparative des traitements d'ultrasonication, d'oxydation Fenton et de ferro-sonication pour la dégradation de la CBZ présent dans les eaux usées et étude de test d'œstrogénicité sur levures (YES)

Une étude comparative des traitements par ultrasons (US), oxydation de Fenton (FO), ferro-sonication (FS) (combinaison d'ultrasonication et d'oxydation Fenton) et procédés d'oxydation avancée (POA) pour la dégradation de la carbamazépine (CBZ) dans les eaux usées (WW) a été réaliseé pour la première fois. Parmi les différents traitements effectués (US, FO et FS), la plus importante augmentation de la demande chimique en oxygène soluble (SCOD) et du carbone organique soluble (SOC) (63 à 86% et de 21 à 34%, respectivement) a été observée au cours du processus de prétraitement de FO, ce qui a abouti a une augmentation de l'élimination de la CBZ (84 à 100%) des eaux usées. L'augmentation de SCOD et SOC pendant les prétraitements de Fenton et de ferro-sonication par rapport au prétraitement à ultrasons était due à une hydrolyse acide et à une oxydation partielle (radicaux hydroxyle) de la matière organique. L'ordre de l'efficacité des méthodes de prétraitement pour l'augmentation de SCOD et SOC et la suppression simultanée de CBZ était: FO3 > FO2 > FO1 > FS3 > FS2 > FS1 > US3 > US2 > US1 et FO3 > FO2 > FO1 >FS3 >FS2 >FS1 > US3> US2> US1, respectivement.

Par ailleurs, l'analyse des sous-produits formés au cours des prétraitements des eaux usées par US, FO et FS a été réalisée en utilisant la diode laser de désorption thermique-ionisation chimique à pression atmosphérique (LDTD-APCI) couplée avec la spectrométrie de masse en tandem (MS/MS). Les analyses par LDTD-APCI-MS/MS analyse ont indiqué la formation de deux sous-produits tel que l' epoxycarbamazepine et l'hydroxycarbamazepine, formés suite à la réaction des radicaux hydroxyle ($OH^{\bullet}$) avec le CBZ au cours des trois pré-traitements. En outre, l'activité oestrogénique des échantillons prétraités par FO et FS contenant la CBZ et ses sous-produits a été analysée en utilisant de test d'œstrogénicité sur levures (YES). La sensibilité et la reproductibilité de l'œstrogénicité sur levures ont été évaluées en mesurant la réponse de la levure à la solution de 17β-estradiol et différents échantillons des eaux usées contenant la CBZ. En se basant sur les résultats de ce test, tous les échantillons prétraités n'ont pas montré une activité oestrogénique.

4.5. Dégradation photocatalytique de la CBZ dans les eaux usées à l'aide de nano-cristaux de TiO_2 et de ZnO

Les photocatalyseurs nanométriques ont attiré beaucoup d'attention en raison de leur grande rapport de surface/volume. Ce travail étudie la dégradation photocatalytique de la carbamazépine (CBZ), un composé pharmaceutique persistant dans les eaux usées, en utilisant des nanoparticules de TiO_2 et de ZnO. Les nanoparticules de TiO_2 et de ZnO ont été caractérisées par des images de microscopie électronique à balayage (SEM). Pour tester la photodégradation de CBZ, un film mince de nanoparticules de TiO_2 et ZnO a été introduit dans le réacteur des eaux usées contenant le contaminant, qui a été photoirradié en utilisant une lampe au mercure.

À des intervalles de temps périodiques, des aliquotes d'échantillons ont été retirés, et les spectres d'absorption et de quantification de CBZ ont été effectués. En utilisant des nanoparticules de TiO_2 et de ZnO, la dégradation de la CBZ dans les eaux usées photo-irradiées durant 120 min, était 100% et 92%, respectivement. Cela peut être attribué à la dégradation oxydative de la CBZ par TiO_2 et ZnO suite à l'excitation du film de semi-conducteurs, provoquant ainsi l'apparition de trous d'électrons. Les trous d'électrons photogénérés oxydent la CBZ à la surface du film de TiO_2 et de ZnO. Cependant, la dégradation de la CBZ dans les eaux usées à l'aide de nanoparticules de TiO_2 était plus élevée que celle à l'aide de nanoparticules de ZnO. Cela était dû à la formation d'un nombre plus grand de trous photo-générés, vue que le pouvoir oxydant de TiO_2 est plus élevé. La capacité photocatalytique du ZnO était prévue égale à celle de TiO_2 comme, ils ont le même intervalle de bande d'énergie (3,2 eV).

PARTIE 5: CONCLUSIONS ET RECOMMANDATIONS

5.1. Conclusions

1. L'étude a démontré que le bisphénol A et la carbamazépine étaient présents dans les effluents (0,41 µg L^{-1} et 261 ng L^{-1}, respectivement) et dans les boues déshydratées (0,29 µg g^{-1} et 15 ng g^{-1}, respectivement) de la station d'épuration des eaux usées De la ville de Québec , ce qui remet en cause la décharge des effluents de cette station dans la rivière Saint-Laurent, qui peut avoir des effets physiologiques sur la faune et aussi la réutilisation des boues d'épuration.

2. Parmi les prétraitements de AH, TH, TAH, TO et TAO (ref. Tableau 1, Chapitre IV, Partie 1), la solubilisation la plus élevée de SS, VSS et de COD a été observée après le prétraitement par TAH. Par contre, la dégradation de BPA la plus élevée était observée après prétraitement par TAO en raison de la contribution de l'oxydation par les radicaux OH• et le traitement thermo-alcalin.
3. Le nombre de cellules viables de *Sinorhizobium meliloti* a augmenté dans les boues prétraitées TH, TAH, TO et TAO et la croissance la plus élevée ($4,4 \times 10^{10}$ UFC mL^{-1}) a été observée dans les boues prétraitées par TAH. Une plus grande activité de laccase a également été observée dans les boues prétraitées par TAH entraînant ainsi une hausse de la biodégradation de BPA (22,9%) par rapport aux boues prétraitées par TH, TO et TAO.
4. Parmi les prétraitements par ultrasons, l'oxydation de Fenton et la ferro-sonication, la solubilisation la plus élevée de SS, VSS, COD et SOC a été observée après prétraitement par ferro-sonication, durant 180 min, ce qui a augmenté la dégradation de BPA (82,7%).
5. Le nombre de cellules viables de *S. meliloti* a augmenté dans les boues prétraitées par US1, US2, US3, FO, FS1 et FS2 (ref. Tableau 1, Chapitre IV, Partie 2) et la croissance la plus élevée ($4,4 \times 10^{9}$ UFC mL^{-1}) a été observée dans les boues pré-traitées par FS2. L'activité la plus élevée de la laccase a été également obtenue dans les boues prétraitées par FS2, entraînant ainsi une hausse de la dégradation de BPA (16,2%) par rapport aux autres boues prétraitées.
6. Les conditions optimales élaborées par le modèle de surface de réponse afin d'obtenir une dégradation maximale de bisphénol A (88%) par prétraitement par ferrosonication des boues sont les suivantes: durée d'ultrasonication = 163 min, concentration de $FeSO_4 . 7H_2O$ =2,71 mg L^{-1}, pH= 2,81 et SS= 22 g L^{-1}.
7. Les conditions optimales mises au point par le modèle de surface de réponse afin d'obtenir une dégradation de bisphénol A de 100%, après la prétraitement des boues d'épuration par ozonation sont les suivantes: SS= 24 g L^{-1}, pH =6,23, dose d'ozone= 26,14 mg g^{-1} et durée d'ozonation=16,47 min.
8. La loi de puissance a décrit les caractéristiques d'écoulement de boues brutes et prétraitées dans toute la gamme de concentration en matière sèche (15 à 35 g L^{-1}). La variation des constantes du modèle de puissance, à savoir la diminution de l'indice de consistance (K) et l'augmentation de l'indice de comportement à l'écoulement (n) signifie l'effet des

prétraitements de ferro-sonication et d'ozonation sur la réduction de la pseudoplasticité des boues.

9. Parmi les prétraitements de ferro-sonication et d'ozonation des boues d'épuration, une diminution de la viscosité et de la taille des particules a été observée dans les boues ozonées avec gamme de concentration de solides (15 à 35 g L^{-1}) et une dégradation plus élevée du bisphénol A (de 92,95 à 100%) a été obtenue dans les boues premières.
10. Parmi les prétraitements par ultrasons, oxydation de Fenton et ferro-sonication, l'augmentation la plus élevée de SCOD et de SOC (63 à 86% et de 21 à 34%, respectivement) a été observée au cours de l'oxydation de Fenton des eaux usées, résultant en une importante élimination de la carbamazépine (84 à 100%).
11. Les résultats du test d'œstrogénicité sur levures ont montré que les eaux usées contenant la carbamazépine prétraité par ultrasons, oxydation de Fenton et ferro-sonication n'étaient pas toxiques pour les levures (non oestrogéniques).
12. En utilisant des nanoparticules de TiO_2 et de ZnO, la dégradation de la CBZ dans les eaux usées photo-irradiés durant 120 min était de 100% et 92%, respectivement.

5.2. Recommandations

Á partir des études sur les traitements physico-chimiques et la biotransformation des eaux usées et des boues d'épuration, la dégradation du bisphénol A et de la carbamazépine, les recommandations suivantes peuvent être envisagées:

1. Des recherches plus approfondies sont nécessaires en particulier sur l'application combinée de la méthode de pré-traitement avec un traitement biologique, afin d'étudier l'effet de synergie, d'identifier la toxicité des sous-produits intermédiaires et d'établir leur durabilité environnementale.

2. Plusieurs travaux de la littérature ont étudié l'effet du processus d'oxydation avancée sur la dégradation des contaminants en état de traces, y compris EDC et PhACs dans l'eau potable, les eaux de surface et les eaux usées et ont négligé les boues d'épuration, qui sont des sous-produits essentiels des stations d'épuration des eaux usées. Donc, des études sont nécessaires pour une application pratique de ces procédés de traitements physico-chimiques sur les boues d'épuration.

3. Néanmoins, basées sur les animaux sont nécessaires pour étudier la toxicité des contaminants sur la santé humaine et aussi l'élimination / dégradation du bisphénol A et de la carbamazépine dans les milieux environnementaux contaminés, y compris les eaux usées et les boues d'épuration.

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CHAPITRE II

ANALYSE ET TRAITEMENT DU BPA ET DE LA CBZ

PARTIE 1

PHYSICO-CHEMICAL PRE-TREATMENT AND BIOTRANSFORMATION OF WASTEWATER AND WASTEWATER SLUDGE- FATE OF BISPHENOL A

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RÉSUMÉ

Le Bisphénol A (BPA) est un perturbateur endocrinien très utilisé dans l'industrie du plastique et du papier. En bout de chaine de production, le BPA se trouve éliminé dans les systèmes aquatiques via les usines de traitement des eaux usées (UTEU). L'identification et la quantification du BPA dans les eaux usées (EU) et les boues des eaux usées (BEU) revêt une importance majeure pour évaluer l'activité endocrine des effluents traités et éliminés dans l'environnement. Plusieurs technologies de traitement, incluant un nombre de techniques de prétraitement telles que l'hydrolyse, l'oxydation Fenton, la peroxydation, l'ultrasonication et l'ozonation ont été développés afin de dégrader le BPA dans les EU et les BEU et pour la production de substances à haute valeur ajoutée issues des BEU. Les produits à haute valeur ajoutée issus des BEU, tels que les biopesticides, bioherbicides, biofertilisants, bioplastiques et les enzymes sont des alternatives biologiques à faible cout pouvant concourir avec les composés chimiques et d'autres composés biologiques à couts élevés. Cependant, ce domaine d'application est discutable en raison de la présence de ces composés organiques dont on a discuté les différentes possibilités de dégradation. Le prétraitement produit un impact sur la rhéologie ainsi que sur la valeur ajoutée potentielle pouvant être produits à partir des BEU. Ces deux éléments ont été étudiés dans cette thèse. Plusieurs techniques analytiques disponibles pour la détection du BPA dans les EU et les BEU sont aussi discutées. La présence de métaux lourds et les possibles comportements thermodynamiques du composé BPA dans les EU et les BEU peuvent avoir une influence majeure sur l'élimination du BPA.

Mots clés : Bisphénol A; Métaux lourds; Prétraitement; Rhéologie; Comportement thermodynamique; Produits à haute valeur ajoutée

ABSTRACT

Bisphenol A (BPA), an endocrine disrupting compound largely used in plastic and paper industry, ends up in aquatic systems via wastewater treatment plants (WWTPs) among other sources. The identification and quantification of BPA in wastewater (WW) and wastewater sludge (WWS) is of major interest to assess the endocrine activity of treated effluent discharged into the environment. Many treatment technologies, including various pre-treatment methods, such as hydrolysis, Fenton oxidation, peroxidation, ultrasonication and ozonation have been developed in order to degrade BPA in WW and WWS and for the production of WWS based value-added products (VAPs). WWS based VAPs, such as biopesticides, bioherbicides, biofertilizers, bioplastics and enzymes are low cost biological alternatives that can compete with chemicals or other cost intensive biological products in the current markets. However, this field application is disputable due to the presence of these organic compounds which has been discussed with a perspective of simultaneous degradation. The pre-treatment produces an impact on rheology as well as value-addition which has been reviewed in this paper. Various analytical techniques available for the detection of BPA in WW and WWS are also discussed. Presence of heavy metals and possible thermodynamical behavior of the compound in WW and WWS can have major impact on BPA removal, which is also included in the review.

Keywords: Bisphenol A; Heavy metal; Pre-treatment; Rheological behavior; Thermodynamical behavior, Value-added products

1. Introduction

The growing population, urbanization and modernization has led to the release of toxic organic compounds into the environment, including the endocrine disrupting compounds (EDCs). Interest in EDCs has risen over the last few years as new, highly sensitive analytical technologies have been developed to be capable of detecting these compounds at extremely low levels (nano and pico-levels) in the environment. These compounds are called "endocrine disruptors" as exposure to them results in disruption of normal endocrine function with possible adverse health impacts.

According to Canada Gazette (2007), bisphenol-A (BPA) is a high priority organic compound for assessment of human health risk as it is considered to present greatest potential for human exposure (GPE) and has been classified by other agencies on the basis of reproductive toxicity. BPA has been identified as EDC by the U.S Environmental Protection Agency (EPA), World Wide Fund for Nature (WWF) (Zhao et al., 2003) and is becoming a social issue of increasing interest (Manfred et al., 2004; Bautista-Toledo et al., 2005). According to EPA, "BPA is an exogenous agent that interfaces with synthesis, secretion, transport, binding, action or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction or behavior". Such concerns have heightened the need for novel and advanced remediation techniques to effectively remove BPA from a variety of contaminated environmental media including water, WW, WWS, sediments and soils.

Sewage entering the WWTPs is increasing in complexity day by day due to the addition of new contaminants which form principal load of the influents. BPA can end up in the municipal and industrial wastewater treatment system, either through direct discharge into sewers or via stormwater run-off. Furthermore, pre-treatment of WW and WWS is adopted to destroy the organic compounds and improve the solubilization of sludge (Weemaes and Verstraete, 1998). Various methods employed for sludge pre-treatment include, mechanical treatment (Tiehm et al., 2001), chemical treatment (Pham et al., 2009), thermo-alkaline treatment (Vlyssides and Karlis, 2004), oxidative treatment (Genc et al., 2002) and radiation treatment (Lafitte-Trouque′ and Forster, 2002). Basically, the choice of either one of these methods depends on the cost of the process and other factors, such as concentrations and volume of the effluent to be treated. Although conventional biological treatments have been reported to be able to reduce some EDCs in WW (Behnisch et al., 2001; Jiang et al., 2005; Ying et al., 2008), a large portion of the

EDCs are in fact removed by adsorption (Marttinen et al., 2003), and their sorption on the sludge causes further concerns for sludge management (Belgiorno et al., 2007).

Recently, WWS has been subjected to reuse for production of value-added products (VAPs) through the route of bioconversion. Bioconversion of WWS into VAPs (biopesticides or other bio-control agents, microbial inoculants, industrial enzymes, bioplastics and other biopolymers) has been envisaged with successful and encouraging results (Brar et al., 2007, 2008 a,b; Verma et al., 2007 a,b; Subramanian et al., 2008). In fact the pre-treatment of WWS and subsequent bioconversion into VAPs allows sludge volume reduction, improvement of sludge quality and potential gains from microbial product sales. In order to protect the ecosystem and end users or create public awareness on beneficial uses of WWS, it must be free from any kind of EDCs including BPA. Hence, it is required to study the fate of BPA during or after pretreatment of WW and WWS and secure an innovative practice of production of WWS based VAPs, free from BPA. The purpose of this review is to provide information on removal of BPA during various pre-treatment processes of WW and WWS and different analytical methods available for detection of the compound in the contaminated medium. This article also reports the relationship between heavy metals, rheology and thermodynamics with BPA removal which plays an important role in its removal, partitioning and transport. Finally, the review also discusses the possibility of BPA removal during WWS based VAPs production and the conventional modes of WWS management.

2. Sources of BPA

BPA has been detected in influent and effluent as well as WWS in WWTPs (Korner et al., 2000; Furhacker et al., 2004) and is released into the environment through sewage treatment effluent (Meesters and Schroder, 2002), landfill leachate (via hydrolysis of BPA from plastics (Wintgens et al., 2003)), or natural degradation of polycarbonate plastics due to moderate water solubility and low vapor pressure. Fig. 1 presents various point and non-point sources of BPA in the environment and its daily contact with the living organisms. BPA is also used as a reactive agent in the production of temperature-sensitive paper with color developing layers. Therefore, paper mill effluents (either paper making or recycling) and recycling paper products, such as toilet paper (contributing significantly to wastewater) must also be considered as a major source for BPA in WW and WWS (Rigol et al., 2002; Gehring, 2004). BPA was also found in groundwaters from agricultural and industrial wells due to leaching of this compound (Latorre et al., 2002). The contaminant may also be transferred from different sources to food via: i) food processing by contact with plastics, resins, lacquers, surfactants, and paints from pipes, gaskets and containers, and ii) migration from packaging and bottling material, envelopes, and printer ink.

Table 1 presents different isomers of bisphenol with their physical and chemical characteristics. Among all the isomers, BPA comes out to be more important and significant on the basis of use and toxicity (Staples et al., 1998). Thus, it provides the base for many studies related to the fate, effects and exposure of the compound. Release of BPA to receiving water streams is minimized by the increased installation of WWTPs depending on the specific treatment conditions (Cespedes et al., 2008). Fig. 2 presents a typical set-up of a WWTP showing the possible steps responsible for BPA release/transformation. The compound is reintroduced into the environment through WWS landfills/dumps leaching, agricultural application and incineration process. The compound in its native or transformed form can, thus, enter the food chain when treated or dewatered WWS is used as fertilizer or soil conditioner.

3. North American scenario

BPA is identified as an organic compound with higher potential for human exposure due to its dispersion and high volume presence in the WW and WWS of North American countries. Approximately 0.78 million tons of BPA are synthesized and used as a raw material for the manufacture of polycarbonate plastic and epoxy resins in the United States per year, leading to BPA designation as a high production volume chemical (Schwartz, 2005). Wide ranging global studies have demonstrated that BPA is present in a diverse range of environmental media (Environment Canada, 2008). The Government of Canada's final assessment of BPA and the accompanying risk management scope document was released on October 18, 2008. BPA has been declared toxic under *Canadian Environmental Protection Act*, both for potential impacts on human health and the damage it could cause to ecosystems.

BPA has been detected in Canadian and U.S. municipal waste treatment products and a high level has been measured in some industrial WWs, most notably those associated with paper and allied products (maximum 0.14923 mg L^{-1}; median 0.00872 mg L^{-1}), chemicals and chemical products (maximum 0.09127 mg L^{-1}; median 0.00150 mg L^{-1}) and commercial laundries (maximum 0.04345 mg L^{-1}; median 0.00656 mg L^{-1}) (Lee *et al.,* 2002).

The results provided by Lee *et al.* (2004) in 1999 and 2000 from four Toronto WWTPs showed evidence that BPA partitioned into the solid and liquid phases within the treatment plants. The measured presence of the BPA in all environmental media, as well as documented presence in Canada and in other parts of the world, indicated that: i) the substance was present in a detection level in WW and WWS and widely distributed in the environment, ii) if the mass balance is taken into consideration, then the rate of entry and quantities entering the environment exceeded the degradation rate, resulting in high detection level, sometimes more than the initial level, particularly in matrix to which it is not directly released, and iii) the substance is stable in metrices, such as WW and WWS and does not break down quickly.

4. Endocrine effects of BPA

Many studies have indicated that BPA is a chemical with a much higher estrogenic potential than previously reported by corporations and regulatory agencies. Recently, significant effects of low doses of BPA were reported in experimental animals including adverse effects in blood levels in animals within and below the average levels in human blood (Nagel et al., 1997; Talsness et al., 2000; NTP, 2001; Melnick et al., 2002; Welshons et al., 2003; vom Sall and Hughes, 2005). Very low doses of BPA were reported to cause proliferation of human prostate cancer cells, cardiovascular disease, type 2 diabetes, and liver-enzyme abnormalities in a representative sample of the adult US population (Wetherill et al. 2002). BPA also accounts for most of the estrogenic activity that leaches from soil to the environment (Vom Saal and Welshons, 2006). Some studies showed that the estrogenicity of BPA is not very high, but given the high concentrations in water, WW and WWS, these may cause deleterious effects on the endocrine system of organisms. The estrogenicity value calculated using the recombinant yeast assay provides a value which can transform "concentration" to "estrogenecity" and thus provide a risk level (Cespedes et al., 2004).

BPA has also been found to have the paradoxical effect to block the beneficial effects of estradiol on neuronal synapse formation (MacLusky et al., 2005) and the potential to disrupt thyroid hormone action (Zoeller et al., 2005). The potential risks of BPA included reproduction and development effects, neurochemical and behavioral effects (Vom Saal et al., 2005). More extensive reviews on endocrine effects can be found in literature (vom Sall and Hughes, 2005; Welshons et al., 2006; vom Sall and Myers, 2008). Although there are not many published studies reporting direct human health effects due to BPA exposure, but its effect on animal suggested that humans may also be affected by BPA through the consumption of contaminated water and foods. There were only two epidemiological studies that potentially linked BPA blood levels with ovarian diseases and pregnancy-miscarriages (Takeuchi et al., 2004; Sugiura-Ogasawara et al., 2005). Thus, BPA has been demonstrated to be toxic and should be given importance on the basis of human health concerns. Nevertheless, more investigation is required to know the carcinogenicity effect of the contaminant on human health and also the removal/degradation of BPA from the contaminated environmental media including water, WW and WWS. Removal/degradation studies represent elaborate analytical methods to precisely estimate the concentration of BPA in these media.

5. Legislation

The federal ministry of health and environment, Canada, announced restriction of imports, sales and advertising of polycarbonate baby bottles containing BPA (CBC news, 2008). In its Risk Management proposal, Health Canada says it plans to further reduce the exposure of newborns and infants under 18 months by banning the use of polycarbonate plastic baby bottles, developing strict limits on BPA in infant formula cans, and developing alternative food packaging. As of the release of National Toxicology Program, U.S. and Health Canada reports in April 2009, ten U.S. states including California, Maryland and New Jersey had legislation pending that would affect the use of BPA and a legislation was introduced that would ban BPA nationally from products for infants (Layton, 2008). In U.S., Chicago was the first city to pass a municipal ban of BPA in baby bottles and sippy cups for children under 3 yrs, which is slated to take effect Jan. 31, 2010.

In the updated 2008 Risk Assessment Report on BPA published in June 2008, the European Commission concluded that products made from BPA, such as polycarbonate plastic and epoxy resins, are safe for consumers and the environment when used as intended (EU Risk Assessment, 2008). On 23rd July 2008, the European Food Safety Authority (EFSA), published an updated opinion on the use of BPA based food contact materials. EFSA concluded that the permanent Tolerable Daily Intake (TDI) of 0.05 mg kg^{-1} body weight (Erickson and Britt, 2008) provided a sufficient margin of safety for the protection of consumer, including sensitive subgroups, such as fetuses and infants. On 10 July 2008, the Norwegian State Pollution Control Authority (SFT) published a recommendation to the Norwegian Ministry of Environment (MoE) to restrict the use of selected substances in consumer products sold in Norway (EU Risk Assessment, 2008). The proposal listed 10 substances recommended for stricter regulation in Norway, and also includes BPA, together with a list of exemptions. Asia, despite being a major manufacturer of products containing BPA, as such do not have any specific regulations for BPA in any type of media or products and this is an important impediment to the global control of BPA.

6. Methods for BPA analysis

Due to the diverse characteristics, the determination of BPA concentration must be carried out in many compartments of the treatment processes and receiving waters. In the WWTPs, influent and effluent are the matrices most often investigated for the detection of the compound. The choice of biological or chemical techniques for determination of BPA level in the medium depends on the required outcome. Biological techniques have been presented in details in several references in literature (Gutendorf and Westendorf, 2001; Tanaka et al., 2001; Gomes et al., 2003) and it is beyond the scope of this review. This review mainly emphasizes the chemical techniques for the determination of BPA as it helps in quantifying the analyte to trace levels (ng L^{-1} or below). The determination of BPA in WW and WWS includes various steps, such as sample collection, extraction, clean-up and quantification. Fig 3 represents a schematic of the various steps required for the analysis of BPA in these media, by using different analytical methods.

BPA has been analyzed mainly by chromatographic methods, and a few analytical methods are available for the determination of BPA in environmental samples. Gas chromatography–mass spectrometry (GC-MS) (Olmo et al., 1997) and liquid chromatography–mass spectrometry (LC-MS) (Motoyama et al., 1999) methods were reported for the determination of BPA in water samples. Rudel et al. (1998) first analyzed the BPA level in WW by using GC-MS method and later, Lee and Peart (2000) analyzed the compound in WW and WWS by using the same analytical method. Table 2 presents in details the detection of BPA, in WW, WWS and river water of different countries, by using different analytical and extraction methods. River water was also included in the Table 2 as most of the treated effluent of WWTPs ultimately ends up in the aquatic stream.

GC/MS as a commonly used method encompasses a drawback that pre-treatment including a derivatization procedure generally requires several hours to a whole day to be completed. On the other hand, the use of HPLC with various detectors only requires a simple and quick pre-treatment method, such as UV, fluorescence and electrochemical detection (Sajiki, 2001). Among the possible detection methods, MS detection showed significant selectivity when the target compound existed in a complex matrix. LC/MS is an effective procedure for environmental analysis since it has a higher detective selectivity than any other detector adopted for HPLC when the analytes existed in complex matrices such as WWS and sediments. LC-MS/MS systems are highly sensitive and specific (Petrovic et al., 2003; Baugros et al., 2008), popular and powerful tool for quantifying polar compounds, such as BPA more so, at ng or pg levels. Comparison of sensitivity between different analytical techniques could be placed in the order: LC-MS/MS > GC-MS/MS > LC-MS. Laser diode thermal desorption (LDTD) is a new approach to analyze emerging contaminants, such as BPA, where the entire sample is introduced in the mass spectrometer by a rapid laser thermal desorption process and is ionized by atmospheric pressure chemical reactions (LDTD-APCI). LDTD technology combines the ultra-fast thermal desorption of a dry sample with the ionization of the produced neutral molecules in the gas phase without solvent, mobile phase or external matrix. A low sample size thermal desorption and the APCI in "dry" environment, provides the LDTD its tremendous analytical speed and analytical performances. The LDTD ion source works with less than 10 μL of sample (typically 2 μL) and it has the option of simultaneous analysis of 96 samples (www.ldtd-ionsource.com). It has been reported to be used for analysis of drug related metabolites in cytochrome P450 inhibition assay incubation sample and determination of benzene, toluene, ethylbenzene and xylenes in ambient air (Wu et al., 2007; Badjagbo et al., 2009). These two studies demonstrate the impeccable capability of LDTD in carrying out precise and solvent less analysis of organic compounds in different matrices with the possibility of analyzing BPA.

The major problem, in quantitative analysis of environmental samples, is the impact on the analyte signals caused by matrix components. This can be overcome by choosing standard addition as the most suitable quantification method for compensating matrix effects evaluated. Thus, the analytical method is in agreement with the environmental concentrations as low as ng kg^{-1}. Generally, extraction must be carried out before determination of BPA by these analytical techniques. Various procedures available for sample extraction comprise, Soxhlet extraction (SE) (Kim et al., 1999), liquid–liquid extraction (LLE) (Varelis and Balafas, 2000), solid phase extraction (SPE) (Kuch and Ballschmiter, 2001), microwave-assisted extraction (MAE) (Pedersen et al., 1999), ultrasonication (Aparicio et al., 2007) and solid-phase microextraction (Salafranca et al., 2003). Extraction of BPA from WWS by Soxhlet extraction technique is time consuming and requires large volumes of organic solvents. In the last few years, new extraction technique has been established in order to reduce the volume of extraction solvents, extraction time and improve the precision of analytes recoveries. The MAE technique has proven to be more efficient than the Soxhlet extraction by curbing solvent consumption and extraction time (Diagne et al., 2002). Moreover, after reviewing different articles based on extraction methods for the analysis of BPA in WWS, it was found that ultrasonication and MAE are the most suitable ones on the basis of time and solvent concentration used. Generally, SPE method is used for the extraction of BPA from WW. SPE utilizes either disks or more common cartridges. Disks reduce sample clogging and have a large surface area for sample contact compared to cartridges. However, with disks, larger volume of solvents is required for analyte elution increasing the overall method duration. Thus, choice of analytical method is an important step to determine the concentration of BPA prior to selection of the pre-treatment or treatment method to eliminate the same.

7. Pre-treatment and BPA degradation

The available data indicated that BPA does not persist significantly under aerobic conditions (Environment Canada, 2008). However, the substance did not degrade or degraded only slowly under conditions of low or no oxygen. Thus, it seems important to treat WW and WWS, for the removal of BPA, as the oxygen level in these media are very low. Many authors extensively investigated and reviewed sludge treatment and disposal, pointing to the challenges involved and future possibilities of reuse (Werther and Ogada, 1999; Neyens and Baeyens, 2003; Liu, 2003). However, the presence of toxic organic compounds in WWS question sludge recycling making its final disposal problematic. If the sludge is applied to soil, the pollutant could remain in the soil for a long time due to the sorption and slow rate of biodegradation (Wilson et al., 1997). Among the treatment technologies, usually pre-treatment methods (thermal, chemical or mechanical) prior to biological degradation, have been developed in order to improve the WWS recycling and reuse. The pre-treatment enhances the hydrolysis of WWS, which reduces the stabilization time and increases the degree of degradation during biological treatment. WWS pre-treatments rupture suspended solids (microbial cells), liberate the nutrients, partially solubilize the suspended solids, increase the soluble chemical oxygen demand, decrease viscosity and improve the overall WWS biodegradability (Barnabé, 2004; Verma et al., 2007c). Mechanical pre-treatment, using mills, homogenizers, ultrasounds and others, where the necessary energy is provided as pressure, translational or rotational energy was found to be very effective in solubilizing microbial cells but turned out to be rather complicated and expensive (Weemaes and Verstraete, 1998).

Ultrasonication, alkaline hydrolysis, thermal hydrolysis (at neutral or alkaline pH) and partial oxidation through Fenton reaction or ozonation are efficient pre-treatment methods that have been reported to increase WWS biodegradability (Muller, 2000a,b; Neyens et al., 2003a,b,c; Liu, 2003; Odegaard, 2004). However, biodegradability might not be improved if generation of inhibitory substances occurs (Delgenes et al., 2000). Therefore, it is important to optimize the methanogenic potential of the waste to be treated by means of an efficient pre-treatment method (substrate become more accessible for the microorganism) to improve the rate and degree of degradation and similar approach applies to biotransformation of WW/WWS into VAPs. An efficient pre-treatment of sludge should preferably result in: i) reduction of sludge volume; ii) an increased biodegradability of the organic matter; iii) destruction of pathogens; iv) possible degradation of organic compounds; and v) potential use of end product for VAPs, incineration, agriculture and landfill. Other processes, such as stabilization, bulking and foaming, conditioning, dewatering and final disposal may be enhanced with different pre-treatment methods in order to improve the overall efficiency of the treatment methods in WWS (Muller, 2000a).

The degradation of BPA in different media including water, WW and WWS, by the application of different pre-treatment methods are presented in Table 3. It is seen that most of the treatment processes are efficient in removal of the pollutant from the medium. The effective removal of BPA is usually limited to physico-chemical methods, such as photo-catalytic oxidation, Fenton oxidation and ozonation (Deborde et al., 2005; Zhan et al., 2006). However, these processes require the addition of catalysts and oxidants to the solution, which may result in high expenses and secondary pollution (Hu et al., 2002; Korshin et al., 2006). The possible fate of BPA during different pre-treatment methods is presented in Table 4. In general, the techniques available for treating organic pollutants in aqueous solution are very diverse and frequently one or more treatment techniques in sequence or parallel are required to completely degrade these compounds.

7.1. Alkaline, thermal alkaline and acid hydrolysis

Sludge hydrolysis is a way to break the major part of the sludge solid fraction into soluble and less complex molecules, and thus has effects on the viscosity and the filterability of sludge (Anderson et al., 2002), which contribute significantly to pathogen destruction and organic compounds (Barjenbruch et al., 1999; Kepp et al., 2000). Alkaline hydrolysis refers to a process in which pH of sludge is increased up to 12 by adding an alkali and maintained for 24 h. Alkaline and thermal alkaline hydrolysis at alkaline pH has the advantage of sanitizing the sludge and enhancing its dewatering properties. The study conducted by Chen et al. (2001) showed that there is a steep decline in the sludge volume when pH was less than 3, which suggested that the centrifugal dewatering becomes more efficient at lower pH. They also concluded that the optimum pH value for dewatering was 2.5 and further decrease in pH does not improve the dewatering capacity. Brooks (1970) observed solubilization of organic matter in WWS and found that the highest yield of hydrolysis can be achieved at 165-180 ^{0}C and holding time (10-30 min) has little influence on the result. Therefore, chemical pre-treatments are known to increase biodegradability, convert biorecalcitrant molecules to readily biodegradable byproducts and/or detoxify the WW by enhancing the mineralization of the organic matter and possible degradation of recalcitrant compounds during biological treatment.

Other studies on hydrolysis pre-treatment suggested that the method may facilitate EDCs biodegradation in subsequent biological treatment as mesophilic anaerobic digestion, sequential biological reactor etc. However, none of the research studies carried out till date reported hydrolysis pre-treatment of WWS with the precise objective of removing or detoxifying BPA.

7.2. Fenton's oxidation and peroxidation

Fenton's oxidation has been exploited in laboratory and WWTPs for stabilization of organic matter including removal of organic contaminants such as pharmaceutical and EDCs (Tekin et al., 2006; Zhang et al., 2008). The process is composed of four stages (Bigda, 1995): pH adjustment, oxidation reaction, neutralization and coagulation, and precipitation. The organic substances are removed during two stages of oxidation and coagulation. Therefore, the most common technique used for the degradation of organic compounds in different contaminant media is Fenton oxidation, which uses iron salt and hydrogen peroxide under acidic conditions (Bidga, 1995).

$$\mathrm{H_2O_2} + \mathrm{Fe^{2+}} \rightarrow \mathrm{OH^-} + \mathrm{HO^\bullet} + \mathrm{Fe^{3+}} \qquad (1)$$

Study by Pere et al. (1993) showed that Fenton's peroxidation of sludge enhances the dewaterability. Neyens et al. (2002, 2003, 2004) studied effects of pH, temperature, reaction time and H_2O_2 concentration on the dewaterability of sludge yielding a considerable reduction in organic and pathogen concentration. The study by Gozmen et al. (2003) demonstrated that conversion of BPA by Fenton's oxidation increased with increasing Fe^{2+}/BPA molar ratio and replacement of ferrous/ferric pair with cuprous/cupric pair resulted in faster BPA degradation. However, ferrous/ferric pair was more efficient in terms of conversion and total mineralization versus the charge utilized. Although Fenton's reagent has high activity in acidic solutions, it would not be suitable as the use of metallic salts as catalysts induces additional pollution. The formation of oxidation by-products, such as phenol, 1,4-Benzoquinone, 1,4-Dihydroxylbenzene, acetate and oxalate during Fenton's peroxidation (Neamtu and Frimmel, 2006a) and 4-Hydroxyacetophenone, p-Hydroquinone, methylbenzofuran, p-Quinone and phenol during

Fenton's oxidation (Katsumata et al., 2004) may be potentially more toxic which remains to be ascertained.

7.3. Ozonation

Ozone is a very strong oxidizing agent, the fact it is commonly used in WWTPs as a clarifying and disinfecting agent. The mechanism of organic matter removal from the contaminated media including water, WW and WWS, is either direct oxidation by ozone or indirect oxidation by $HO^{\bullet}$ radicals that are formed by the decomposition of ozone in alkaline conditions.

$O_3 + OH^- \rightarrow O_2 + HO_2^{\bullet}$ (2)

$HO_3 \rightarrow HO^{\bullet} + O_2$ (3)

WWS pre-treatment by ozonation causes the hydrolysis and partial oxidation of the organic matter. Therefore, partial *ozonation* is adopted in many treatment plants to avoid a complete oxidation. Ozonation is usually appended with biological treatment process for further treatment of sewage effluent in WWTPs. Some studies have been carried out on the behavior of micropollutants including BPA, pharmaceuticals, and antibiotics during ozonation (Ternes et al., 2003; Huber et al., 2003, 2005; Kim et al., 2007). To remove BPA from the sewage influent prior to their accumulation in sludge is becoming necessary, especially if the agricultural application of WWS is taken into consideration.

Alum et al. (2004) carried out a comparative study on degradation of BPA in water and WW. They monitored the concentration of BPA in WW during 120 min ozonation at conditions similar to those of drinking water. They found that, within 2 s, 99% of BPA was degraded with an *initial* ozone concentration of 30 μM. They also observed that the level of estrogenecity of BPA within the medium increased, decreased and then became stable during initial contact, subsequent contact and after 60 min, respectively. The results signified that an estrogenic response was present in the medium due to the formation of oxidation by-products which were not detected in this study. Various by-products of BPA normally obtained during complete ozonation include, p-tert-butylphenol, 2-methyl-2,3-dihydrobenzofuran, hydroquinone and n-butyl-acetate, none of which are classified as endocrine disruptors (Gultekin et al., 2009). Therefore, partial ozonation can be investigated as a possible strategy which may or may not result in the formation of stable by-products (potential EDCs). Further, the cost of partial ozonation will be relatively lower resulting in enhanced biodegradability and simultaneous degradation.

7.4. Ultrasonication

Several authors have reported the successful application of ultrasonication as pre-treatment process for sludge stabilization (Gonze et al., 1999; Khanal et al., 2007; Jin et al., 2009). Ultrasonication has proven to be an efficient hybrid method (Gogate et al., 2004) for the pre-treatment (Blume and Neis, 2004) and treatment of hydrocarbon contaminated water, being successfully applied for degradation of aliphatic (Hong et al., 1999), aromatic (Nikolopoulos et al., 2004; Mahamuni and Pandit, 2006), polycyclic aromatic compounds (Psillakis et al., 2004) and halogenated hydrocarbons (Drijvers et al., 1999). The $HO^{\bullet}$ and $HO_2^{\bullet}$ radicals generated from water and oxygen dissociation during ultrasonication can be represented by the following Equation:

$$H_2O \rightarrow H + HO^{\bullet} \qquad (4)$$

$$H + O_2 \rightarrow HO_2^{\bullet} \qquad (5)$$

$$O + H_2O \rightarrow 2HO^{\bullet} \qquad (6)$$

$$H + O_2 \rightarrow O + HO^{\bullet} \qquad (7)$$

The hydromechanical shear forces produced by ultrasonic cavitations disrupt the cells in sludge, leading to release of organic substances of sludge into the liquid phase. The literature on BPA degradation, by ultrasonication in WW and WWS is limited. Kitajima et al. (2006) studied the effect of dissolved gasses during sonication of BPA (Table 3) and demonstrated that the rate of degradation was highest in the presence of O_2 and lowest in the presence of N_2 in a comparative study between O_2, Ar, air and N_2. The study demonstrated that radial formation is enhanced in the presence of O_2. Ioan et al. (2007) observed that Fenton process was much faster in the presence of ultrasound as compare to its absence in terms of degradation of BPA and the rate enhanced with increasing concentration of Fe(II) and decreasing pH. The effect of saturating gas (O_2, Ar, air), BPA concentration (0.15-460 μmol L^{-1}), ultrasonic frequency (300-800 kHz) and power (20-80 W) on BPA degradation was studied by Torres et al. (2008). They observed that, for a 118 μmol L^{-1} BPA solution, the best performance was obtained at 300 kHz, 80 W and with oxygen as a saturating gas. They also observed various intermediate products during BPA degradation, such as monohydroxylated BPA, 4-isopropenylphenol, quinine of monohydroxylated BPA, dihydroxylated BPA, quinine of dihydroxylated BPA, monohydroxylated-4-isopropenylphenol and 4-hydroxyacetophenone. After 2 h, these intermediates were converted into biodegradable aliphatic acids.

The characteristics of the investigated sludge itself exerted magnitudinal effect on the final efficiency of pre-treatment (Weemaes and Verstraete, 1998), therefore, sometimes it is difficult to compare the results reported by different researchers. It was observed that oxidation processes are efficient in removing the estrogenic activity of BPA in contaminant media but in some cases, a residual activity still remains after the treatment so that the compound might become more toxic than the parent one. Nevertheless, limited studies carried out till date report partial breakdown products and/or intermediates of BPA after pre-treatment which could be potentially more toxic. Therefore, systematic studies are required to explore the degradation of toxic intermediates after WWS pre-treatment and subsequent biological treatments, such as digestion, composting or biotransformation into VAPs. In addition to many factors that affect BPA removal from WW and WWS, such as type of pre-treatment process, degree of pre-treatment, concentration of BPA and ultimate formation of by-products, presence of heavy metals in WW and WWS; complex rheology and thermodynamics can also affect the BPA removal via partitioning/complexation.

8. Other factors affecting BPA transformation during pre-treatment/bioconversion

8.1. Presence of heavy metals

WW and WWS from industrial wastewater treatment processes and from municipal treatment facilities that receive substantial industrial water and stormwater inputs are enriched in heavy metals and refractory organic species. Research on the fate of BPA in WW including abundant organic substances and heavy metals is important to investigate any adverse effect of BPA on living things (Sajiki, 2001; Kang et al., 2006; Vandenberg et al., 2007). Heavy metals and surface active compounds are commonly coexisting with BPA in contaminated surface water and groundwater systems. Cadmium and lead are well detected in the natural water body, particularly in heavily contaminated WW and in hazardous wastes areas. It has been observed that cadmium causes oxidative damage to cells (Figueiredo-Pereira et al., 2002) and some antioxidants are capable of reversing the deleterious effects of the metal (Almazan et al., 2000).

The photochemical behavior of metal ions presents an important contribution to the transformation of organic pollutants in the aqueous environment. Zhou et al. (2004) observed that the photooxidation efficiencies were dependent on the pH values and ferric/oxalate concentration ratios (Fe(III)/Ox) in the water. Meng et al. (2006) studied the oxidation of phenols catalyzed by a copper (II)-schiff base complex in aqueous solution. Metal ions are important group of enzymatic activity modulators and have been observed, in certain instances, to impact on enzyme activity and treatment efficiency. Kim and Nicell (2006) observed the impacts of Mn(II), Fe(III), Co(II), Ni(II), Cu(II) and Zn(II) ions on BPA conversion in WW with reaction conditions: 25 °C, pH 5.0 (25 mM acetate buffer), 120 μM BPA, 0.15 UmL^{-1} laccase, with a reaction time of 1 h at concentrations extending up to 1.0 mM. They observed that solutions treated in the presence of Co(II), Zn(II), Ni(II), and Mn(II) showed no significant difference in BPA conversion relative to controls. Therefore, these ions are not expected to pose problems for the treatment of real wastewaters with laccase. However, solutions treated in the presence of Cu(II) and Fe(III) experienced reduced BPA conversion relative to controls. From the above literature, it may be assumed that there must be interaction between heavy metal and BPA within the medium and BPA may become more toxic when coming in contact with heavy metal. BPA can also synergize with the heavy metal cadmium, leading to increasing harm to the organisms (Koponen and Kukkonen, 2002). It may combine with heavy metal, form a BPA-metal complex in sewage as WW and WWS is a warehouse of different heavy metals (Lazzari et al., 2000; Alvarez et al., 2002; Singh et al., 2004; Wong and Selvam, 2006; Pathak et al., 2009) and become adsorbed to the sludge. The assumption reaction is presented below:

$$\mathrm{BPA} + \mathrm{metal} \leftrightarrow \mathrm{BPA} - \mathrm{metal\ complex} + \mathrm{BPA} + \mathrm{metal} \qquad (8)$$

$$\mathrm{BPA} - \mathrm{metal\ complex} = ([\mathrm{BPA}_{\mathrm{initial}}] + [\mathrm{metal}_{\mathrm{initial}}]) - ([\mathrm{BPA}_{\mathrm{final}}] + [\mathrm{metal}_{\mathrm{final}}]) \quad (9)$$

The elaborate mechanism of adsorption of complex to the sludge is described in thermodynamics section.

8.2. Rheology of WW and WWS

Sorption and desorption are important factors in estimating the transport, transformation, degradation and fate of BPA in the environment. There have been a number of studies focused on the adsorption/partition behavior of BPA (Akio et al., 2002; Nakanishi et al., 2002; Fent et al., 2003; Clara et al., 2004; Manfred et al., 2004; Zhao et al., 2008). Meanwhile, sorption and desorption also become key parameters for its transport in WWTPs for both WW and WWS. However Fernandez et al. (2009) observed that, 52-100% removal of BPA in a aerobic WWTP was obtained with no accumulation in sludge which showed high solubility of BPA with no partitioning to sludge. This study can be taken as an isolated study as many articles presented the more partitioning of BPA to solid as compare to liquid part due to its high $\log K_{ow}$ value (Staples et al., 1998).

Rheology aids to achieve a better understanding of the flow properties of WW and WWS. WW/WWS flows can be Newtonian or non-Newtonian, but all studies so far have demonstrated non-Newtonian regime (Brar et al., 2006, 2007, 2008; Verma et al., 2007; Pham et al., 2009). Non-Newtonian sludge flows are first encountered in wastewater treatment processes (e.g. activated sludge wastewater treatment processes) in which they are linked with hydrodynamic phenomena, such as stirring or settling and to mass oxygen transfer. Rheological characterization of sludges represents one of the best examples of a fundamental property that has also been correlated to real time processes. In fact, the importance of the apparent viscosity of sludges on their behavior in different processes of both wastewater treatment (in particular on the hydrodynamic and transfers phenomena in aeration and settling tanks) and sewage sludges treatment (disposal, dewatering, physico-chemical conditioning, anaerobic digestion, etc.) cannot be ruled out.

The hydrodynamic phenomenon in a typical unit operation can be easily modeled using apparent viscosity measurements as it influences the flow properties. Further, these measurements can also help in predicting the settling of flocs, oxygen transfer (for an aeration process) and pumping set installations etc. (Battistoni, 2000; Mikkelsen, 2001). WW and WWS suspensions are invariably non-Newtonian fluids, the shear rate being non-linearly related to the shear stress. The most commonly used Equations to represent the non-Newtonian behaviour of sludges suspensions are Ostwald or power law Eq. (10), Sisko Eq. (11), Bingham Eq. (12), Herschel–Buckley Eq. (13) or Casson Eq. (14).

$$\tau = K\gamma^{m} \qquad (10)$$

$$\tau = \eta_{\infty}\gamma + K\gamma^{m} \qquad (11)$$

$$\tau = \tau_{Y}^{b} + \eta_{B}\gamma \qquad (12)$$

$$\tau = \tau_{Y}^{hb} + K\gamma^{m} \qquad (13)$$

$$\sqrt{\tau} = \sqrt{\tau_{Y}^{C}} + \sqrt{\eta_{C}\gamma} \qquad (14)$$

As can be seen from the Eq 10 and 11, the shear thinning of the suspension occurs when *m* < 1. As per the plasticity models given in the value of the yield stress corresponds to the stress needed to be applied to overcome the cohesion Van-der-Waals forces and induce the suspension to flow (Courraze and Gouillard, 1991). Presence of yield stress interferes with mass and heat transfers as noted by Magnin and Piau (1989) and it further increases with the increase in solid volume fraction. Thus, the presence of yield stress will also influence the transport of

BPA. This kind of yield stress effect on organic contaminant transport has been demonstrated in soil clay barriers, packed beds (Balhoff and Thompson, 2004; Lewis et al., 2008).

The rheological changes can also be due to change in surface chemistry of flocs or suspension forming WW / WWS. The changes in surface chemistry can be brought out by pre-treatment and or value-addition (microbial transformation). Pre-treatment processes, such as ultrasonication, Fenton oxidation, hydrolysis, among other are known to bring about a change in the surface chemistry of WW/WWS (proteins and polysaccharides) (Zhang et al., 2006; Stasinakis, 2008). The apparent viscosity value influences the global coefficient of mass transfer in an inverse relation (Schluter and Dekwer, 1992). Such a rheological characterization is essential to achieve a better understanding of the structure and interparticle interactions inside flocs that is needed to optimize all kind of processes involving sludges. These processes are notably the separation processes (settling, dewatering) the oxygen or substrate mass transfers for value-added products and finally the transformation processes for sewage sludges reuse and of all, transport of organic compounds, mainly the partitioning (adsorption-desorption profiles). In fact, the slow mass transfer may limit the overall bioavailability and rates of biodegradation of sorbed BPA systems.

Particle size and surface chemistry are critical to the rheological behavior of WW/WWS (raw/pre-treated/biotransformed). As particle size decreases, surface effects, such as dispersion and flocculation become increasingly of practical concern. Similarly with decreasing particle size, those factors which affect surface behavior, such as surface charge and adsorbed species, become increasingly important. In fact, our previous studies have demonstrated that pre-treatment can result in change in particle size (larger formation of fine particles) (Brar et al., 2006, 2007, 2008; Verma et al., 2006, 2007; Ha et al., 2009). The scavenging of organic compounds is largely influenced by the particle size distribution (Marttinen et al., 2003). Thus, the decrease in particle size on pre-treatment would result in large number of small particles per unit volume of the medium so that the organic compound will be adsorbed on these large surface flocs. Further, during value-addition, different enzymes produced by the microorganisms (as discussed later) will have improved access to the organic compound, such as BPA and finally favor biodegradation of the compound.

Thus, rheology is a conglomeration of two important parameters, apparent viscosity and particle size which will impact the removal of organic compound, such as BPA in several ways: a) decrease in apparent viscosity during pre-treatment will enhance degradation; b) decreased viscosity will increase oxygen transfer during microbial fermentation to produce value-added products and aid in biodegradation; c) decreased particle size during pre-treatment will enhance BPA adsorption and desorption and thus degradation; and d) decreased particle size will improve access by microbial enzymes during value-addition and thus biodegradation.

8.3. Thermodynamics

8.3.1. Adsorption Kinetics

A kinetic model has been proposed for the adsorption of BPA-heavy metal complex to the sludge in the sewage. This model can be helpful to understand the mechanisms of the complex adsorption and evaluate performance of the adsorbent (WWS) on the basis of removal of the compounds. Though the Lagergren model may limitedly reflect the true nature of adsorption kinetics, but it may be considered due to its simplicity and good fit. The kinetics of adsorption of complex to sludge was assumed to be pseudo-first-order and pseudo-second-order. The pseudo-first-order Lagergren model (Lagergren, 1898) is expressed as:

$$\frac{dq}{dt} = K_1 (q_e - q_t) \qquad (15)$$

where; q_e (mg g^{-1}) and q_t (mg g^{-1}) are the amounts of the BPA-metal complex adsorbed on the adsorbent at equilibrium and at any time t, respectively; and K_1(min^{-1}) is the Lagergren rate constant of the first-order adsorption. The model is based on the assumption that the rate is proportional to the number of free sites. Integrating Eq (1) with boundary conditions $q_t = 0$ at t = 0 and $q_t = q_t$ at t = t, yields the linearized version of the model, Equation 2:

$$\ln(q_e - q_t) = \ln q_e - K_1 t \qquad (16)$$

The second ordered kinetic equation based on the assumption that adsorption follows a second order mechanism, whereby the rate of adsorption is proportional to the square of the number of unoccupied sites, can be represented as:

$$\frac{dq}{dt} = K_2 (q_e - q_t)^2 \qquad (17)$$

Where; K_2 (g mg^{-1}min^{-1}) is the second order rate constant. Integrating Eq (3) from t = 0 to t = t and $q_t = 0$ to $q_t = q_t$ and linearization yields Equation 18:

$$\frac{t}{q_t} = \frac{1}{K_2 {q_e}^2} + \frac{t}{q_e} \qquad (18)$$

q_e and K_2 can be obtained from the slope and intercept of the plot of t/q_t versus t, respectively. It is not necessary to determine q_e independently for this model.

8.3.2. Adsorption Isotherm

It is necessary to study the adsorption isotherm in order to know the mechanism of adsorption of BPA- metal complex to the WWS. Adsorption isotherms are plots of the adsorbate (BPA-metal complex) uptake (q_e) (according to Eq (1)) versus the equilibrium concentration (C_e) of the residual adsorbate remaining in the solution (sewage). Langmuir and Freundlich isotherm model may be used to establish a relationship between the amount of complex adsorbed on WWS and its equilibrium concentration on sewage. The Langmuir isotherm model equation, Equation 5 (assumption: the adsorbent surface is homogenous and contains only one type of binding site so that the energy of adsorption is constant) is presented as (Langmuir, 1918):

$$q_e = \frac{Kq_{max}C_e}{1 + Kq_{max}} \qquad (19)$$

Where; q_{max} is the maximum amount of the BPA-heavy metal complex per unit mass of adsorbent when all binding sites are occupied and K (L mg^{-1}) is the constant related to free energy or net enthalpy of adsorption. The linear form of Langmuir model can be presented as Equation 20:

$$\frac{C_e}{q_e} = \frac{C_e}{q_{max}} + \frac{1}{Kq_{max}} \qquad (20)$$

The constants q_{max} and b are evaluated from the slope and intercept of the linear plot of C_e/q_e versus C_e. The Freundlich model (assumption: heterogeneous adsorbent surface with different energy of adsorption) can be presented as Equation 21 (Freundlich, 1906):

$$q_e = K_f C_e^{\frac{1}{n}} \qquad (21)$$

Where; K_f (mg g^{-1}) and n are Freundlich constants related to adsorption capacity and intensity respectively. The linear form of Freundlich model is represented as Equation 22:

$$\ln q_e = \ln K_f + \frac{1}{n}\ln C_e \qquad (22)$$

The constants K_f and 1/n can be evaluated from the intercept and slope of linear plot of $\ln q_e$ versus $\ln C_e$, respectively.

8.3.3. Adsorption thermodynamics

In order to calculate whether the adsorption process is spontaneous or not, thermodynamics parameters including Gibbs free energy (ΔG^0), enthalpy change (ΔH^0) and entropy change (ΔS^0) will be considered. ΔG^0 calculated by Equation 23:

$$\Delta G^0 = -RT \ln K_d \qquad (23)$$

Where; R is the universal gas constant (8.314 J mol^{-1} K^{-1}), T is the temperature (K), and K_d is the distribution coefficient. The K_d value can be calculated using Equation 23 (Unuabonah et al., 2007).

$$K_d = \frac{q_e}{C_e} \qquad (24)$$

Where q_e and C_e are the equilibrium concentration of BPA-heavy metal complexes on adsorbent (mg L^{-1}) and in the solution (mg L^{-1}), respectively. Relation between ΔG^0, ΔH^0 and ΔS^0 can be expressed by Equation 25;

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \qquad (25)$$

Eq (25) can be written as

$$\ln K_d = \frac{\Delta S^0}{R} - \frac{\Delta H^0}{RT} \qquad (26)$$

ΔH^0 and ΔS^0 can be calculated from the slope and intercept, of the plot of $\ln K_d$ versus 1/T respectively. Calculation of thermodynamic parameters will help us understand, whether BPA-metal complex adsorption to WWS is physisorption (role of physical reactions), chemisorption (role of chemical reactions) or a combination of two. By using the adsorption kinetics, isotherm and thermodynamics, it is possible to calculate the process conditions (pH, temperature, time among others) at which the optimum concentration of BPA-metal complex can be adsorbed to the WWS. This will help to determine the possibility of using a pre-treatment process to remove/desorb/transform the BPA-metal complex from WW and WWS.

9. BPA removal during production of VAPs from WWS

There exist three major options for complete sludge management as control at source by process modification; improvement in handling, processing and disposal; and reuse to produce VAPs. Development of processes for the production of VAPs, such as biopesticides, microbial inoculants or industrial enzymes through bioconversion of raw or pre-treated WWS has undergone a substantial progress over the last decade. Value addition to WWS by utilizing it as a raw material for commercially important products, such as biopesticides (Brar et al., 2004; Verma et al., 2005), biofertilizers (Rebah et al., 2002), bioplastics (Yan et al., 2008) and enzymes (Tyagi et al., 2002; Bezawada et al., 2009) is a novel sustainable approach of sludge management.

For final product formulation, although WW and WWS are low cost raw material and sometimes reduce the need for numerous additives (Brar et al., 2006a,b, 2007), the presence of BPA and other organic pollutants in WW and WWS questions the benefits of production of VAPs. During WW treatment, BPA tends to accumulate in WWS due to its hydrophobic nature (Table 1) that favors adsorption onto suspended solid and organic matter. Developments of WWS treatment or value-added processes need to consider removal of the pollutant as it is closely related to WWS beneficial uses. It is important that removal studies must include the fate of the compound as toxic intermediates may be generated during treatment processes that may cause a serious threat to the ecosystem once released to the environment. For bioconversion of WWS into unconventional VAPs, such as biopesticides and microbial inoculants, enzyme production during microbial growth can simultaneously detoxify or degrade toxic compounds. However, the extent to which the microorganisms can degrade the organic pollutant, especially, the degree needs to be determined. In other words, as to if they can be innocuously transformed to non-toxic by-products.

9.1. Capacity of microorganisms to degrade BPA

The possible method of BPA removal also involves the application of microorganisms or their enzymatic systems for the degradation of BPA. Many bacteria are capable of degrading BPA in WW (Spivack et al., 1994). Study by Staples et al. (1998) and Furhacker et al. (2004) showed that, more than 90% of BPA was removed from the influent and effluent of wastewater treatment process by using microorganisms present in WW. Lobos et al. (1992) isolated a Gram-negative aerobic bacterium designated as strain MV-1 from sludge collected from a WW treatment plant at a plastic manufacturing facility. The study showed that MV-1 strain utilized BPA as the sole carbon and energy source and the major pathway produced two primary metabolites, 4-hydroxyacetephenone and 4-hydroxybenzoic acid, and the minor pathway also produced two primary metabolites, 2,2-bis(4-hydrozyphenyl)-1-propanol and 2,3-bis(4-hydroxyphenyl)-1, 2-propanediol. Moreover, from total carbon analysis for BPA, they suggested that 60% of the carbon was mineralized to CO_2, 20% was associated with the bacterial cells and 20% concerted to soluble organic compounds. Ike et al. (1995) isolated *Pseudomonas paucimobilis* FJ-4 from sludge from a WW treatment plant at an epoxy resin manufacturing facility. They reported that only 4-hydroxyacetephenone among four metabolites showed slight estrogenic activity compared with BPA. BPA biodegradation by fungi is limited and caused mainly by lignin-degrading enzymes, such as manganese peroxidase and laccase, which are produced by white rot basidiomycetes fungi (Tsutsumi et al., 2001).

The indigenous microbial flora of WWS can produce panoply of enzymes, such as fungal or bacterial peroxidases, tyrosinases and laccases, which form a group of phenol-oxidising enzymes that degrade or detoxify organic pollutants (Verma et al., 2007b; Subramanian et al., 2008). There have been several reports on the enzymatic treatment of WW using oxidative or hydrolytic enzymes (Ying et al., 2002; Torres et al., 2003; Ikehata et al., 2004; Auriol et al., 2006). An enzyme-catalyzed polymerization and precipitation process has been widely studied as a new method for the treatment of aqueous phenols (Wu et al., 1994), with the bulk of the work reported using peroxidases.

Several researchers proposed that laccases might be useful for removing phenolic contaminants from water or WW instead of peroxidases (Bollag et al., 1988). Laccases have multiple copper atoms in their active sites and utilize molecular oxygen as the oxidant for a variety of phenols, including BPA, to form corresponding reactive quinones (Tanaka et al., 2001). Panda and Gowrishankar (2005) reviewed the role of esterases in detoxification and degradation of organic pollutants, viz., plastics, polyesters, polyethylene glycol adipate, etc. However, application of esterases for WW treatment has not been widely reported yet. Researchers have the option to produce this enzyme from *Streptomyces* sp. (Nishimura and Inouye, 2000), *Pseudomonas* sp. (Kim et al., 2002), *Bacillus* sp. (Kim et al., 2004), *Lactobacillus* sp. (Choi and Lee, 2001), *Thermoanaerobacterium* sp. (Shao and Wiegel, 1995), *Micrococcus* sp. (Fernandez et al., 2004), *Ophistoma* sp. (Calero-Rueda et al., 2002), *Pencillium* sp. (Horne et al., 2002), *Aspergillus* sp. (Giuliani et al., 2001), *Humicola* sp. (Htzakis et al., 2003) and *Sporotrichum* sp. (Topakas et al., 2003). In fact, panoply of these enzymes is also being produced by the microorganisms that are used in value-addition of WW/WWS.

9.2. Value-addition and BPA degradation

Microbial enzymes secreted by commercial microorganisms during value-addition would play a pivotal role in degradation of BPA. Thus, it is interesting to select microorganism cultivable in WWS to obtain VAPs and concomitantly produce enzymes to detoxify or degrade BPA. Table 5 presents some microorganisms with their enzymatic system for the production of VAPs using WWS as a raw material. The value-added product microorganisms possess the potential enzyme system to detoxify or degrade BPA. However, the possibility of generating toxic intermediates should be considered as they might persist in the final product. Hence, it is very important to study the fate of BPA during VAPs production which includes the selection of potential microorganisms, identification of less or more toxic intermediates and the elucidation of the degradation mechanism involved.

WWS based VAPs production processes use different bacterial and fungal strains as value-added producers. Among them, *Trichoderma*, *Rhizobium* and *Bacillus* sp. show higher potency to grow in WW and WWS and produce microbial derivatives used for bio-control and bio-bleaching processes (Brar et al., 2007, 2008 a,b; Verma et al., 2007 a,b). However, *Trichoderma* sp., have not been well exploited due to lack of commercially viable processes, as most of the current processes utilize costly raw materials and have less spore yield, an important factor for their success as bio-control agents (BCAs). Alam and Fakhru'l-Razi, (2003) mentioned about using *Trichoderma* sp. for WW and WWS treatment to degrade organic matter and toxic organic compounds but they did not employ the waste matter as raw material for production of VAPs. Study by Verma et al. (2007a,b) demonstrated that, *Trichoderma viride* is a promising microorganism which produces laccases in WWS and could potentially degrade or detoxify organic pollutants including BPA. *Rhizobium sp.*, *Sinorhizobium meliloti* cells are used as microbial inoculants for leguminous cultures and as BCA for agricultural crops. Laccases produced by *S. meliloti* (Rosconi et al., 2005), possess the potential to degrade or detoxify organic compounds, especially with phenolic groups present in WWS simultaneously during bioconversion of WWS into VAPs. *Bacillus sp.*, can produce enzyme, such as oxidases, dehydrogenases and peroxidases able to degrade organic pollutants in WW and WWS. *Bacillus thuringiensis* produces tyrosinases that could potentially degrade phenolic compounds (Donova et al., 2005; Quan et al., 2005). *Bacillus thuringiensis* bayed biopesticides have been found to degrade an EDC, Dimethyle patholate in a concomitant mode during biopesticides production (Brar et al., 2009). Thus, the value-added product forming microorganisms, such as *Rhizobium sp.*, *Bacillus sp.* and *Trichoderma sp.* do possess the arsenal of enzyme system which can efficiently degrade the organic compounds, such as BPA.

10. Fate of BPA in WWS based VAPs during post application

WWS originated from WWTPs, are rich in organic materials as well as nitrogen and phosphorous, making it an appropriate substrate to be used in agriculture as fertilizer or soil conditioner. WWS is generally disposed by conventional means as in agriculture (land application), incineration, landfills and by other lesser means, such as advanced treatment, beneficial use etc. Conventional options for sewage sludge disposal are being progressively restricted on the soils as they contain (organic and inorganic) pollutants and lack environmental sustainability. The draft European Directive (EC, 2000) revising the 1986/278 Directive on sludge agricultural use imposes stricter limits on heavy metals, organic toxicants and pathogens. Although, the most widely used current disposal practice is landfilling, but this mode will not be sustainable at current or projected levels due to increasing competition for landfill space, higher cost, more stringent environmental standards, and implementation of policies to promote recycling (Metcalf and Eddy, 2003). As a consequence, interest in sludge incineration is increasing as it offers three main advantages: volume reduction of upto 80–90%, destruction of organic micropollutants and pathogens, and energy recovery. The reduction in sludge volume by incineration produces secondary environmental pollution and is cost intensive. During land spreading, some of the pollutants may be transferred to the soil and groundwater resulting in the subsequent transfer to the food chain restricting their future uses. Hence, the adverse environmental effects arising from these conventional uses and presence of toxic compounds in sludge make the way for production of WWS based VAPs, an option that has many advantages, such as market entry of alternate eco-products, lowered greenhouse gases, carbon sequestration among others. One major problem associated with post application of WWS is related to the presence of contaminants, such as toxic organic and inorganic compounds and pathogens (Smith and Tibbett, 2004). EDCs, such as BPA, nonylphenols, alkylbenzene sulfonates are less likely to adsorb to humus and are more easily degraded as compare to polycyclic aromatic hydrocarbons, polychlorinated biphenyls which may adsorb and accumulate due to their low water solubility. However, despite the unlikely contamination of the soils and groundwater by BPA, still its water solubility can enhance the transport in the downstream aquifers. Thus, pertinent detailed studies are required as BPA might enter the soil and hence the groundwater.

Re-use of sludge may also lead to re-circulation of these pollutants to human food supplements and to animal feed via soil as a mediator. Relating the results obtained from spiking soil with BPA to sludge applied organic compounds may be problematic due to influences of the sludge matrix, as sludge applications stimulate soil microbial activity through the addition of nutrients. The ubiquitous presence of *Bacillus* sp. may further enhance degradability of these compounds in the soils, yet concrete and environmentally controlled bioreactor studies are required in this context. In addition to this, there may be ingestion of BPA by the soil inhabiting invertebrates, such as earthworms which can further enhance the degradation or lead to the formation of products which are potentially more toxic (Kizilkaya, 2004). No study to this extent has been carried out so far in details in literature.

11. Conclusions and future challenges

Pre-treatment of WW and WWS involves physico-chemical reactions to increase sludge biodegradability which can be further reused for development of VAPs. The presence of different organic pollutants including BPA, question the reuse and recycle of the sludge. It is essential to obtain knowledge on the efficacy of different pre-treatment methods, used for the removal of BPA and its intermediate by-products, from the contaminated media including WW and WWS. Research has shown that pre-treatment methods, such as ozonation, ultrasonication and Fenton's oxidation, which generate hydroxyl radicals, are promising methods for the destruction of BPA from WW and WWS. However, more investigation is required particularly on the combined application of pre-treatment method with biological treatment to make advantage of synergistic effects, identify the toxicity of intermediate by-products and establish their environmental sustainability. WW and WWS reuse and recycle guidelines have to be upgraded to take into consideration the fate of BPA and other emerging pollutants present in the contaminated media.

Fate studies should focus on the removal pathways and the identification of other metabolic intermediates with potential toxicity especially on intermediate with metals and other organic compounds. Rheology and thermodynamic analysis can play a key role. Therefore, the need of more detailed analytical and toxicological data is crucial at this juncture.

The change in scenario to value-addition of WWS these days has posed altogether different type of challenges especially with respect to their registration and end-use. The value-addition of sludge has resulted in the development of various products, such as biofertilizer, biopesticides, bioherbicides, bioplastics and biocontrol agents. Value-added product microorganisms, such as *B. thuringiensis*, *S. meliloti* and *T. viride* produce enzymes (laccases, esterases, tyrosinases, oxidases, hydrolases) that could potentially degrade BPA and its toxic intermediates in a simultaneous fashion nipping two problems, disposal and toxicity. WWS based VAPs free of BPA, toxic intermediates and other toxic organic pollutants is important to protect the ecosystem, ease commercialization of final product and to spread public acceptance of WWS derived value-added products. Therefore, multidisciplinary studies, involving analytical chemistry, biochemistry, environmental sciences, biology and engineering are key to determine the effects of these value-addition processes on the fate of BPA and its intermediate by-products in WWS.

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Table 1. Physico-chemical characteristics of different isomers of BPA

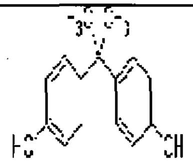
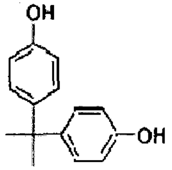
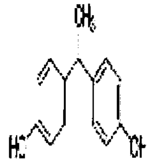
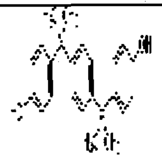
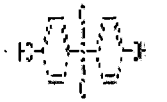
	BPA	BPB	BPE	BPF	BPP	BPS
Structure						
Molecular formula	$C_{15}H_{16}O_2$	$C_{16}H_{18}O_2$	$C_{14}H_{12}O_2$	$C_{13}H_{12}O_2$	$C_{24}H_{26}O_2$	$C_{12}H_{10}O_4S$
Molar mass	228.29 g mol^{-1}	242.312 g mol^{-1}	212.24 g mol^{-1}	200.23 g mol^{-1}	346.46 g mol^{-1}	250.27 g mol^{-1}
Density	1.20 g cm^{-3}	1.33 g cm^{-3}	—	1.174 g cm^{-3}	—	1.36 g cm^{-3}
Water solubility	120-200 mg L^{-1} (20-25 ^{0}C)	Insoluble	—	360 mg L^{-1}	—	1000 mg L^{-1} (20 ^{0}C)
Melting point	158-159 ^{0}C	125 ^{0}C	123-127 ^{0}C	162-164 ^{0}C	193-195 ^{0}C	245-250 ^{0}C
Boiling point	220 ^{0}C at 4mm Hg	412 ^{0}C	383 ^{0}C	—	—	423 ^{0}C
Vapour pressure	87 Pa at 190 ^{0}C	2.66x10^9 Pa (25 ^{0} C)	—	—	—	—
log K_{ow}	3.32	3.27	—	2.91	—	2.15
Appearance	White Crystalline Solid	Odorless slightly brown solid	—	—	—	White to light pink powder
Uses	production of adhesives, protective coatings, powder paints, automotive lenses, thermal paper, paper coatings, protective window glazing, building materials, compact disks, as a developer in dyes, optical lenses and for encapsulation of eletrical and electronic parts	manufacture of phenolic resins	as the monomer for the production of polycarbonate plastics and epoxy resins	in coatings, adhesives, tank linings, flooring, filament winding, casting and pultrusion	reagent for high performance polymer research, colour former	oxidizing agents

Table 2. Country-wise detection level of BPA by using different analytical techniques and extraction methods

Country	Sample	Analytical Techniques	Extraction method	Level of detection	Reference
Canada	Sewage	GC-MS	SPE	1.28 µg L^{-1} (in) 0.18 µg L^{-1} (ef)	Lee et al., 2005
	Sewage	HPLC-MS-MS	SPE	3.78 -74.38 ng g^{-1} (d.w)	Chu et al., 2005
	Sludge	GC-MS	SFE	0.45 µg g^{-1}	Lee and Peart, 2000
Mexico	Wastewater	GC-MS	SPE	0.712-2.86 µg L^{-1}	Gibson et al., 2007
Austria	Sludge	GC-MS	Ultrasonication, SPE	5.8 µg L^{-1}	Fürhacker et al., 2004
Germany	Wastewater	GC-MS	SPE	12205 ng L^{-1} (in) 7625 ng L^{-1} (ef)	Hohne and Puttmann,2008
	Wastewater	GC-MS	SPE	0.4-1 µg L^{-1}	Barun et al., 2003
	Spiked sewage sludge	GC-MS	SE, SFE, ASE	0.125 µg g^{-1}	Meesters and Schroder, 2002
	Sludge	GC-MS/MS	SE	0.004-1.363 mg kg^{-1}	Fromme et al., 2002
	Sludge	HPLC	SE	< LOD	Naassner et al., 2002
South Germany	River water	HRGC-(NCI)-MS	SPE	16 ng L^{-1}	Kuch and Schmiter, 2001
	Sewage	GC-MS	SPE	0.542 µg L^{-1} (in) 0.162 µg L^{-1} (ef)	Korner et al., 2000
Spain	Wastewater	GC-MS/MS	SPE	2.06 µg L^{-1}	Fernandaz et al., 2009
	Wastewater	GC-MS/MS	SPE	0.5 ng L^{-1}	Gomez et al., 2007
	Sewage	LC-ESI-MS	SPE	0.06–1.51 µg L^{-1}	Cespedes et al., 2006
	Wastewater	GC-MS	SPE	---	Ballesteros et al., 2006

Country	Sample	Analytical Techniques	Extraction method	Level of detection	Reference
Spain	Sludge	LC-ESI-MS	Sonication	130 ng g^{-1}	Petrovic and Barcelo, 2000
Sweden	Wastewater	GC-MS	Polystyrene divenylbenzene adsorbent	490 ng L^{-1}	Larsson et al., 1999
Greece	Wastewater	GC-MS	SPE, Sonication	0.15 µg L^{-1}	Stasinakis et al., 2008
	Wastewater and Sludge	GC-MS	SPE and Sonication	0.16 µg L^{-1} (in) 0.22 µg L^{-1} (ef)(city), 1.01 µg L^{-1} (in) 0.13 µg L^{-1} (ef)(hospital), <LOD (in and ef)(University) and 0.62µg g^{-1}	Gatidou et al., 2007
China	River water	GC-MS	SPE	43.5-639.1 ng L^{-1}	Gong et al., 2008
	Sewage	MEC	SPE	32.73 µg L^{-1}	Wu et al., 2008
	Sewage and Sludge	GC-MS	ASE and Ultrasonic	2.5-11.4 ng L^{-1} and 0.3-2.1 ng g^{-1}	Gang et al., 2005
France	Wastewater	GC-MS and GC-MS-MS	SPE	450 ng L^{-1}	Jeannot et al., 2002
Singapore	Sludge	LC-MS-MS	SE and SPE	0.01-2.05 µg L^{-1} (pilot-scale) upto1.20 µg L^{-1} (lab-scale)	Hu et al., 2007
Japan	Sludge and Wastewater	GC-MS	Ultrasonic and SPE	300 ng L^{-1}	Zhang et al., 2008
	River water	LC-MS	CST	5 ng L^{-1}	Watabe et al., 2005
USA	Sludge	LC-MS	SPE	0.31-6.25 µg L^{-1}	Jackson and Sutton, 2008
			SPE	0-147.2 ng L^{-1}	Zhang et al., 2007

Country	Sample	Analytical Techniques	Extraction method	Level of detection	Reference
Switzerland	River water	GC-MS	SPE	9-76 ng L^{-1}	Voutsa et al., 2006
Portugal	River water	GC-MS	SPE	10.7 µg L^{-1}	Ribeiro et al., 2009
	Wastewater and Sludge	LC-MS/MS	SPE and Sonication	0.15-1.55 µg L^{-1}	Mauricio et al., 2006
Italy	Wastewater	HPLC-ESI-MS-MS	SPE	NA	Roda et al., 2006
	River water	HPLC-MS/MS	SPE	0.494 µg L^{-1}	Vigano et al., 2006
The Netherland	River water	GC-MS/MS	SPE	21 µg L^{-1} (summer) and < LOD (spring & autumn)	Belfroid et al., 2002
UK	Crude and Treated wastewater	GC-MS	SPE	1.2 µg L^{-1} and 0.046 µg L^{-1}	Jiang et al., 2005
Republic of Korea	Sewage and River water	GC-MS	CH_2Cl_2 100 mLx3	46.1 µg L^{-1} and 39.4 µg L^{-1}	Ko et al., 2007
	Wastewater	LC-MS/MS	SPE	1 ng L^{-1}	Kim et al., 2007

In: Influent, ef: Effluent, LOD: Limit of detection, HRGC: High-Resolution Gas Chromatography, MEC: Micellar Electrokinetic Chromatography, NCI: Negative Chemical Ionisation, SE: Soxhlet Extraction, SFE: Supercritical Fluid Extraction, ASE: Accelerated Solvent Extraction, LLE: Liquid-liquid Extraction, SPE: Solid Phase Extraction, CLLE: Continuous Liquid-liquid Extraction, CST: Column Switching Technique

Table 3. Effect of pre-treatment/treatment methods on BPA removal

Sample	Pre-treatment/Treatment method	Concentration	Process Condition Parameters	Removal (%)	Reference
Wastewater	Fenton and sono-Fenton	25 mg L^{-1}	25±5 ^{0}C, pH 4-6.5, 50-130 min	---	Ioan et al., 2007
	Photodegradation	520 μM	7 mg L^{-1}(H_2O_2), 1.4-2.5 mg L^{-1} ($FeSO_4.7H_2O$), 43-47 kHz	28	Neamtu and Frimmel, 2006
	Ultrafiltration, Pre-oxidation and Ozone disinfection	12, 5.2 and 5.5 ng L^{-1}	optical path lengh 4 cm , 298 ^{0}K,15 W, 254 nm, 23 L min^{-1} (flow rate), 1.3-8.7 mg L^{-1} (O_3), 0.0625-3.5 mg L^{-1} (H_2O_2)	48, 57 and 61	Snyder et al., 2006
	TiO_2 based photocatalytic suspended system	100 mg L^{-1}	1 h, pH 6	63	Kaneco et al., 2004
		20 mg L^{-1}	1h, pH 3 and 1h, pH 10	95 and 99	Chiang et al., 2004
	Photo-Fenton	10 mg L^{-1}	25±1 ^{0}C, 990 W, 0.5 mW cm^{-2}(LI)	>90	Katsumata et al., 2004
	Ultrasonication	---	pH 6.8-7.5, 20±2 ^{0}C, 500 kHz, 0-100 W	---	Gonze et al., 1999
Sewage	Ozonation	326 ng L^{-1}	0.1 mg L^{-1} DO_3, 0.2 L min^{-1} (flow rate)	100	Zhang et al., 2008
	Primary clarifier and settle tank	1.776 mg L^{-1}	pH 7 to 2.5	88	Korner et al., 2000
Water	Sonochemical degradation	0.022 μmol L^{-1}	300 kHz, 80 W	55	Petrier et al., 2009
	Ozonation	100±1.4 μM	20± 2 ^{0}C, pH 6.5	100	Deborde et al., 2008
	Sequential helio-photo-Fenton and Sonication	0.002 μmol L^{-1}	22 ± 2 ^{0}C, 300 kHz, 80 W, 830 W m^{-2} (II)	---	Torres et al., 2008
	Ultrasonic cavication and Fentons reaction	118 μmol L^{-1}	pH 7.6, 300 kHz, 80W and ferrous sulphate (100 μmol L^{-1}), H_2O_2	---	Torres et al., 2008

Sample	Pre-treatment/Treatment method	Concentration	Process Condition Parameters	Removal (%)	Reference
	Solar UV-irradiation	520 μmol L^{-1}	pH 7.2-7.8, 1000 W, 290-400 nm	2	Espinoza et al., 2007
	Oxidation	1 mg L^{-1}	300 min, 25 ^{0}C, 20 W black light (359 nm, 0.4 mW cm^{-2})	99	Nomiyama et al., 2007
	Photodegradation	50 mg L^{-1}	pH 7 and 10,20 min, lamp (254 nm)	7.1 and 89.7	Wang et al., 2007
	Photodegradation	520 μM	volume of irradiated solution750 ml, optical path lengh 4 cm, 298K, 15 W, 254 nm	35	Neamtu and Frimmel,2006
	O_2-accelerated Sonolysis	50 mg L^{-1}	20 ^{0}C, 491 kHz, 120 W	---	Kitajima et al., 2006
	Photocatalysis (UV and TiO_2)	10 mg L^{-1}	pH 6, lamp (40 W, 144 μW cm^{-2}, 253.7 nm)	100	Thiruvenkatachari et al., 2005
	Photooxidation	2 mg L^{-1}	pH 3.5±0.05, 125 W, 365 nm	90.2	Zhou et al., 2004
	Chlorination and Ozonation	201 ng L^{-1}	1-24 h, 20 ^{0}C, pH 7.5 and 20 ^{0}C, 258 nm	>99	Alum et al., 2004
	Solar photocatalytic	100 μg mL^{-1}	pH 2-10, 10-70 ^{0}C, 0-1000 mg (TiO_2), 0-1.7 Mw cm^{-2} (LI), 60 min	100	Kaneco et al., 2004
	Photocatalysis	20 mg L^{-1}	pH 3, *120 min*, 20 W, *355 nm*	100	Chiang et al., 2004
	UV photolysis and UV/hydrogen peroxide	---	1 kW, 200-400 nm	8.5 and 19	Rosenfeldt and Karl, 2004
	Fentons reagent	0.7 mM	0.20 dm^3(V), 4.28 ($[Fe^{2+}][BPA]^{-1}$)	82	Gozmen et al., 2003
	Ozonation	1-10 mg L^{-1}	pH 2,7,12, 25-30 mg L^{-1} (O_3)	100	Lee et al., 2003
	Photodegradation	0.1 mM	mercury lamp (75W, 2.5 mW cm^{-2}, 360 nm)	---	Watanabe et al., 2003

Sample	Pre-treatment/Treatment method	Concentration	Process Condition Parameters	Removal (%)	Reference
Aqueous medium	US/O_3 and US and O_3	100 µg L^{-1}	25±0.5 ^{0}C, 20 kHz	100, 34.6, 63	Guo and Feng, 2009
	Ozonation	23-57 µM	22±1 ^{0}C, pH 7, 0.8 L min^{-1},	99	Garoma and Matsumoto,2009
	Sonochemical	0.50 mM	10,3 and 2 h, 404 kHz, 3.5,9 and12.9 Kw m^{-2}	100	Inoue et al., 2008
	Ultrasonication	10,40,60,100 µM	300 kHz, 0.19 W Ml^{-1} (UP)	48-94	Gultekin and Ince.,2008
	Photodegradation	2 mg L^{-1}	pH 6, 4 h,70 µmol L^{-1}(Cu^{2+}),15 mg L^{-1}(AsA), high pressure mercury lamp and daylight lamp	59 and 10	Zhange et al., 2008
	H_2O_2-assisted photoelectrocatalytic oxidation	11.2 mg L^{-1}	8 W, 0.68 mW cm^{-2}, 365 nm and 110 W, 48.9 mW cm^{-2}, 450-650 nm	68-99	Xie and Li, 2006
	O_3 and O_3/UV AOT	0.0004 mmol mL^{-1}	pH 5.25, 15 W, 280 nm, sphereclone 5 µm	100	Irmak et al., 2005
	Ozonation	---	pH 2.5-10.5, 20±2 ^{0}C,	>95	Deborde et al., 2005
	Chlorination	1 mg L^{-1}	10.24 and 1 mg L^{-1} (chlorine),5 and 60 min	100 and 90.7	Yamamoto and Yasuhara,2002
	Photocatalysis	90 µg L^{-1}	1h, 15 W (black blue lamp), 0.24 mW cm^{-2} (LI)	98	Nakashima et al., 2002
Distilled water	Photocatalysis	175 µmol L^{-1}	20 h, 200 W (Hg-Xe lamp), 10 mW Cm^{-2} (LI)	100	Ohko et al., 2001

Table 4. Possible fate of BPA in WW and WWS during different pre-treatment methods

Pre-treatment Method	**Possible Fate of BPA**
Alkaline Hydrolysis	Hydrophobicity of BPA may convert to hydrophilicity. It may undergo chemical transformation and hence get partitioned in different phases accordingly
Thermal Alkaline Hydrolysis	Process can split and decompose a major part of the sludge solid fraction into soluble and less complex molecules, as a result BPA gets partitioned which may produce destruction of the compound.
Acid Hydrolysis	Similar to alkaline and thermal hydrolysis, it can transform the compound into simple moieties.
Ultrasonication	BPA at the water interface can be thermally decomposed and a large amount of reactive radicals are generated via the thermal dissociation of water. These may initiate a series of radical reactions resulting in the decomposition of pollutant generating compounds with lower molecular weight and lower toxicity.
Fenton Oxidation	The substance may oxidize, coagulate and mineralize, as a result reduce its estrogenic activity within the solution and potentially less or more toxic organic compounds may be formed.
Partial Ozonation	BPA may react with ozone or OH radical produce by decomposition of ozone in aqueous solution and transformed to simple molecules.

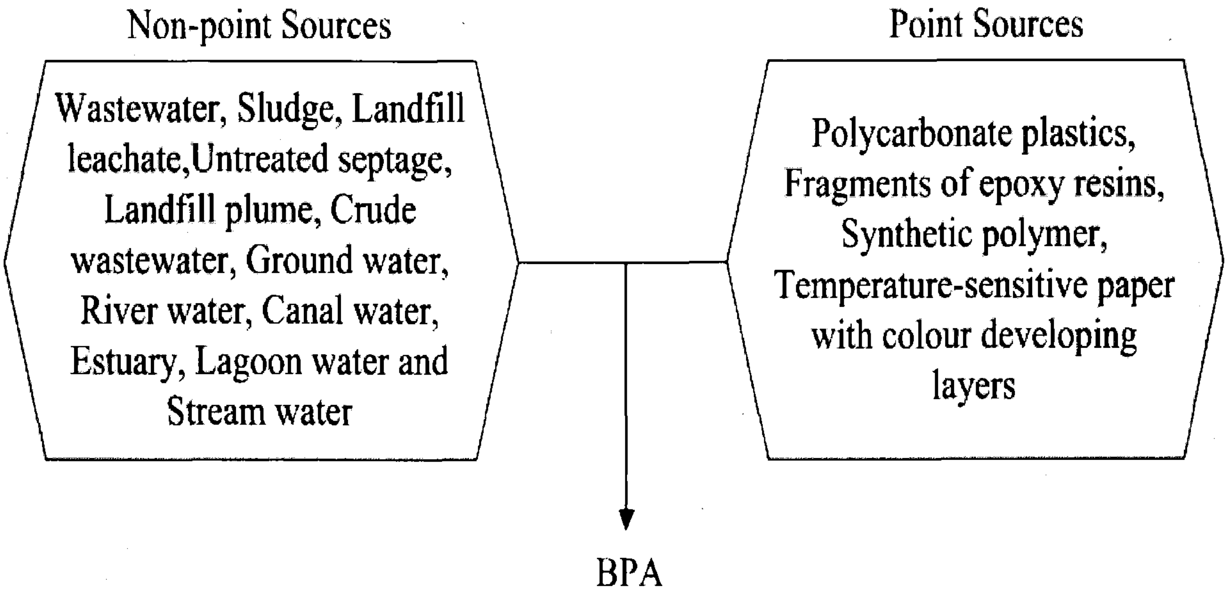


Figure 1. Different point and non-point sources of BPA

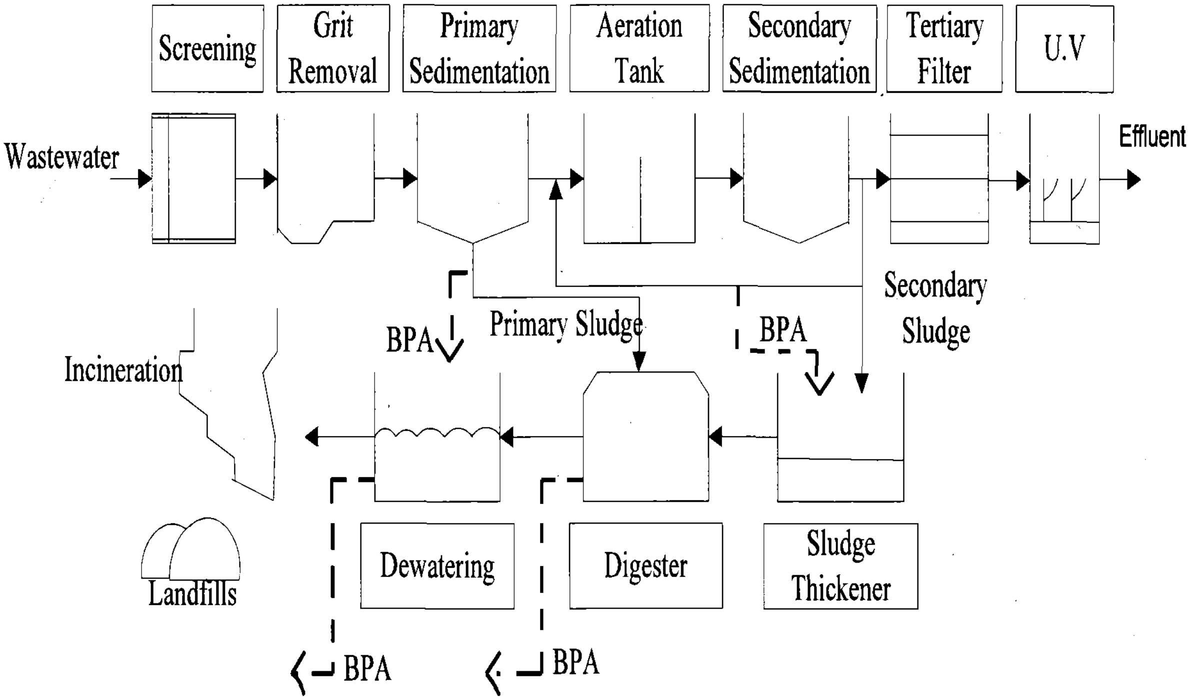


Figure 2. Typical set-up of a WWTP showing WWS production and the arrows with dotted lines indicate BPA release/transformation during various process steps of sludge production

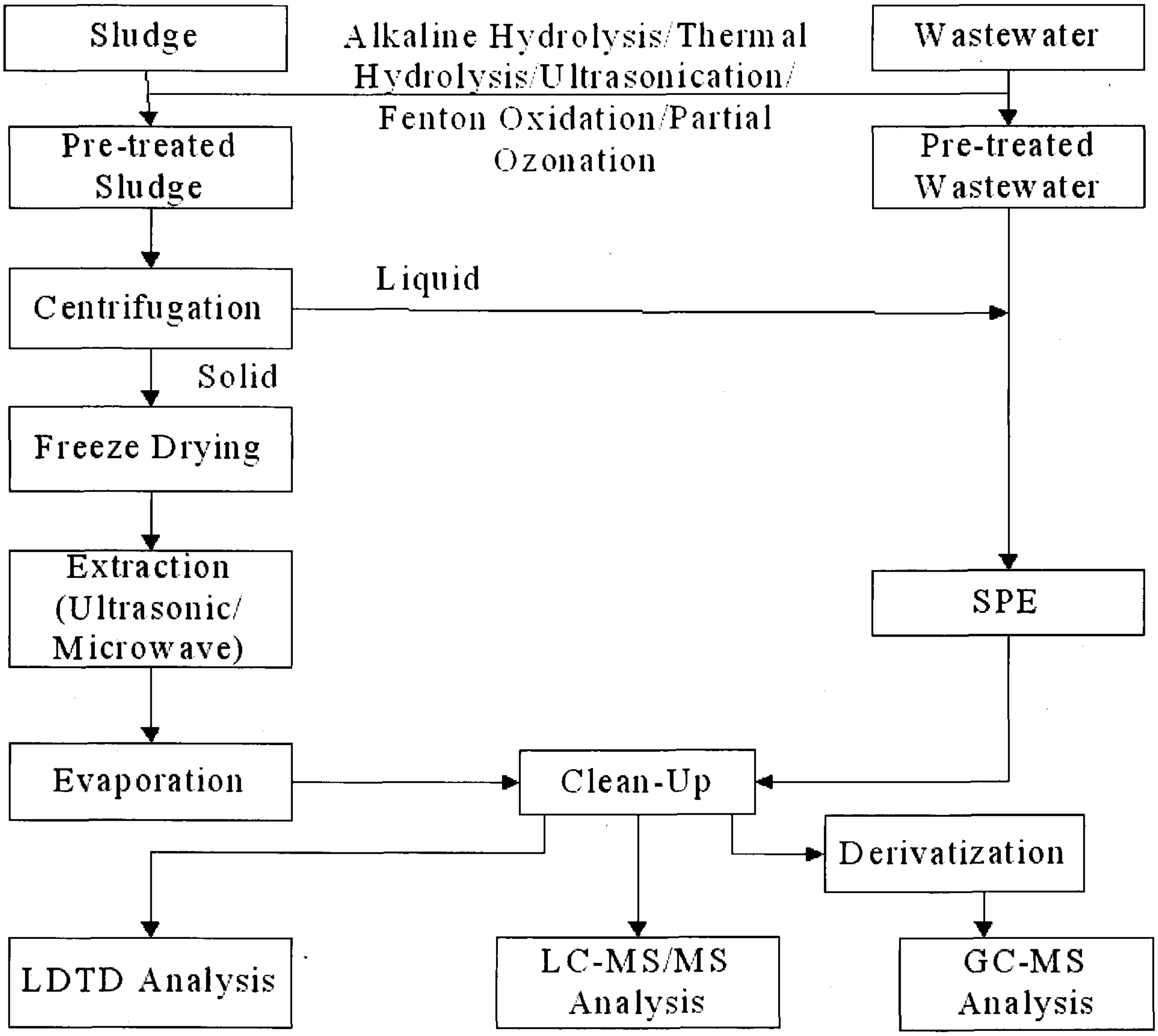


Figure 3. Analytical procedure schematic diagram for analysis of BPA in WW and WWS

PARTIE 2

ANALYSIS AND TREATMENT OF CARBAMAZEPINE IN WASTEWATER AND WASTEWATER SLUDGE: A REVIEW

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ENVIRONMENTAL INTERNATIONAL (UNDER REVIEW)

RÉSUMÉ

Les composés pharmaceutiques actifs (CPA) sont considérés comme polluants émergeants du fait de leur persistance et de l'augmentation de leurs concentration dans les écosystèmes aquatiques. Un des plus fréquemment rencontré est la carbamazepine (CBZ). Celui-ci est émis par des sources variées, comme les stations d'épuration (WWTP). La mesure de la toxicité des effluents de WWTP, comme les boues d'épuration (WWS) et les eaux usées (WW) rejetées dans l'environnement est des plus importantes. En effet, du fait de la réutilisation des BE comme produits à valeur ajoutée, notamment dans l'agriculture pourrait-être remis en cause du fait du risque potentiel pour les écosystèmes et les utilisateurs. Par conséquent, le besoin de développer les connaissances sur les effets et les méthodes de traitement des CPA est primordial. De nombreuses technologies, comme les procédés d'oxydation avancés (POA) ont été développées dans le but de dégrader la CBZ contenue dans les BE et les EU. Ceux-ci utilisent les radicaux libres qui permettent d'oxyder les composés non biodégradables et persistants en différent sous-produits, idéalement, stables et inertes. Cette revue de littérature vise à dresser l'état des connaissances sur la CBZ et, plus précisément, sur ses impacts écologiques, ainsi que sur les méthodes analytiques et les POA permettant la mesure et le traitement de la CBZ dans les BE et les EU.

Mots Clefs : Carbamazepine; Analyse de risque; Quantification; Procédés d'oxydation avancés; Eaux Usées; Boues d'épuration

ABSTRACT

Pharmaceutically active compounds (PhACs) are considered as emerging environmental problem due to their continuous input and persistence to the aquatic ecosystem even at low concentrations. Among them, carbamazepine (CBZ) has been detected at the highest frequency, which ends up in aquatic systems via wastewater treatment plants (WWTPs) among other sources. The identification and quantification of CBZ in wastewater (WW) and wastewater sludge (WWS) is of major interest to assess the toxicity of treated effluent discharged into the environment. Furthermore, WWS has been subjected for re-use either in agricultural application or for the production of value-added products through the route of bioconversion. However, this field application is disputable due to the presence of these organic compounds and in order to protect the ecosystem or end users, data concerning the concentration, fate, behavior as well as the perspective of simultaneous degradation of these compounds is urgently necessary. Many treatment technologies, including advanced oxidation processes (AOPs) have been developed in order to degrade CBZ in WW and WWS. AOPs are technologies based on the intermediacy of hydroxyl and other radicals to oxidize recalcitrant, toxic and non-biodegradable compounds to various by-products and eventually to inert end products. The purpose of this review is to provide information on persistent pharmaceutical compound, carbamazepine, its ecological effects and removal during various AOPs of WW and WWS. This review also reports the different analytical methods available for quantification of CBZ in different contaminated media including WW and WWS.

Keywords: Carbamazepine; Risk analysis; Quantification; Advanced oxidation processes; Wastewater; Wastewater sludge

1. Introduction

Large volumes of pharmaceuticals are used for the prevention, diagnosis and treatment of diseases in human and animals. Pharmaceutically active compounds (PhACs) have become a subject of great interest to environmental researchers worldwide (Hao et al., 2007). The worldwide average per capita consumption of pharmaceuticals per year is estimated to be about 15 g and in industrialized countries, the value is expected to be in between 50 to 150 g (Alder et al., 2006). Due to their extensive use, presence in the aquatic environment and their potential for impacts on wildlife and humans, it is becoming an important environmental issue (Kolpin et al., 2002). To date, most attention has been focused on identification, fate and distribution of PhACs in municipal wastewater treatment plants (WWTPs), which are commonly found at very low concentrations (ppb level or low) (Radjenovic et al., 2009). Many chemical, physical and biological factors may affect the fate of PhACs in WWTPs, including adsorption on biosolids, pH, ionic strength of the sewage, and the physico-chemical properties of PhACs. Hydrophilic compounds may remain in the dissolved form in the aqueous phase of the WWTP effluent, or hydrophobic substances may bind to the biosolids. Hence, these compounds may enter the environment through the discharge of WWTP effluents into receiving water or they may enter the environment in association with biosolids that are ultimately disposed to agricultural lands.

Carbamazepine (CBZ) was the most frequently detected pharmaceutical residue in water bodies (Zhang et al., 2008). CBZ (5H-dibenzo[b,f]azepine-5-carboxamide) is a drug sold under the commercial name Tegratol. CBZ is used alone or in combination with other medications to control certain types of seizures. It is also used to treat trigeminal neuralgia (a condition that causes facial nerve pain). CBZ extended-release capsules (Equetro brand only) are used to treat episodes of mania (frenzied, abnormally excited or irritated mood) or mixed episodes (symptoms of mania and depression that happen at the same time) in patients with bipolar I disorder (manic-depressive disorder; a disease that causes episodes of depression, episodes of mania, and other abnormal moods) (Prajapati et al., 2007). CBZ is in a class of medications called anticonvulsants. It works by reducing abnormal electrical activity in the brain. CBZ is also sometimes used to treat mental illnesses, depression, posttraumatic stress disorder, drug and alcohol withdrawal, restless leg syndrome, diabetes insipidus, certain pain syndromes, and a disease in children called chorea (Miao and Metcalfe, 2003). The physico-chemical properties of CBZ are summarized in Table 1.

Following human administration (excreted unchanged and/or as metabolities with feces and urine), CBZ has been detected in wastewater (WW) and wastewater sludge (WWS). Studies in Europe and North America have shown that CBZ is one of the most frequently detected pharmaceuticals in WWTP effluents and in river water (Ternes, 1998; Heberer, 2002; Metcalfe et al., 2003). Researchers considered that CBZ could be a “witness molecule” confirming the presence and persistence of drugs in water bodies (Clara et al., 2004). Furthermore, pre-treatment of WW and WWS is adopted to destroy the organic compounds and improve the solubilization of sludge (Weemaes and Verstraete, 1998). Various methods employed for sludge pre-treatment include, mechanical treatment (Tiehm et al., 2001), chemical treatment (Bougrier et al., 2006), alkaline and thermo-alkaline treatment (Vlyssides and Karlis, 2004), oxidative treatment (Huber et al., 2003), photocatalytic treatment (Doll and Frimmel, 2004) and radiation treatment (Lafitte-Trouque and Forster, 2002). However, the choice of either one of these pre-treatment methods depends on the cost of the process and other factors, such as concentration and volume of the effluent to be treated.

As WWTPs provide the first and perhaps the most important opportunity for removing CBZ that are destined for discharge into the environment, it is important to characterize the fate of CBZ during the treatment of municipal WW and WWS. Furthermore, in order to protect the ecosystem and end users or create public awareness on beneficial uses of WWS, it must be free from any kind of organic compounds including CBZ. Hence, it is required to study the fate of CBZ during or after pre-treatment of WW and WWS and secure an innovative practice for discharge of WW to river and sludge recycling and reuse. Further, the measurement problems associated with quantification of these pharmaceutical compounds including CBZ in WW and WWS is to detect the analyte in trace levels (ng L^{-1} or below) and to avoid the impact on the analyte signals caused by matrix components. The purpose of this review is to provide information on persistent pharmaceutical compound, carbamazepine, its ecological effects and removal during various pre-treatment and treatment processes (advanced oxidation processes (AOPs)) of WW and WWS. This review also reports the different analytical methods available for quantification of CBZ in different contaminated media including WW and WWS.

2. Sources of carbamazepine and its metabolites

The quantities of pharmaceuticals including CBZ consumed in a region or in the world aid in the estimation of their occurrence in the aquatic environment. Annually, about 1014 tons of CBZ is consumed worldwide (estimated value is in accordance with Intercontinental Marketing Services

(IMS) Health data: 942 tons of CBZ were sold in 2007 in 76 major countries which are believed to account for 96% of the global pharmaceutical market) and this yields more than 30 tons of CBZ which have to be removed from effluents. In Canada, approximately 28 tons of CBZ was sold as prescriptions in 2001 (Zhang et al., 2008).

Transformation of anti-epileptic drug, CBZ in the human body has been extensively studied. Once administered, the absorption of CBZ in the body is slow and unpredictable with approximately 2-3% of the applied dose discharged via urine (Elmquist et al. 1991). Approximately 72% of orally administered CBZ is absorbed, while 28% is unchanged and subsequently discharged through the faeces (RxList, 2006). Studies indicate that the CBZ concentration in the blood plasma peaks about 4 to 8 h after ingestion, but it may take upto 26 h for the CBZ to take effect (Guneysel et al., 2008). After it is absorbed, CBZ is heavily metabolized by the liver, leaving only 1% of dosage in an unaltered form. CBZ is metabolized by the cytochrome P450 system in the liver, producing several metabolites (Guneysel et al., 2008). These metabolites can act to inhibit the pharmaceutically active form of CBZ. The metabolites of this drug undergo enterohepatic cycling and are finally excreted with urine. Three key metabolic pathways have been listed (Breton et al., 2005), with the main route being the formation of carbamazepine-10, 11- epoxide (CBZ-EP), a pharmacologically active compound with anticonvulsivant properties. Further, CBZ-EP is metabolized into 10, 11-dihydro-10, 11- trans-dihydroxy-carbamazepine (DiOH-CBZ). Later, DiOH-CBZ leads to the formation of 9-hydroxymethyl-10-carbamoylacridan. A second metabolic route involves the production of hydroxylated compounds, such as 2-hydroxycarbamazepine (2-OH-CBZ) and 3-hydroxycarbamazepine (3-OH-CBZ). The third metabolic route leads to iminostilbene (IM) formation. Furthermore, within leukocytes, CBZ and IM are metabolized into oxidative products, including acridine (AI) and acridone (AO), which are known to be genotoxic (Bleeker et al., 1999). Other drugs can also interact with CBZ by affecting cytochrome P450 3A4 (CYP3A4) activity (Guneysel et al., 2008). Some of these interactions, for example the usage of simethicone with CBZ may result in toxicity to patients (Guneysel et al., 2008).

Due to extensive use of CBZ in day to day life, the parent compound and some of its metabolites are subsequently released to WWTPs. Due to the high production level and pharmacokinetic behavior of CBZ during normal therapeutic use (e.g., half-life, urinary and fecal excretion, and metabolism), the presence of CBZ and its metabolites have been detected in WWTPs and they were considered to be major source of discharge of CBZ to the environment (Daughton and Ternes, 1999). CBZ has been detected in influent and effluent as well as WWS in WWTP and is released to environment through sewage treatment effluent (Ternes, 1998). CBZ was found in most of the Canadian WWTP effluents at concentrations up to 2.3 µg L^{-1} (Metcalfe et al., 2003), and in German wastewaters up to 6.3 µg L^{-1} (Ternes, 1998). Pharmaceuticals, including CBZ are continuously introduced into the environment and are prevalent at small concentrations (Kolpin et al., 2002), which ultimately affect the water quality and potentially impact drinking water supplies, ecosystem and human health (Heberer, 2002). Effluents from WWTPs contain a variety of pharmaceuticals including CBZ, which are not completely removed in treatment processes and are released into receiving water systems (Carballa et al., 2004). Furthermore, instead of being discharged, effluents are being reused increasingly in many parts of the world, especially in arid and semi-arid countries, for irrigation and/or for aquifer replenishment in coastal regions where aquifers are subjected to overuse. Spreading WW on soil during reuse may result in the transfer of some PhACs including CBZ and their metabolites. Recent studies have revealed that CBZ was present in wastewater-irrigated soil with concentrations ranging from 0.02 to 15 ng g^{-1} dry matter (Kinney et al., 2006). Therefore, it is necessary to treat the effluents containing PhACs including CBZ adequately before discharging or treating the water for drinking purposes by the water treatment plants.

Recently, a hydroxylated metabolite of CBZ, i.e. DiOH-CBZ, has been observed to occur in higher concentrations in WW than the parent compound (Miao and Metcalfe, 2003; Hummel et al., 2006). Other CBZ metabolites, such as CBZ-EP and 2-hydroxycarbamazepine have been detected in wastewater effluents (Leclercq et al., 2009). These studies illustrate that metabolites derived from the human metabolism or microbial activities must be studied in order to gain greater insight into the behavior of pharmaceuticals including CBZ in WW and WWS treatment.

Release of CBZ to receiving water streams is minimized by the increased installation of WWTPs depending on the specific treatment conditions (Kim et al., 2007). The compound is reintroduced into the environment through WWS landfills/dumps leaching, agricultural application and incineration process. The compound in its native or transformed form can thus enter the food chain when treated or dewatered WWS is used as fertilizer or soil conditioner. Furthermore, the octanol-water partitioning coefficient (Log *K*ow) and the distribution coefficient (K_d) between water and secondary sludge of CBZ is 2.45 and 1.2 L kg^{-1}SS, respectively, far from the value 500 L kg^{-1}SS required for significant sorption onto sludge. Therefore, the bulk of CBZ remains associated with the aqueous phase. Further, the researchers assumed that the incineration of WWS can mineralize the presence of small fraction of CBZ and it may be absent from the ash residues. However, if incineration was discontinued for beneficial end use of sludge, such as land application (agriculture) and land spreading, the presence of CBZ might raise question in sludge reuse with further possibility of contamination of groundwater aquifers.

3. Effects– flora and fauna

The concern for human health and potential ecological impacts of PhACs including CBZ even at concentrations as low as ng L^{-1} are becoming a major focus of scientific research (Ferrari et al., 2003). According to the basal cytotoxicity concept, majority of chemicals cause toxicity by means of basal cytotoxicity, while a clear minority causes toxicity by interference with either organ-specific cell functions or extracellular bodily functions. According to this reductionist view, the toxicity of a compound can be broken down into a number of elements, each of which can be identified and quantified in appropriate model systems (Ekwall, 1994). This makes pharmaceuticals a group of environmental pollutants to be taken into account in environmental risk assessment procedures. The occurrence and fate of PhACs in the aquatic environment has been recognized as one of the emerging issues in environmental chemistry (Heberer, 2002). PhACs including CBZ are often released directly into the environment after use owing to a low removal rate in WWTPs, affecting the aquatic ecosystem (Andreozzi et al., 2002). Since PhACs are designed with the intention of performing a biological effect (Henschel et al., 1997), this concept may be very important for these specific substances in terms of fate and effects toward nontarget organisms in the environment, even at low concentrations. Therefore, the probability of the environmental risk from these compounds cannot be ruled out, as it was demonstrated for the conventional "priority" pollutants, especially those acutely toxic pesticides and industrial intermediates displaying persistence in the environment (Daughton and Ternes, 1999). Therefore, it is necessary to evaluate the impact of PhACs including CBZ on the ecosystems where they are present.

Bioassays have typically been used to evaluate CBZ toxicity by exposing test organisms to specific concentrations of this compound. These bioassays are then used to calculate the predicted no-effect concentrations (PNEC) and the results are then compared to the measured environmental concentrations (MEC) (Ferrari et al., 2003; Younghee et al., 2007). Risk characterization is an estimation of the incidence of the adverse effect occurring in an environmental compartment as a result of actual or predicted exposure to a substance. It generally involves a risk quotient calculated between the highest MEC and the PNEC, the environmental level at which no adverse effect on ecosystem function is to be expected.

Younghee et al. (2007) studied the acute toxicity of CBZ on *Vibro fischeri* (a marine bacterium), *Daphnia magna* (a freshwater invertebrate) and *Oryzias latipes* (the medaka fish) via half maximum effective concentrations (EC_{50}) analysis. They observed that EC_{50} values for *V. fischeri* and *D. magna* were 52.5 mg L^{-1} (5 min exposure) and 76.3 mg L^{-1} (96 h exposure), respectively. The lethal doses of CBZ for medaka fish were determined as ranging between 15 to 35 mg L^{-1}. Study by Jos et al. (2003) showed that CBZ was not expected to produce acute toxic effects on the aquatic biota (EC_{50}4.5–383.5 mg L^{-1}). According to the results of their research and the present European legislation on the classification and labeling of chemicals (92/32/EEC), they classified CBZ as "R52/53 Harmful to aquatic organisms and may cause long term adverse effects in the aquatic environment."

Ferrari et al. (2003) studied the toxic effects of CBZ on bacteria, algae, microcrustaceans and fish and observed that CBZ had a limited acute ecotoxicity on the tested organism. Study by Andreozzi et al. (2002) showed no toxicity effect of CBZ on algae *Ankistrodesmus braunii* and also found that the concentration of CBZ progressively decreased in the culture of the algae. They observed that, after 60 days of experiment, over 50% of CBZ had been removed from the medium. Furthermore, no CBZ was observed in the cells of *Ankistrodesmus braunii* during the course of experiments. The author concluded that CBZ was taken up by algal cells and entered into biochemical processes. Dussault et al. (2008) examined the toxicity of CBZ to the midge, *Chironomus tentans* and the freshwater amphipod *Hyalella azteca* (benthic invertebrates) in 10-d waterborne exposures. The results indicated that LC_{50} and EC_{50} of CBZ ranged from 9.9 to 47.3 mg L^{-1}. Other studies about CBZ toxicity have shown similar toxicity to species from various taxa, and measured toxicity values varied from a 7-d EC_{50} of 25.5 mg L^{-1} for the macrophyte *Lemna minor* (Cleuvers, 2003) to a 24-h LC_{50} of 140 mg L^{-1} for the fairy shrimp *Thamnocephalus platyurus* (Nalecz-Jawecki and Persoone, 2006).

CBZ acute toxicity experiments with aquatic non-target organisms indicate EC_{50} concentrations in the mg L^{-1}range (Ferrari et al., 2003), which is much higher than the concentrations found in surface or effluent waters. However, physiological studies with lower, hence environmentally relevant concentrations and sensitive endpoints have shown that CBZ can adversely affect exposed species. Martin-Diaz et al. (2009) reported significant effects of CBZ on biotransformation and antioxidant response, as well as decreased transcript levels of genes encoding proteins involved in the multi-xenobiotic-resistance (MXR) at 0.1–10 µg L^{-1} CBZ in the mussel, *Mytilus galloprovincialis.* Thus, there is evidence that CBZ can impact non-target organism in many ways even at very low concentrations and that more studies are needed to highlight and identify potential effects. Contardo-Jara et al. (2011) studied the adverse effect of CBZ on the invertebrate species *Dreissena polymorpha muscles. They observed that* the bioconcentration factor was highest for mussels exposed to the lowest CBZ concentrations, with 90-fold higher tissue concentration, respectively, after seven days. CBZ caused a significant increase in gill mRNA level of hsp70 after only one day exposure, evidencing the potential of CBZ to immediately provoke a stress condition and assumingly protein damage in gills. After longer exposure, mussels displayed down-regulated mRNA levels of heat shock protein 70 (hsp70) and superoxide dismutase (SOD) in gills, as well as of metallothionein (MT) and P-glycoprotein (P-gp) in the digestive gland, hinting on an inhibitory character of CBZ. Malarvizhi et al. (2012) studied CBZ induced enzymatic stress in gill, liver and muscles of a common carp, *Cyprinus carpio*. They concluded that CBZ induced alterations in the activities of glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and lactate dehydrogenase (LDH) in various organs of fish.

Furthermore, only few studies had used the bioactivity of algae to evaluate the eco-toxicological impact of CBZ (Ferrari et al., 2003; Vernouillet et al., 2010). A large number of ecological indicators and test organisms have been proposed in the past studies, among which green algae are highly sensitive to the contamination of aquatic environments (Eguchi et al., 2004). They are considered as indicators of bioactivity for compounds such as PhACs (Vernouillet et al., 2010). A recent study carried out by Zhang et al. (2012) on eco-toxicological effect of CBZ on *Scenedesmus obliquus* and *Chlorella pyrenoidosa* showed that CBZ could significantly inhibit the growth of the two algae in almost all treatment groups. They observed from the EC_{50} values that CBZ had a relatively limited acute toxic effect (0–144 h) on *S. obliquus* and *C. pyrenoidosa*, while, chronic tests (5–30 d) displayed higher toxicity. Chlorophyll a synthesis was nearly all inhibited by CBZ exposure. However, SOD and catalase (CAT) activities in acute tests of both algae were markedly promoted. Furthermore, the effect of CBZ on the environment is still a mystery as results vary greatly in different studies that have addressed its toxicological impact. A study by Cunningham et al. (2010) on human health risk assessment of CBZ in surface waters of North America and Europe concluded that CBZ and its major metabolites have high margins of safety (MOS) (> 1) and thus should have no appreciable risk to human health through environmental exposures. Nevertheless, more animal based studies required to know the toxic effect of the contaminant on human health and also the removal/degradation of CBZ from the contaminated environmental media including water, WW and WWS. Removal/degradation studies represent elaborate analytical methods to precisely estimate the concentration of CBZ in these media.

4. Methods for analysis of carbamazepine in wastewater and wastewater sludge

Monitoring of PhACs including CBZ in the aquatic environment is progressively becoming a priority for the government agencies and regulatory agencies as well as the general public. One of the major limitations in the analysis of these contaminants remains the lack of methods for quantification of low concentrations. The prerequisite for proper risk assessment and monitoring of these PhACs including CBZ in water, WW and WWS is the availability of multiresidual methods that permit measurement at the low ng L^{-1} level (or even much lower concentrations). Due to the diverse characteristics, the quantification of CBZ concentration must be carried out in many compartments of the treatment processes and receiving waters. In the WWTPs, influent and effluent are the matrices most often investigated for the detection of the compound. The choice of biological or chemical techniques for quantification of CBZ in the medium depends on the required outcome. The quantification of CBZ in WW and WWS includes various steps, such as sample collection, extraction, clean-up and quantification.

The analysis of CBZ in WW and WWS is a challenge due to the presence of the contaminant in trace levels and the complexity of the matrix. Exhaustive extraction, effective clean-up and sensitive detection are necessary to make a successful quantification of CBZ in WW and WWS. Generally, extraction must be carried out before quantification of CBZ by different analytical techniques. Liquid-liquid extraction (LLE) (Rudel et al., 1998), solid-phase extraction (SPE) (Gasser et al., 2011; Mohapatra et al., 2012), and solid-phase microextraction (SPME) (Gil-Garcia et al., 2009) have been generally used to extract CBZ in WW. Among all the extraction methods, SPE has the advantages as: a) relatively less solvent is used; b) convenient operation and; c) good reproducibility (Laven et al., 2009). However, recently Yu et al. (2012) studied the analysis of CBZ in municipal wastewater using ultrahigh performance liquid chromatography-tandem mass spectrometry without SPE pre-concentration. They observed a strong signal enhancement of CBZ for non-SPE pre-concentrated influent and effluent samples. This study can be taken as an isolated study as several articles have presented strong signal enhancement by using SPE. However, more studies are required in order to conclude the signal enhancement with and without SPE during analysis of CBZ in WW.

Further, some of the most frequently used methods to extract CBZ from WWS are Soxhlet extraction (SE) (Sena et al., 2010), supercritical fluid extraction (SFE) (Moneghini et al., 2001), accelerated solvent extraction (ASE) (Jelic et al., 2011; Mohapatra et al., 2012), microwave-assisted extraction (MAE) (Mohapatra et al., 2012), and ultrasonic extraction (Yu and Wu, 2012; Mohapatra et al., 2012). In 1994, automated Soxhlet extraction (Soxtec, commercially) was approved by the US Environmental Protection Agency (USEPA) as a standard method. The extraction was carried out in three stages, boiling, rinsing and solvent recovery. However, extraction of CBZ from WWS by Soxhlet extraction technique is time consuming and requires large volumes of organic solvents. As an alternative to Soxhlet extraction, ultrasonic extraction has also been widely used for the extraction of CBZ from WWS. The cavitation process produced by the ultrasound bath reduces considerably the extraction time used in Soxhlet extraction method. However, some studies represented ultrasonic extraction as less reproducible as compared to Soxhlet (Luque-Garcia and Luque de Castro, 2003). Further, SFE was introduced in the 1980s as an alternative extraction method with the advantages of reduced solvent consumption and extraction time compared to the Soxhlet and ultrasonic extraction technique (Farre et al., 2010). Carbon dioxide is commonly used as a fluid and methanol is added as organic modifier for extraction of organic compounds including CBZ from WWS. However, there are very few applications of SFE technique for the extraction of CBZ from WWS.

In the last few years, various new extraction techniques have been established in order to reduce the volume of extraction solvents, extraction time and improve the precision of analyte recoveries. The MAE and ASE technique has proven to be more efficient than the Soxhlet extraction by curbing solvent consumption and extraction time (Mohapatra et al., 2012). MAE utilizes microwave energy, which is a non-ionizing radiation that causes molecular motion by migration of ions and rotation of dipoles to heat the solvent and sample for extracting pollutants including CBZ from WWS. MAE is gaining an important role in sample preparation techniques because it offers greatly reduced usage of organic solvents and extraction times and also increases sample numbers through the use of multi-vessel systems that allow simultaneous extraction of multiple samples.

Further, ASE is performed at temperatures in the range of 40-200 °C to enhance the speed of elution and pressures in the range of 1000-2500 psi to keep the solvents in liquid state. The increase on the extraction temperature can promote higher analyte solubility by increasing both solubility and mass transfer rate. Besides, the high temperature decreases the viscosity and surface tension of the solvents, which helps to reach areas of the matrices more easily, improving the extraction rate (Ramos, 2012). This technique gained wide acceptance since it provided quantitative extraction with short extraction time. The technique is also simple to learn and apply in laboratory. Moreover, after reviewing different articles based on extraction methods for the analysis of CBZ in WWS, it was found that ASE is the most suitable technique on the basis of time and solvent concentration used and also for high reproducibility (Mohapatra et al., 2012).

To date, numerous analytical procedures have been developed for quantification of CBZ in WW and WWS and often include the use of chromatography (gas or liquid) coupled to mass spectrometry (MS) or tandem MS (MS/MS). Separation techniques include gas chromatography (GC) and liquid chromatography (LC), while for detection, MS is the technique most widely employed. However, due to the low volatility of CBZ (polar PhACs), GC-MS analysis requires an additional derivatization step, which makes sample preparation laborious and time consuming and also increases the possibility of contamination and errors. Moreover, CBZ is thermolabile and decomposes during GC analysis (CBZ forms iminostilben as degradation product) (Ternes, 2001). However, a recent study by Yu and Wu (2012) showed the possibility of quantification of CBZ in WWS using GC-MS method after derivatization with *N-tert*-butyldimethylsilyl-*N*-methyltrifluoroacetamide (MTBSTFA). On the other hand, the use of high performance liquid chromatography (HPLC) with various detectors only requires a simple and quick pre-treatment method, such as UV, fluorescence and electrochemical detection. Recently, Chong and Jin (2012) used HPLC for quantification of high concentration CBZ (5000 μg L^{-1}) in hospital WW. However, in order to attain quantitation limits within the lower ng L^{-1} range (or below) for CBZ in the environmental matrices (eg. WW and WWS), advanced techniques such as LC-MS/MS or LC-electrospray ionization tandem MS (LC-ESI-MS/MS) are essential (Ternes, 2001).

As a result, use of LC-MS/MS and LC-ESI-MS/MS for analysis of CBZ in WW and WWS is increasing. Ternes (2001) indicated that LC-MS/MS is the technique of choice for assaying polar PhACs including CBZ and their metabolities, when reviewing the principal methods for the analysis of PhACs in aqueous environmental samples. LC-MS/MS systems are highly sensitive and specific (Petrovic et al., 2003), popular and powerful tool for quantifying acidic pharmaceuticals, such as CBZ in WW more so, at ng or pg levels (Bahlmann et al., 2012). Further, LC-ESI-MS/MS has commonly been applied as a selective and sensitive tool for the detection CBZ in WW and WWS because of the polar, thermolabile and non-volatile nature of the contaminant (Clara et al., 2004; Miao et al., 2005; Kim et al., 2007; Gebhardt and Schroder, 2007; Calisto et al., 2011; Lee et al., 2012). Niessen (1998) reported that approximately 95% of LC-MS work uses ESI or atmospheric pressure chemical ionization (APCI) as the ionization source, however ESI is most commonly used in the quantification of CBZ in environmental matrices such as WW and WWS. Table 2 presents in details the detection of CBZ in different environmental matrices of different countries, by using different analytical and extraction methods.

Hence, using LC-MS with ESI is a suitable technique since it does not require any derivatization procedure and the identification and quantification can be performed in one single step. However, one of the main drawbacks of using LC-MS/MS for the analysis of trace organics including CBZ in complex matrices (e.g. WW and WWS) is the matrix effect. The matrix effect is caused by the high concentration of matrix ionizable components, such as natural organic matter, salts, ion-paring agents, non-target and target contaminants that can interfere with the ionization processes, especially when ESI interface is used (Liang et al., 2003). Matrix effects are observed when the signal intensity of an analyte detected in a field sample is different from the signal intensity detected in a solvent solution (Miao and Metcalfe, 2003; Hernando et al., 2004) and may result in a signal suppression or enhancement, leading to low sensitivity and inaccurate results. Therefore, to ensure the reliability of the performed method, the evaluation of the matrix effect is widely considered in the literature as a part of the method validation.

There are several analytical approaches that are used in the literature for reducing potential matrix interferences. The best approach to compensate for matrix effects is the use of isotopically labeled internal standards, which elute from the separation column at a similar retention time and undergo similar conditions in the ionization source. Recent studies investigating the fate of CBZ in WW and WWS have incorporated the use of internal standards into their methods to correct the matrix effects (Clara et al., 2004; Miao et al., 2005; Kim et al., 2007; Calisto et al., 2011). Matrix effects can also be addressed by using a standard addition approach, improving clean-up and extraction procedures for removal of interfering components, reducing the flow rate of the sample matrix into the ionization source and decreasing the injection volume (Hernando et al., 2004; Gomez et al., 2006).

Although sample preparation steps for analysis of CBZ in WW and WWS are time consuming, the LC step also requires several minutes, increasing the overall analysis time and data generation. Several alternative techniques, such as laser diode thermal desorption (LDTD) (Mohapatra et al., 2012), direct analysis in real time (DART) (Cody et al., 2005), desorption electrospray ionization (DESI) (Takats et al., 2004), and atmospheric pressure matrix-assisted laser desorption/ionization (AP MALDI) (Laiko et al., 2000) coupled to tandem MS have eliminated the use of an LC step prior to detection. These techniques helps in reducing analysis time and sample pre-treatment and overcome the traditional use of LC which composes use of expensive organic solvents, cost of maintenance of chromatography pumps and replacing chromatography columns.

LDTD is a new approach to analyze emerging contaminants, such as CBZ in different environmental matrices including WW and WWS. This innovative design allows a rapid laser thermal desorption of the analyte under atmospheric condition followed by ionization using APCI and the introduction of ions into the MS system, thus eliminating the need of liquid mobile phase to carry the sample and all the limitations that the mobile phase may induce. LDTD technology combines the ultra-fast thermal desorption of a dry sample with the ionization of the produced neutral molecules in the gas phase without solvent, mobile phase or external matrix. A low sample size thermal desorption and the APCI in "dry" environment provides the LDTD its tremendous analytical speed and analytical performances. The LDTD ion source works with less than 10 µL of sample (typically 2 µL) and it has the option of simultaneous analysis of 96 samples (www.ldtd-ionsource.com). LDTD-APCI-MS/MS analysis has been recently developed to enhance the high throughput capacity in MS by reducing LC-MS/MS runs of 5 to 30 min to 10 to 30 s in LDTD-APCI-MS/MS run (Mohapatra et al. 2012). LDTD-APCI-MS/MS was applied for the analysis of endocrine disruptor compounds (EDCs), steroid hormones and PhACs including CBZ in WW and WWS (Fayad et al., 2010; Viglino et al., 2011; Mohapatra et al., 2012). Further, advancement made in the development of analytical techniques have allowed researchers for quick detection and quantification of PhACs including CBZ in WW and WWS prior to selection of the pre-treatment or treatment method to eliminate the same.

5. Advanced oxidation processes (AOPs) for carbamazepine degradation from wastewater and wastewater sludge

Investigations found that CBZ is persistent and its removal efficiency by the WWTPs is mostly below 10% (based on 19 published studies) (Zhang et al., 2008). However, a study by Paxeus (2004) showed a removal efficiency of 53% from WWTPs. The researcher assumed that the unusually high content of silicon oil probably enhanced the removal via extraction of CBZ by the silicon oil attached to the sludge. The low removal efficiency of CBZ by WWTPs was due to resistance to biodegradation at low concentrations and less attachment to sludge (Ternes et al., 2004). Interestingly, some studies showed higher effluent CBZ concentration as compared to influent (Joss et al., 2005; Vieno et al., 2007).

Further, many authors extensively investigated and reviewed WWS treatment and disposal, pointing to the challenges involved and future possibilities of reuse (Neyens and Baeyens, 2003; Liu, 2003). However, the presence of toxic organic compounds including CBZ in WWS question sludge recycling making its final disposal problematic. If the sludge is applied to soil, the pollutant could remain in the soil for a longer time due to the sorption and slow rate of biodegradation (Stamatelatou et al., 2003). Among the treatment technologies, usually pre-treatment methods (thermal, chemical or mechanical) prior to biological degradation have been developed in order to improve the WWS recycling and reuse. The pre-treatment enhances the hydrolysis of WWS, which reduces the stabilization time and increases the degree of degradation during biological treatment. WWS pre-treatments rupture suspended solids (microbial cells), liberate the nutrients, partially solubilize the suspended solids, increase the soluble chemical oxygen demand, decrease viscosity and improve the overall WWS biodegradability (Verma et al., 2007). However, the choice of either one of these pre-treatment methods depends on the cost of the process and other factors, such as concentrations and volume of sludge to be treated. Mechanical pre-treatment using mills, homogenizers, ultrasounds and others, where the necessary energy is provided as pressure, translational or rotational energy was found to be very effective in solubilizing microbial cells but turned out to be rather complicated and expensive (Weemaes and Verstraete, 1998).

Advanced oxidation processes (AOPs) have recently emerged as an important class of technologies for the oxidation and destruction of a wide range of organic pollutants including CBZ from WW and WWS (Chong and Jin, 2012). Table 3 presents the advantages and disadvantages of AOPs and biological treatment applied for the degradation of organic compounds including CBZ from WW and WWS. AOPs involve *in situ* generation of highly reactive species, such as the hydroxyl radical ($OH^{\bullet}$), which is the most powerful oxidizing agent after fluorine with an oxidation potential of 2.80 V (Parsons and Williams, 2004). Hydroxyl radical is non-selective in nature and thus readily attacks a large group of organic chemicals including CBZ to either totally mineralize them or convert them to less complex products. Over the past 30 years, research and development concerning AOPs has been immense particularly for two reasons, such as the diversity of the technologies involved and the areas of potential application. Different AOPs methods include heterogeneous and homogenous photocatalysis based on near ultraviolet (UV) or solar visible irradiation, electrolysis, ozonation, ultrasonication (US), Fenton's reagent, and wet air oxidation (WAO), while less conventional but evolving processes include ionizing radiation, microwaves, pulsed plasma and the ferrate reagent. The degradation of CBZ in different media including water, WW and WWS by the application of different AOPs is presented in Table 4.

Depending on the properties of waste stream to be treated and the treatment objective itself, AOPs can be employed either alone or coupled with other physico-chemical or biological processes. Process coupling is conceptually beneficial usually leading to improved treatment efficiencies. In general, the techniques available for treating organic pollutants including CBZ from WW and WWS are very diverse and frequently one or more treatment techniques in sequence or parallel are required to completely degrade these compounds.

5.1. Fenton and photoassisted Fenton processes

Fenton's oxidation has been exploited in laboratory and WWTPs for stabilization of organic matter including removal of organic contaminants, such as PhACs (Zhang et al., 2008). Homogenous oxidation with the Fenton reagent occurs in the presence of ferrous or ferric ions with hydrogen peroxide via a free radical chain reaction which produces hydroxyl radicals. The organic substances are removed during two stages of oxidation and coagulation.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet \qquad (1)$$

This is a very simple method of producing hydroxyl radicals without any special reactants or apparatus. Fenton's process is an attractive oxidative system for the treatment of WW and WWS due to the fact that iron is very abundant and is a non-toxic element and hydrogen peroxide is easy to handle and is environmentally safe. Process efficiency is closely related to the solution pH whose optimal values are between 2 and 4 as well as the H_2O_2:catalyst ratio in the feed.

Study by Pere et al. (1993) showed that Fenton's oxidation of WWS enhances the dewaterability. Neyens et al. (2004) studied effects of pH, temperature, reaction time and H_2O_2 concentration on the dewaterability of sludge yielding a considerable reduction in organic and pathogen concentration. The oxidation of CBZ by a biological Fenton-like system mediated by the white-rot fungus *Trametes versicolor* was investigated by Marco-Urrea et al. (2010). They observed more than 80% degradation of CBZ after 6 h of incubation. Ghauch et al. (2011) studied the degradation of aqueous CBZ by an improved Fenton's process (ultrasonic/Fe^0/H_2O_2). They observed that the Fe^0/H_2O_2 ultrasonic process is a promising technique for oxidative removal of CBZ (> 80%) under mild conditions. From different experiments, they concluded that ultrasonic/Fe^0/H_2O_2 system is an improved Fenton process for the treatment of pharmaceutical effluents. Li et al. (2012) observed complete removal of CBZ from WW by Fenton treatment carried out using a 20 mg L^{-1} Fe (II) concentration and a 2.5

H_2O_2/Fe (II) molar ratio. These different studies showed that Fenton oxidation process as a pre-treatment improved the removal of pharmaceuticals including CBZ from WW and appears to be an effective solution to achieve compliance with the legislation with respect to discharge in a determined receptor medium.

Further, photoassisted Fenton process is an extension of Fenton process which takes advantage from UV-VIS light irradiation at wavelength values higher than 300 nm. The rate of degradation of organic pollutants with Fenton like reagents is strongly accelerated by irradiation with UV-VIS light. In this process, the photolysis of Fe^{3+} complexes allows Fe^{2+} regeneration and the occurrence of Fenton reactions due to the presence of H_2O_2 (Eq. 2-3).

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^\bullet \qquad (2)$$

$$Fe^{3+} + H_2O + h\nu \rightarrow Fe^{2+} + OH^\bullet + H^+ \qquad (3)$$

Klamerth et al. (2010) studied the degradation of CBZ at µg L^{-1} initial concentration by photo-Fenton in WWTP effluent. They observed that the degradation of CBZ with an initial concentration of 100 µg L^{-1} was found to depend on the presence of CO_3^{2-} and HCO_3^- (hydroxyl radicals scavengers) and on the type of water (simulated water, simulated effluent wastewater and real effluent wastewater), but is relatively independent of pH, the type of acid used for release of hydroxyl radicals scavengers and the initial H_2O_2 concentration used. Further, De la Cruz et al. (2012) studied the degradation of CBZ by UV and neutral photo-Fenton in WW under following conditions: UV-light emitting at 254 nm (UV_{254}) alone, dark Fenton ($Fe^{2+,3+}/H_2O_2$) and photo-Fenton ($Fe^{2+,3+}/H_2O_2$/light). They observed more than 97% of removal of CBZ and photo-Fenton treatments employing UV_{254}, 50 mg L^{-1} of H_2O_2, with and without adding iron (5 mg L^{-1} of Fe^{2+} added or 1.48 mg L^{-1} of total iron already present) gave the best results. The applicability of photo-Fenton to degrade CBZ was also studied by Bernabeu et al. (2012) and they observed complete removal of pollutant after 120 min of irradiation time.

Furthermore, in a comparision of Fenton and photo-Fenton process for degradation of CBZ, Dai et al. (2012) observed that an approximately 20% increase in CBZ removal efficiency occurred with the UV/Fenton process as compared with the Fenton process. Sires and Brillas (2012) reviewed the emerging different electrochemical technologies for separation and degradation of PhACs including CBZ from aqueous sample and concluded that the solar photo-Fenton process is foreseen as one of the most promising methods to handle this environmental concern.

Furthermore, an improvement of photoassisted Fenton process is the UV-VIS/Ferrioxalate/H_2O_2 system which has been recently demonstrated to be more efficient than photo-Fenton for the degradation of organic pollutants. Ferrioxalate is the oldest and best known photo-active example of Fe^{3+}-polycarboxylate complexes. Irradiation of ferrioxalate in acidic solution generates carbon dioxide and ferrous ions Fe^{2+} free or combined with oxalate which in combination with H_2O_2 provides a continuous source of Fenton's reagent (Eq. 4-6).

$$[\mathrm{Fe^{III}(C_2O_4)_3}]^{3-} + h\nu \rightarrow [\mathrm{Fe^{II}(C_2O_4)_2}]^{2-} + \mathrm{C_2O_4}^{\bullet -} \qquad (4)$$
$$\mathrm{C_2O_4}^{\bullet -} + [\mathrm{Fe^{III}(C_2O_4)_3}]^{3-} \rightarrow [\mathrm{Fe^{II}(C_2O_4)_2}]^{2-} + \mathrm{C_2O_4}^{2-} + 2\mathrm{CO_2} \qquad (5)$$
$$\mathrm{C_2O_4}^{\bullet -} + \mathrm{O_2} \rightarrow \mathrm{O_2}^{\bullet -} + 2\mathrm{CO_2} \qquad (6)$$

Klamerth et al. (2011) studied the modified photo-Fenton process for degradation of CBZ in WWTPs and observed that the oxalate-enhanced process provided satisfactory CBZ degradation but low residual pH of the treated water.

From different studies, it is observed that the application of Fenton or photo-Fenton processes for degradation of PhACs including CBZ from WW and WWS deal with homogenous reaction system. Use of ferrous or ferric salts usually suffers two major drawbacks: (i) low pH range of operation to avoid the formation and simultaneous precipitation of iron oxyhydroxides, and (ii) need to recover dissolved ions from the treated solution, hence require an additional treatment stage. Further, the oxalate used in an improved photo-Fenton process is not a catalyst and is consumed during the process, thus raising the cost of maintaining the process. In this respect, the immobilization of Fenton catalyst on a heterogeneous matrix would enable its use under non-controlled pH condition as well as its easy recovery from the treated media, which may be a step for future investigation.

5.2. Ozonation

Ozonation is one of the most widely investigated techniques of AOPs owing to the fact that it is commonly used in large number of water and WW treatment plants as a clarifying and disinfecting agent. Special attention is given to WWTPs since PhACs usually exit the secondary treatment unaffected and, therefore, they need to be treated in subsequent stages. During ozonation, the degradation of the target compounds can be initiated by the direct reaction with hydroxyl radical which is generated with chain reactions including initiation, propagation and termination steps (Eq. 7-11).

$$\mathrm{O_3} + \mathrm{OH^-} \rightarrow \mathrm{O_2^{\bullet -}} + \mathrm{HO_2}^{\bullet} \qquad (7)$$
$$\mathrm{O_3} + \mathrm{O_2^{\bullet -}} \rightarrow \mathrm{O_3^{\bullet -}} + \mathrm{O_2} \qquad (8)$$
$$\mathrm{HO_3} \rightarrow \mathrm{HO}^{\bullet} + \mathrm{O_2} \qquad (9)$$
$$\mathrm{O_3} + \mathrm{HO}^{\bullet} \rightarrow \mathrm{HO_4^{\bullet}} \qquad (10)$$
$$\mathrm{HO_4^{\bullet}} \rightarrow \mathrm{HO_2}^{\bullet} + \mathrm{O_2} \qquad (11)$$

Ozonation is particularly appropriate for treatment of WW and WWS containing bio-refractory and/or toxic organic pollutants including PhACs (Ternes et al., 2003). Ozone has strong cell lytic activity, and can kill the microorganisms found in activated sludge and further oxidize the organic substances released from the cells. The process of sludge ozonation is generally described by the sequential decomposition reactions of floc disintegration, solubilization, and the subsequent oxidation of the released organics into carbon dioxide (mineralization). Carballa et al. (2007) studied the influence of ozone pre-treatment on sludge anaerobic digestion and simultaneous removal of pharmaceutical and personal care products. They observed that an ozone dose of 20 mg O_3 g TSS^{-1} was able to remove up to 60% of CBZ. Further, a study by Wert et al. (2009) on effect of ozone exposure on the oxidation of CBZ in WW showed that CBZ with second order reaction rate constant with O_3 (kO3) > 10^5 M^{-1} s^{-1} and $OH^{\bullet}$ (k_{OH}) > 10^9 M^{-1} s^{-1}, was removed (> 95%) independent of water quality when the O_3 exposure was measurable (0–0.8 mg min L^{-1}). The removal of CBZ during sand filtration and ozonation at a municipal sewage treatment plant was studied by Nakada et al. (2007 and they observed the combination of treatment with activated sludge treatment resulting in removal efficiency varies in between 8.3 to 81%. Rosal et al. (2010) studied the removal of CBZ from WW through biological treatment followed by ozonation. They observed that ozonation with doses lower than 130 μM resulted in 100% removal of CBZ. Zimmermann et al. (2011) studied the kinetics of oxidation and disinfection processes during ozonation in a full-scale reactor treating secondary wastewater effluent for seven ozone doses ranging from 0.21 to 1.24 g O_3 g^{-1} dissolved organic carbon (DOC). They observed that substances reacting fast with ozone, such as CBZ (kP, O_3 > 10^4 M^{-1} s^{-1}), was eliminated within the gas bubble column, except for the lowest ozone dose of 0.21 g O_3 g^{-1} DOC. A study by Reungoat et al. (2012) on removal of CBZ from WWTP effluent by ozonation showed that more than 90% removal of pollutant is possible by ozonation. Lee et al. (2012) studied the feasibility of ozone and biofiltration as an alternative to reverse osmosis (RO) for removing pharmaceutical and personal care products (PPCPs), including CBZ from treated WW. They concluded that ozone doses of 4–8 mg L^{-1} were nearly as effective as RO for removing micropollutants. When wider environmental impacts such as energy consumption, water recovery, and waste production are considered, ozone/biofiltration may be a more desirable process than RO for removing PPCPs and other trace organics from treated WW.

Further, a combined oxidation process usually has higher reaction efficiency than an individual oxidation process because of the enhanced generation of hydroxyl radicals. Moreover, treatment performance is enhanced if ozone is combined with light irradiation (O_3/UV) (Irmak et al., 2005) or hydrogen peroxide (O_3/H_2O_2) (Huber et al., 2003). UV enhances the generation of hydroxyl radicals and H_2O_2 prevents the recombination of electrons and holes, thus leading to an effective degradation of many organic pollutants including CBZ (Klavarioti et al., 2009). Although H_2O_2 reacts very slowly with the ozone molecule in aqueous solution, its conjugate base (HO_2^-) can rapidly react with molecular ozone, thereby initiating the formation of hydroxyl radicals in two steps (Eq. 12-13).

$$H_2O_2 + H_2O \rightarrow HO_2^- + H_3O^+ \qquad (12)$$
$$HO_2^- + O_3 \rightarrow OH^\bullet + O_2^- + O_2 \qquad (13)$$

The rate constant between HO_2^- and O_3 was measured to be 5.5×10^6 M^{-1} s^{-1}, as compared to 7.0×10^1 M^{-1} s^{-1} between OH^- ion and O_3. The results showed that even at a very low concentration, the HO_2^- ion will be very effective in initiating ozone decomposition and facilitating the formation of hydroxyl radicals. Further, during O3/UV process, UV photons activate ozone molecules, thereby facilitating the formation of hydroxyl radicals. The reaction mechanism starts with activating the ozone molecule by UV to form oxygen radicals, which then combine with water to form hydroxyl radicals (Eq. 14-15).

$$O_3 + H_2O + h\nu \rightarrow O_2 + H_2O_2 \qquad (14)$$
$$2O_3 + H_2O_2 \rightarrow 2OH^\bullet + 3O_2 \qquad (15)$$

Giri et al. (2010) studied the degradation of common PPCPs including CBZ in a mixed solution by AOPs. They demonstrated that ozone-based techniques rather than ultraviolet radiation-based techniques were very powerful for simultaneous removal of the compounds efficiently. Moreover, ozonation combined with ultraviolet radiation was the most appropriate technique for simultaneous removal of the tested compounds efficiently. Pisarenko et al. (2012) studied the effect of ozone and ozone/peroxide on removal of CBZ from water reuse applications. They demonstrated that the addition of peroxide expedited the formation of hydroxyl radicals and yielded greater removal of CBZ compared to ozone alone. Im et al. (2012) studied the optimization of CBZ removal in O_3/UV/H_2O_2 system using a response surface methodology with central composite design. They observed the optimal ratio of O_3 (mg L^{-1}): H_2O_2 (mg L^{-1}): UV (mW cm^{-2}) were 0.91:5.52:2.98 for complete removal of CBZ from aqueous medium.

5.3. Ultraviolet–Hydrogen peroxide (UV/H_2O_2)

UV/H_2O_2 process is an AOP for the effective oxidation and destruction of toxic and refractory organics from water, WW and WWS. Under UV irradiation, H_2O_2 photolyzed to form two hydroxyl radicals which then react with organic contaminants or undergo a H_2O_2 decomposition-formation cycle (Eq. 16-18).

$$H_2O_2 + h\nu \rightarrow 2OH^\bullet \qquad (16)$$
$$H_2O_2 + OH^\bullet \rightarrow H_2O + HO_2 \qquad (17)$$
$$HO_2 + HO_2 \rightarrow H_2O_2 + O_2 \qquad (18)$$

Unlike ozone, H_2O_2 has an exceptionally low molar absorption within the wavelength range of 200-300 nm. Two commercially available lamps are most commonly used: low pressure (LP) and medium pressure (MP) mercury vapor lamps. LP lamps emit UV light in a narrow band at 254 nm. MP lamps emit a broad spectrum of light between 200–800 nm. Generally, direct photodegradation of PhACs is more effective using MP lamps as compared to LP lamps (for the same UV dose), but hydroxyl radical generation using H_2O_2 is more effective with LP lamps (Ijpelaar et al., 2010). MP UV lamps were found to be superior as compared to LP lamps to degrade PhACs including CBZ by UV/H_2O_2 process (Pereira et al., 2007). Although UV/H_2O_2 treatment may be accomplished with either LP or MP lamps, it is possible that different mechanisms will drive oxidation using LP or MP lamps due to differences in photodegradation versus formation of hydroxyl radicals.

Kim et al. (2009) studied photodegradation of PPCPs including CBZ in WW by using UV and UV/H_2O_2 treatment. They observed that during UV/H_2O_2 treatment, most of the PPCPs including CBZ were degraded by more than 90% by UV irradiation for 30 min (UV dose: 691 mJ cm^{-2}), indicating that H_2O_2 addition during UV treatment was highly effective for improving the degradation of PPCPs by UV, even though much higher UV dose is necessary comparing to UV disinfection only. Further, Kim et al. (2009a) studied the performance of UV and UV/H_2O_2 processes for the removal of pharmaceuticals detected in secondary effluent of a WWTP. They observed that for UV/H_2O_2 process, 90% removal efficiency could be accomplished in 39 pharmaceuticals including CBZ at UV dose of 923 mJ cm^{-2}, indicating that it will be possible to reduce UV energy required for the effective pharmaceuticals removal by the combination of H_2O_2 with UV process. Rosario-Ortiz et al. (2010) studied the evaluation of UV/H_2O_2 treatment for the oxidation of PhACs including CBZ in WW. They observed more than 90% removal of CBZ and also concluded that UV/H_2O_2 treatment for the removal of PhACs from WW was a function of not only the concentration of effluent organic matter (EfOM) but also its inherent reactivity towards hydroxyl radicals. The removal of PhACs also correlated with reduction in ultraviolet absorbance at 254 nm (UV_{254}), which offers utilities a surrogate to assess pharmaceutical removal efficiency during UV/H_2O_2 treatment. Kohler et al. (2012) studied the elimination of pharmaceutical residues in biologically pre-treated hospital WW using advanced UV *irradiation technology*. They observed that the effect of UV irradiation for degradation of PhACs including CBZ from WW was enhanced with varying dosages of H_2O_2 and the “conventional” cost-benefit analysis of the different technology options taking into account both electrical energy consumption and pharmaceutical removal efficiency, clearly revealed better performance of low pressure UV lamps as AOP.

5.4. Heterogeneous photocatalytic processes

Heterogeneous photocatalytic processes use certain metal oxides that can readily generate hydroxyl radicals on the surface of particles when absorbing UV light. Many catalysts have been so far tested, although only TiO_2 in the anatase form seems to have the most interesting attributes, such as high stability, commercially available at various crystalline forms, good performance and low cost (Zhang et al., 1994). The anatase form of TiO_2 has low band-gap energy (approximately 3.2 eV), which is almost equivalent to 400 nm wavelength of light. Hence, the most commonly used heterogeneous photocatalytic processes include TiO_2-UV and TiO_2-H_2O_2-UV. The principal reaction mechanism (Eq. 19-24) includes illumination of an aqueous TiO_2 suspension with irradiation with energy greater than the band-gap energy of the semiconductor generated valence-band holes ($\mathrm{h}^+_{\mathrm{TiO_2}}$) and conduction-band electrons ($\mathrm{e}^-_{\mathrm{TiO_2}}$). Holes and electrons may either undesirably recombine liberating heat or make their separate ways to the surface of TiO_2, where they can react with species adsorbed on the catalyst surface. Valence band holes can react with water and the hydroxide ion to generate hydroxyl radicals, while electrons can react with adsorbed molecular oxygen reducing it to superoxide radical anion which reacts with protons to form peroxide radicals.

$$\mathrm{TiO_2} + h\nu \rightarrow \mathrm{h}^+_{\mathrm{TiO_2}} + \mathrm{e}^-_{\mathrm{TiO_2}} \quad (19)$$
$$\mathrm{h}^+_{\mathrm{TiO_2}} + \mathrm{H_2O} \rightarrow \mathrm{OH}^\bullet_{\mathrm{TiO_2}} + \mathrm{H}^+ \quad (20)$$
$$\mathrm{h}^+_{\mathrm{TiO_2}} + \mathrm{OH}^- \rightarrow \mathrm{OH}^\bullet_{\mathrm{TiO_2}} \quad (21)$$
$$\mathrm{e}^-_{\mathrm{TiO_2}} + \mathrm{O_2} \rightarrow \mathrm{O}^-_{2,\mathrm{TiO_2}} \quad (22)$$
$$\mathrm{O}^-_{2,\mathrm{TiO_2}} + \mathrm{e}^-_{\mathrm{TiO_2}} \rightarrow \mathrm{O}_2^{2-} \quad (23)$$
$$\mathrm{O}_2^{2-} + \mathrm{H}^+ \rightarrow \mathrm{HO_2} \quad (24)$$

Rizzo et al. (2009) investigated the degradation kinetics and mineralization of an urban WWTP effluent with a mixture of PhACs including CBZ by TiO_2 photocatalysis process. They concluded that the mineralization rate (evaluated in terms of total organic carbon (TOC) measurements) in WW contaminated with pharmaceuticals including CBZ was found to be really slow ($t_{1/2}$ = 86.6 min) compared to that of the same pharmaceutical mixture in distilled water ($t_{1/2}$ = 46.5 min). They demonstrated that the difference in results was probably due to the interference of radical scavengers, such as carbonates which typically occur in high concentrations in WW.

Further, a major limitation for the use of TiO_2 as photocatalyst is its low photoefficiency due to the short lifetime of the generated holes and electrons pairs and the necessity of energy rich light. To improve the photoefficiency of TiO_2, researchers have investigated doping TiO_2 with metals or ions to trap the charge carriers for the hindrance of recombination and to shift the band-gap into the region of visible light (Ma et al., 2008). Another approach to improve photoefficieny is to address the poor adsorption of target substances on TiO_2. For efficient degradation, the target compounds need to be very close or directly attached to the TiO_2 surface as the generated radicals have a lifetime of less than 10 µs (Hoigné, 1997). Ziegmann and Frimmel (2010) studied the photocatalytic degradation of CBZ using conglomerated TiO_2 and powdered activated carbon (PAC) in aqueous suspension. They observed higher synergistic effect for CBZ, caused by rapid adsorption on the PAC surface followed by diffusion to the TiO_2 surface and photocatalytic degradation. Laera et al. (2011) studied an integrated membrane bioreactor (MBR)-TiO_2 photocatalysis process for the removal of CBZ from simulated pharmaceutical industrial effluent. They demonstrated that the adaptation of recycling ratio of 4:1 resulted in the removal of 95% CBZ. Further, the effluent chemical oxygen demand (COD) reduction, sludge yield increase and respirometric tests suggested that the oxidation products were mostly biodegradable and that they did not inhibit the microbial activity. These studies evidenced the advantages of the heterogeneous photocatalytic processes for the degradation of CBZ from WW and WWS. Further, Laera et al. (2011a) studied the photocatalytic activity of TiO_2 nanofibers in simulated and real WWTP effluents for degradation of CBZ. They observed the fact that a higher catalyst loading is required to obtain higher performance of TiO_2. The nanofiber offers the advantage of being easily separated by settling or microfiltration, allowing for a simpler use in continuous operation and higher removal of CBZ.

From an economic point of view, heterogeneous photocatalysis process is likely to benefit from the use of renewable energy sources to power the process. In this direction, solar photocatalysis has gained considerable attention and several studies reported the use of natural (Munoz et al., 2006; Coleman et al., 2007) or simulated (Mendez-Arriaga et al., 2008) irradiation for PhACs including CBZ degradation from WW. Doll and Frimmel (2004) studied the light-induced degradation of CBZ under simulated solar irradiation in aqueous medium suspended with different TiO_2 materials such as P25 and Hombikat UV100. They observed higher photocatalytic degradation of CBZ was obtained with P25 as compared to Hombikat UV100. Achilleos et al. (2010) studied the efficiency of solar and UV photocatalysis with suspended TiO_2 to degrade CBZ from WW. They carried out experiment under different operating conditions, such as catalyst type (six commercially available titanium oxide samples were tested) and concentration

(50–3000 mg L^{-1}), initial CBZ concentration (5–20 mg L^{-1}), solution pH (3–10), and the addition of hydrogen peroxide (0.07–1.4 mM) and observed better performance (higher degradation) under UV and H_2O_2 condition. From the different studies, it was concluded that solar photocatalysis involved substantially lower environmental impact but it was less efficient for the degradation of target compound as compared to photocatalysis under UV. Further, future efforts should be directed to develop more active photocatalyst to increase UV quantum efficiency and create innovative technologies to prevent the potential loss of photocatalysts.

5.5. Ultrasonic irradiation

The application of ultrasonic irradiation or sonolysis for remediation of water, WW and WWS is an area of increasing interest. However, ultrasonic irradiation has received less attention than other AOPs on the basis of water and WW treatment for hazardous chemical destruction. This is also reflected in the few publications concerning pharmaceutical treatment including CBZ using ultrasonic irradiation. Several authors have reported the successful application of ultrasonic irradiation as pre-treatment process for sludge stabilization (Khanal et al., 2007; Jin et al., 2009). The chemical effects of ultrasound are derived from acoustic cavitation, i.e., the formation, growth and implosive collapse of cavitation bubbles in a liquid. Extreme temperatures of several thousand degrees and pressures of several hundred atmospheres are developed locally within the bubbles during their collapse with these bubbles serving as hot spot microreactors in an otherwise cold liquid. Destruction of chemicals is usually achieved through a combination of pyrolytic reactions occurring inside or near the bubble and hydroxyl radical-mediated reactions occurring in the bulk liquid (Eq. 25-28).

$$H_2O \rightarrow H + HO^{\bullet} \qquad (25)$$
$$H + O_2 \rightarrow HO_2^{\bullet} \qquad (26)$$
$$O + H_2O \rightarrow 2HO^{\bullet} \qquad (27)$$
$$H + O_2 \rightarrow O + HO^{\bullet} \qquad (28)$$

Naddeo et al. (2009) studied the fate of PhACs including CBZ in contaminated urban WW effluent under ultrasonic irradiation. They studied several operating conditions, such as power density (25–100 W L^{-1}), initial substrate concentrations (2.5–10 mg L^{-1}), initial solution pH (3–11), and air sparging for the evaluation and understanding of the process. They also monitored the degradation (as assessed by measuring UV absorbance), generation of hydroxyl radicals (as assessed measuring H_2O_2 concentration), mineralization (in terms of total organic carbon (TOC) and COD removal), and aerobic biodegradability (as assessed by the (biochemical oxygen demand)$_5$/COD ratio) during sonication. They observed that the degradation of CBZ is enhanced at increased applied power densities, acidic conditions and in the presence of dissolved air.

Furthermore, the characteristics of the investigated WW and WWS itself exerted magnitudinal effect on the final efficiency of AOPs, therefore sometimes it is difficult to compare the results reported by different researchers to make a comparison of AOPs. From different studies, it was concluded that the main mechanism that involves AOPs is the generation of hydroxyl radicals to facilitate the oxidation of organic compounds including CBZ. However, despite these similarities, different AOPs required different radical initiators to generate hydroxyl radicals. Because of this difference, the cost to generate hydroxyl radicals varies greatly for different AOPs. The following points may be considered for a correct comparison of AOPs: (1) additional equipment or material of construction; (2) light wasting; (3) inhibition due to presence or production of different scavengers; (4) influence of pH; (5) mass transfer limitations; and (6) cost evaluation.

Among the different AOPs studied for the degradation of CBZ from WW, it was observed that the ozone based techniques (O_3, O_3/H_2O_2, O_3/UV) and UV/H_2O_2 process have gained the widest application. The UV/H_2O_2 process has a distinct advantage because of its simplicity. The only chemical required is H_2O_2, which is easily available, easily stored and precisely fed according to the process demand. Thus, the UV/H_2O_2 process is well suited to small systems that required no additional equipment or material of construction, minimum maintenance, and intermittent operation, or both. Further, O_3/H_2O_2 and O_3/UV system require the transfer of ozone molecule from the gas stream to liquid bulk, within the liquid bulk and from liquid bulk to target compound. The overall process of absorption with reaction can develop according to different regimes. However, the O_3/UV process is considered less favorable than the O_3/H_2O_2 and UV/H_2O_2 processes. It has been reported that the rate of ozone consumption per unit volume in case of O_3/UV process can be so high that mass transfer limited regime for ozone absorption is established thus resulting in a decrease of quantum efficiencies and an increase of operating cost (Ollis et al., 1993). Furthermore, only few studies have reported the overall cost analysis of AOPs (Hirvonen et al., 1998) and due to their dependence on the various factors, such as effluent type, WWTP characteristics and the type of target compound, their direct comparison often produces difficulties. The results of these studies indicated that the overall costs of AOP processes are comparable with those of well established technologies of pollutant removal.

6. Conclusions and future challenges

The occurrence and fate of PhACs including CBZ in the environment, particularly in aquatic media have received considerable attention by the scientific community during the last two decades. Due to the higher consumption of CBZ, approximately 30% of an oral CBZ dose is excreted unaltered in urine and faeces together, while its urine metabolite, DiOH-CBZ, accounts for around 30% of oral dosage and potentially persistent in water bodies. According to the concentration of carbamazepine found in environmental samples, a calculated ratio between the measured environmental concentration (MEC) and the predicted no-effect environmental concentration (PNEC), showed that CBZ is not expected to produce acute toxic effects on the aquatic biota. However, chronic effects and synergistic effects with other chemicals cannot be excluded and should be further investigated.

Several advanced analytical methods have been developed and optimized in recent years for WW and WWS matrices, in order to obtain better precision and sensitivity and to quantify CBZ present in trace concentrations in these matrices. Different extraction methods, such as derivatization and clean-up have been investigated in order to minimize the matrix effects during the quantification of CBZ in WW and WWS by HPLC, GC-MS, GC-MS/MS, LC-MS or LC-MS-MS. Likewise, new ultrafast methods have been developed in order to minimize the sample preparation time, analysis time, sample pre-treatment and cost by eliminating column use and reducing solvent consumption. Despite the current availability of advanced detection instrumentation, the rapid and accurate quantification of CBZ in complex environmental matrices, such as WW and WWS continues to be a major and fascinating challenge because of the low concentration (ppb or below) and the complexity of the matrices. Further improvements are needed in the field of instrumentation and method development in order to lower the limit of detections (LODs).

CBZ is ineffectively removed by WWTPs and have removal efficiency less than 10% in most cases which paved the way for the installation of different treatment methods, mainly AOPs for its complete removal from WW and WWS. Research has shown that AOPs, which generate very active oxidative species, such as hydroxyl radicals, are promising tools for the destruction of PhACs including CBZ from WW and WWS. However, major attention should be paid in future to fill some specific gap which exists for these techniques in the areas, such as identification of reaction intermediates, development of rate expressions based on established reaction mechanisms, identification of scale-up parameters and criteria for cost effectiveness with simultaneous higher degradation of the target compound. Furthermore, more research is required particularly with hybrid processes to make advantage of synergistic effects and to investigate the impacts of interfering agents and chemical structures before proposing advanced oxidation as a viable solution to the treatment of PhACs including CBZ from WW and WWS.

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Table1. Physico-chemical and pharmacological properties of CBZ

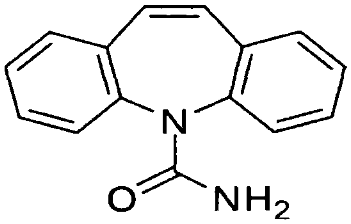
Structure	
Molecular formula, CAS No. and molecular weight	$C_{15}H_{12}N_2O$, 298-46-4 and 236.27 g mol^{-1}
Water solubility[a]	17.7 mg L^{-1} (20 °C)
Log K_{ow} (octanol-water partitioning)[a]	2.45
Henry's Law Constant[a]	1.09 X 10^{-5} Pa m^3 mol^{-1} (25 °C)
p*K*a	Neutral
Melting Point	189-193 °C
Usage	Analgesic, anticonvulsant, antimanic agent
Elimination half-life[b]	25-65 h
Appearance	White, light yellowish powder
Toxicity	Mild ingestion cause vomiting, drowsiness, ataxia, slurred speech, nystagmus, dystonic reactions, and hallucinations. Severe intoxications may produce coma, seizures, respiratory depression and hypotension
Affected organisms	Human and aquatic organisms

Table 2. Country-wise detection level of CBZ by using *different analytical techniques and extraction methods*

Country	Sample type	Extraction method	Clean-up/Pre-concentration	Analytical Techniques	Level of detection	Reference
Canada	WW and WWS	UAE, ASE, MAE	SPE	LDTD-APCI-MS/MS	12 ng L^{-1} (WW) 3.4 ng g^{-1} (WWS)	Mohapatra et al. 2012
	Surface water	---	SPE	LC-MS/MS	2 ng L^{-1}	Daneshver et al. 2012
	WW	---	SPE	GC-MS/MS	18 ng L^{-1}	Gagnon and Lajeunesse, 2012
	Surface water	---	SPE	GC-MS	0.01 µg L^{-1}	Waiser et al. 2011
	WW and Surface water	---	SPE	LC-ESI-MS/MS	2 ng L^{-1}	Viglino et al. 2008
	Surface water	---	SPE	LC-MS/MS	2 ng L^{-1}	Yargeau et al. 2007
	WW	---	SPE	HPLC-MS	20 ng L^{-1}	Brun et al. 2006
	WW and Surface water	---	SPE	LC-ESI-MS/MS	0.1 ng L^{-1} and 0.05 ng L^{-1}	Hua et al. 2006
		---	SPE	LC-MS/MS	---	Gagné et al. 2006
	WW	PLE	SPE	LC-ES-MS/MS	0.15 µg kg^{-1}	Miao et al. 2005
	WW and Biosolid	---	SPE	LC-MS/MS	0.8 to 4.8 pg	Miao and Metcalfe, 2003
	WW and Surface water	---	SPE	LC-MS/MS	0.5 µg L^{-1} (in), 0.1 µg L^{-1} (ef)	Metcalfe et al. 2003
	WW	---	SPE	LC-ESI-MS/MS	5 ng L^{-1} and 1 ng L^{-1}	Metcalfe et al. 2003a
Mexico	WW	---	SPE	GC-MS	0.14 ng L^{-1}	Chavez et al. 2011
Austria	WW	---	SPE	HPLC-MS/MS	1 ng L^{-1}	Fenz et al. 2005
	WW	---	SPE	LC-MS/MS	10 ng L^{-1}	Clara et al. 2004

Country	Sample type	Extraction method	Clean-up/Pre-concentration	Analytical Techniques	Level of detection	Reference
Germany	WW and Surface water	---	SPE	HPLC-MS/MS	10 ng L^{-1}	Scheurer et al. 2011
	WW	---	SPE	LC-MS/MS	0.0001 μg L^{-1}	Gebhardt and Schroder, 2007
	WW	---	SPE	GC-MS	0.05 μg L^{-1}	Bernhard et al. 2006
	WW and Surface water	---	SPE	LC-MS/MS	0.01 μg L^{-1}	Zuehlke et al. 2004
	WW	---	SPE	LC-MS/MS	0.05 μg L^{-1}	Ternes et al. 2003
	WW and River water	---	SPE	LC-MS/MS	0.05 μg L^{-1} and 0.03 μg L^{-1}	Ternes, 1998
Spain	WW and WWS	UAE	SPE	HPLC	0.05 ng L^{-1} and 14 μg kg^{-1}	Martin et al. 2012
	WW	---	SPE	GC-MS	---	Reyes-Contreras et al. 2012
	WW and WWS	ASE	SPE	HPLC-QLIT-MS/MS	2 ng L^{-1} and 0.2 ng g^{-1}	Jelic et al. 2011
	WWS	ASE	SPE	LC-MS/MS	1 μg kg^{-1}	Nieto et al. 2010
	WW	---	SPE	LC-QTRAP-MS	1 ng L^{-1}	Rosal et al. 2010
	WW and WWS	ASE	SPE	LC-MS	15.8 ng L^{-1} and 0.04 ng g^{-1}	Radjenovic et al. 2009
	WW and Surface water	---	SPME	LC-DAD	---	Garcia et al. 2009
	WW	---	SPE	HPLC-DAD	0.04 μg L^{-1}	Santos et al. 2009
	WW and WWS	UAE	SPE	LC-MS/MS	74 ng L^{-1} and 20 ng g^{-1}	Carballa et al. 2007
	WW	---	SPE	GC-MS	22 ng L^{-1}	Matamoros et al. 2007
	WW	---	SPE	GC-MS/MS	0.7 ng L^{-1}	Gomez et al. 2007
	WW	---	SPE	LC-MS/MS	0.6 ng L^{-1}	Radjenovic et al. 2007
		---	SPE	HPLC-DAD	0.04 μg L^{-1}	

Country	Sample type	Extraction method	Clean-up/Pre-concentration	Analytical Techniques	Level of detection	Reference
Spain	WW	---	SPE	GC-MS	22.2 ng L^{-1}	Carballa et al. 2004
Sweden	WW	---	SPE	LC-MS	---	Andreozzi et al. 2003
Denmark	WWS	Centrifugation	SPE	LC-MS/MS	---	Horsing et al. 2011
Greece	WW	---	SPME	GC-MS	---	Antoniou et al. 2009
	WW	---	SPE	LC-MS	---	Andreozzi et al. 2003
Portugal	WW and Surface water	---	SPE	ELISA and LC-MS/MS	---	Calisto et al. 2011
	Surface water	---	SPE	LC-MS/MS	0.03 ng L^{-1}	Madureira et al. 2010
	WW	---	SPE	Voltammetric sensor	40/nM	Veiga et al. 2010
China	WW	---	SPE and without SPE	LC-MS/MS	30-30000 ng L^{-1} and 0.24-240 ng L^{-1}	Yu et al. 2012
	Surface water	---	SPE	HPLC-Diode array	0.1 to 0.2 µg L^{-1}	Zhou et al. 2011
	WW and Surface water	---	SPE	HPLC-MS/MS	0.5 ng L^{-1} and 0.1 ng L^{-1}	Yu et al. 2011
	Surface water	---	SPE	GC-MS	< 10 ng L^{-1}	Zhao et al. 2010
	WW	---	SPE	LC-MS/MS	---	Zhou et al. 2010
France	WW	---	SPE	HPLC-MS/MS	0.02 µg L^{-1}	Martin Ruel et al. 2012
	WW	---	SPE	LC-MS	2.4 ng L^{-1}	Leclercq et al. 2009
	WW	---	SPE	LC-MS	---	Andreozzi et al. 2003
Norway	WW and Surface water	---	SPE	HPLC-MS/MS	1 ng L^{-1}	Langford and Thomas, 2011
Switzerland	Surface water	---	SPE	GC-MS	1.5 ng L^{-1}	Tixier et al. 2003

Country	Sample type	Extraction method	Clean-up/Pre-concentration	Analytical Techniques	Level of detection	Reference
Japan	WW	---	SPE	LC-MS/MS	11 mg L^{-1}	Okuda et al. 2008
	WW	---	SPE	GC-MS	6 ng L^{-1}	Nakada et al. 2006
USA	WWS	ASE	SPE	LC-MS/MS	---	Hyland et al. 2012
	WW and WWS	ASE	SPE	HPLC-MS/MS	2.2 ng L^{-1} and 2.9 µg kg^{-1}	Gao et al. 2012
	WW	---	SPE and XAD	GC-MS	12 ng L^{-1} (in) and 7 ng L^{-1} (ef)	Yu and Wu, 2011
	WW	---	SPE	HPLC-MS/MS	10 ng L^{-1}	Nelson et al. 2011
	WWS	---	SPE	LC-MS/MS	---	Garmon et al. 2011
	WW	---	SPE	CE-UV	17.2 ppb	Gibbons et al. 2011
	Biosolids	ASE	SPE	LC-MS/MS	2.9 µg kg^{-1}	Ding et al. 2011
	WW	---	SPE	LC-MS/MS	10 ng L^{-1}	Yang et al. 2011
	Biosolids	ASE	SPE	LC-MS/MS	5.6 µg kg^{-1}	McClellan and Halden, 2010
	WW	---	SPE	GC-MS	30 ng L^{-1}	Bisceglia et al. 2010
	WW	---	SPE	LC-MS	0.01-0.15 pg $µL^{-1}$	Celiz et al. 2009
	WW and Surface water	---	SPE	LC-MS/MS	0.71 ng	Bartelt-Hunt et al. 2009
	WW and Biosolids	USE	SPE	LC-ESI-MS/MS	0.00085 µg L^{-1} and 0.90 µg kg^{-1}	Spongberg and Witter, 2008
	WW	---	SPE	LC-MS/MS	0.003 µg L^{-1}	Conkle et al. 2008
Israel	WW	---	SPE	GC-MS and LC-MS/MS	100 ng L^{-1} and 0.1 ng L^{-1}	Gasser et al. 2011
Romania	WW	---	SPE	GC-MS	---	Moldovan et al. 2009
India	Surface water	---	SPE	GC-MS	1.6 ng L^{-1}	Ramaswamy et al. 2011

Country	Sample type	Extraction method	Clean-up/Pre-concentration	Analytical Techniques	Level of detection	Reference
India	WW	---	Filtration	GC-MS	---	Sreekanth et al. 2009
Ireland	WW	---	SPE	LC-MS/MS	0.20 µg L^{-1}	Lacey et al. 2012
	WWS	ASE	SPE	LC-MS/MS	3 ng g^{-1}	Barron et al. 2008
Italy	WW and Surface water	---	SPE	HPLC-MS/MS	< 1 ng L^{-1}	Castiglioni et al. 2006
	WW	---	SPE	HPLC-MS/MS	1.3 ng L^{-1}	Castiglioni et al. 2005
	WW	---	SPE	LC-MS	---	Andreozzi et al. 2003
Australia	WW	---	SPE	GC-MS	0.19 µg L^{-1}	Allinson et al. 2012
	WW	---	SPE	HPLC	---	Chong and Jin, 2012
	WW	---	SPE	HPLC	---	Chong et al. 2011
UK	WW	---	SPE	LC-MS/MS	0.05 ng L^{-1}	Zhou et al. 2009
	Surface water	---	SPE	LC-ESI-MS/MS	0.1 ng L^{-1}	Kasprzyk-Hordern et al. 2008
The Netherlands	Surface water	---	SPE	LC-MS/MS	---	Teunissen et al. 2012
Republic of Korea	Aqueous medium	---	SPE	HPLC	---	Im et al. 2012
	WW	---	SPE	LC-MS	0.088 ng L^{-1}	Behera et al. 2011
	WW	---	SPE	LC-MS/MS	0.001 µg L^{-1}	Sim et al. 2011
	WW	---	SPE	LC-MS	0.004 µg L^{-1}	Sim et al. 2010
	Surface water	---	SPE	LC-MS/MS	---	Yoon et al. 2010
	Surface water and WW	---	SPE	LC-MS/MS	1.0 ng L^{-1}	Kim et al. 2007

Country	Sample type	Extraction method	Clean-up/Pre-concentration	Analytical Techniques	Level of detection	Reference
Tunisia	Water and Soil sample	MAE	SPE	LC-MS	0.25 ng g^{-1}	Fenet et al. 2012
Turkey	WW	---	SPE	LC-ESI-MS/MS	0.17 ng L^{-1}	Muz et al. 2012
	WW	---	SPE	HPLC-ESI-MS/MS	0.12 ng mL^{-1}	Komesli et al. 2012
Costa Rica	Surface water	---	SPE	LC-MS/MS	1.3 ng L^{-1}	Spongberg et al. 2011
Taiwan	WW and Surface water	---	SPE	LC-MS/MS	0.2 ng L^{-1}	Chen et al. 2008

in : Influent, ef: Effluent, LOD: Limit of detection, ASE: Accelerated Solvent Extraction, LLE: Liquid-liquid Extraction, SPE: Solid Phase Extraction, LLE: Liquid-liquid Extraction

Table 3. Advantages and disadvantages of advanced oxidation and biological treatment of organic pollutants including carbamazepine

Advanced oxidation technologies	Biological treatment technologies
Advantages	**Advantages**
1. Complete oxidation possible	1. Partial mineralization
2. Can handle large recalcitrant molecules	2. Eco-friendly
3. Easy process operability	3. Low capital and operating costs
4. Ability to handle fluctuating flow rates and compositions	4. True destruction of organics, versus mere phase separation
5. Absence of secondary wastes	5. Simultaneous removal of reduced inorganic compounds, such as sulphides and ammonia, and total nitrogen removal possible through denitrification
	6. Operational flexibility to handle a wide range of flows and wastewater characteristics
	7. No use of chemicals
	8. Require less energy inputs
Disadvantages	**Disadvantages**
1. Higher capital and operating costs	1. Digestion rates are slow, in general days or weeks, requiring large storage tanks.
2. Difficult to manage utilities in wastewater treatment plants	2. Microorganisms must be fed constantly, or they will die off. If they die off, re-acclimating the microorganisms to the waste stream may take several days
3. Radical scavenging which may lead to lowered degradation efficiency	3. Changing from one organic removal compartment to another can greatly reduce or eliminate effective treatment.
4. Simultaneous removal of inorganics not reported	
5. Operational flexibility is not feasible	
6. Often uses harsh chemicals	
7. Energy input is higher	

Table 4. Effect of advanced oxidation processes for carbamazepine removal from different media including water, wastewater and wastewater sludge

Sample	Pre-treatment/Treatment method	Concentration	Process Condition Parameters	Removal (%)	Reference
Wastewater	Photocatalysis	5000 μg L^{-1}	TiO_2 nanofiber, UV-254 nm, pH 7, 4 h reaction time	78	Chong and Jin, 2012
	Fenton oxidation	1 μg L^{-1}	20 mg L^{-1} Fe (II), H_2O_2/Fe (II) = 2.5, pH 3	100	Li et al. 2012
	UV/H_2O_2	0.26-1 mg L^{-1}	H_2O_2: 10 mg L^{-1}, medium pressure UV	---	Keen et al. 2012
	Photocatalysis	5000 μg L^{-1}	TiO_2, UV lamp (11 W), pH 7.41, 3.5 h reaction time	23	Chong et al. 2011
	MBR-TiO_2 Photocatalysis	10 mg L^{-1}	TiO_2, UV-360 nm, 6 h reaction time	95	Laera et al. 2011
	Photocatalysis	100 μg L^{-1}	TiO_2, solar UV power (30 W m^{-2}), pH unadjusted, 120 min irradiation time	100	Miranda-Garcia et al. 2011
	Ozonation	---	0.25, 0.5, 1.0, 2.0, 3.0, 4.0 and 5.0 mg L^{-1} O_3, exposure time: 20 min	100	Nanaboina and Korshin, 2010
	UV/H_2O_2	210 and 320 ng L^{-1}	2, 5, 10, 15 and 20 mg L^{-1} H_2O_2, UV- 300 to 700 mJ cm^{-2}, temperature 18 °C, 6 to 20 min irradiation time	10 to 90	Rosario-Ortiz et al. 2010
	Photo-Fenton	100 and 5 μg L^{-1}	5 mg and 20 mg L^{-1} Fe, 50 mg L^{-1} H_2O_2, pH 3, solar UV radiation (< 400 nm), 150 min reaction time	100	Klamerth et al. 2010
	Ozonation and UV/H_2O_2	1 μM	1.5 mM O_3, 1 h reaction time; H_2O_2 (0.2-5 mM), UV-254 to 320 nm	100	Lee and Gunten, 2010
	Nonthermal Plasma (NTP)	219 ng L^{-1}	Generator condition: frequency 500 Hz, voltage 8 kV and circulation rate 8 L min^{-1}, skid energy: 3.8 to 4.8 kWh m^{-3}, O_3 (2 g m^{-3})	> 90	Gerrity et al. 2010
	UV/H_2O_2	1000 μg mL^{-1}	UV-254 nm, H_2O_2: 100 μg mL^{-1}	> 60	Rao and Belorkar, 2010

Sample	Pre-treatment/Treatment method	Concentration	Process Condition Parameters	Removal (%)	Reference
Wastewater	Ultrasonication	2.5 and 5 mg L^{-1}	Power density (25-100 W L^{-1}), pH 3-11, temperature 24 °C, 60 min irradiation time	42	Naddeo et al. 2009
	Photocatalysis	5 mg L^{-1}	Catalyst loading (0.8 g TiO_2 L^{-1}), UV-350 nm, 120 min irradiation time, pH 8.1	85	Rizzo et al. 2009
	O_3 and O_3/H_2O_2	10-430 ng L^{-1}	65-80 mg L^{-1} O_3, O_3:H_2O_2 = 1.0:0.5 mg mg^{-1}	55	Wert et al. 2009
	Ozonation	2.3 to 33.3 ng L^{-1}	Ozone dose: 3 mg L^{-1}, ozonation time 27 min	> 81	Nakada et al. 2007
	Ozonation	10-250 ng L^{-1}	Ozone dose : 4.9, 7.3 and 8.7 mg L^{-1}, ozonation time: 18 min	> 98	Lei and Snyder, 2007
	O_3, O_3/UV and H_2O_2/UV	1.4 µg L^{-1}	2.6 g O_3 h^{-1} (reaction time 1 or 3 min), 15 W medium pressure mercury lamp, temperature 10-15 °C, 0.5 mL H_2O_2 L^{-1}	> 97.5	Gebhardt and Schroder, 2007
	Ozonation	0.5-2 µM	Ozone dose: 1.2-2.4 mg L^{-1}, pH 8, temperature 22 °C	---	Buffle et al. 2006
	Ozonation, UV/H_2O_2 and TiO_2 Photocatalysis	7070 µg L^{-1}	Ozone dose: 36 dm^3 h^{-1}, UV-254 nm, H_2O_2 : 5-10 mM, TiO_2 : 0.3 g L^{-1}, pH 7.6	100, 100 and 80	Andreozzi et al. 2004
	O_3/UV and O_3/H_2O_2	2.1 µg L^{-1}	Ozone dose: 5-15 mg L^{-1}, H_2O_2 : 10 mg L^{-1}, low pressure UV-254 nm	100	Ternes et al. 2003
Wastewater Sludge	Ozonation	400 µg L^{-1}	Ozone dose: 20 mg O_3.g TSS^{-1}	60	Carballa et al. 2007
Water	Hydrodynamic-Acoustic-Cavitation	5 µg L^{-1}	Ultrasound, amplitude 30-125 µm, 25 °C, 15 min, orifice plates (diameter 12 mm and thickness 2 mm)	> 96	Braeutigam et al. 2012
	O_3/UV/H_2O_2	---	O_3 (0.91 mg L^{-1}), UV (2.98 mW cm^{-2}),5.52 mg $L^{-1}$$H_2O_2$	100	Im et al. 2012

Sample	Pre-treatment/Treatment method	Concentration	Process Condition Parameters	Removal (%)	Reference
Water	UV and UV/H_2O_2	8.48 mg L^{-1}	UV doses (300-700 mJ cm^{-2}), H_2O_2 doses (0-10 mg L^{-1}), pH 5	< 20	Teunissen et al. 2012
	UV, UV/H_2O_2, Fenton, UV/Fenton and Photocatalysis (UV/TiO_2)	4.2-42.3 µM	UV: 172 mJ cm^{-2}, H_2O_2: 5 and 10 mM, ferrous ions: 0.5, 1.0 and 2.0 mM at pH 3.5, TiO_2: 0.5, 100, 1.5 and 2.0 g L^{-1} at pH 6.5,	12.2 40.65 67.8 86.9 and 70.4	Dai et al. 2012
	UV/H_2O_2 and UV/Chlorine	1 µg L^{-1}	UV-254 nm, 1 mg L^{-1} Cl_2, 5 mg L^{-1} H_2O_2, pH 7, temperature 20-22 °C	60 and 23	Sichel et al. 2011
	Ozonation	0.5 ng L^{-1}	Ozone dose : 1-1.3 mg L^{-1}, ozonation time: 10-20 min, pH 7.5	100	Vieno et al. 2007
	Ozonation	0.3-3.8 ng L^{-1}	Ozone dose : 1.5 to 2 mg L^{-1}, 20 min ozonation time, pH 7.5	100	Hua et al. 2006
	Photocatalysis	10 mg L^{-1}	TiO_2 (1 g L^{-1}), medium pressure UV, pH 2-12	30	Molinari et al. 2006
	Photodegradation	25 mg L^{-1}	UV: 313, 366, 406, 436, 546 and 578 nm, addition of 15 g L^{-1} Cl_2	>60	Chiron et al. 2006
	Photocatalysis	4.3 mg L^{-1}	TiO_2 (P25 and Hombikat UV100), solar UV simulator (1000 W), 9 h irradiation time	> 90	Doll and Frimmel, 2005
	Ozonation	54 ng L^{-1}	0.1 to 2 mg L^{-1} O_3, 20 min reaction time, temperature 20 °C,	100	Mcdowell et al. 2005

Sample	Pre-treatment/Treatment method	Concentration	Process Condition Parameters	Removal (%)	Reference
Water	Ozonation	80 µM	Ozone dose: 0.1-2 mg L^{-1}, pH 7	> 90	Huber et al. 2003
	Ozonation	0.5 mM	1 mg L^{-1} O_3, pH 5.5, 4 to 60 min ozonation time	100	Andreozzi et al. 2002
Aqueous Medium	Dieletric Barrier Discharges	20 mg L^{-1}	Ozone concentration (40 mg L^{-1}), power injected (0.7 W (*ex situ*) and 12 W (*in situ*)), time of reaction (3 min (*ex situ*) and 60 min (*in situ*))	100 (*ex situ*) 81 (*in situ*)	Liu et al. 2012
	Ultrasonic/Fe^0/H_2O_2	10 mg L^{-1}	Ultrasonication (40 kHz), Fe^0 (0.44-3.57 mM), H_2O_2 (12.5-100 mM), 21 °C, pH 3, 5, 7, and 9, 1 h reaction time	100	Ghauch et al. 2011
	Photocatalysis	17.7 mg L^{-1}.	TiO_2, ZnO, UV-254, 313, 364, 405, 436, 546 and 578 nm, room temperature	100	Martinez et al. 2011
	Direct Photodegradation	9.5 mg L^{-1}	Solar irradiation, 290-400 nm, 24 h irradiation time, pH 2.9, 4.0, 5.8 and 9.0	> 80	Calisto et al. 2011
	Photocatalysis	9 mg L^{-1}	UV-300 nm, TiO_2 (100 mg L^{-1}), temperature 20 °C, 2 h irradiation time	93	Ziegmann and Frimmel, 2010
	Ozonation, UV/H_2O_2 and O_3/TiO_2	1 mg L^{-1}	Ozone dose: 2 mg L^{-1}, UV-254 nm, H_2O_2: 11.03 mM, temperature 25 °C,	< 20, < 40 and 100	Giri et al. 2010
	Photocatalysis	4.3 mg L^{-1}	TiO_2 (P25 and Hombikat UV100), solar UV simulator (1000 W), temperature 22 °C, pH 6.5	---	Doll and Frimmel, 2004

Table 5. List of by-products formed during degradation of carbamazepine by different AOPs

AOP	By-products	Summary	Reference
UV and UV/H_2O_2	10, 11-epoxycarbamazepine and acridine	Minimal relationship between the transformation products and H_2O_2 dose and the production of intermediate products was non-sequential	Lekkerkerker-Teunissen et al. 2012
UV/H_2O_2	Hydroxycarbamazepine and trihydroxycarbamazepine	The oxidation by-products of carbamazepine containing a hydroxyl or carbonyl group can be fully mineralized by a mixed bacterial inoculum present in wastewater treatment plant	Keen et al. 2012
Ultrasonic/Fe^0/H_2O_2	Hydroxycarbamazepine and trihydroxycarbamazepine	By-products were unstable and disappear after 10 min of reaction	Ghauch et al. 2011
Photocatalysis (UV/TiO_2/ZnO)	10, 11- dihydro-carbamazepine-10, 11-epoxide	Stable intermediate	Martinez et al. 2011
Direct Photodegradation (solar irradiation)	Acridine; acridone or 9-hydroxy-acridine; acridine-9-carbaldehyde; and dihydroxycarbamazepine isomers	Acridine known to have mutagenic and carcinogenic activities	Calisto et al. 2011

AOP	By-products	Summary	Reference
Photodegradation (UV)	Acridone; hydroxy-(9*H*, 10*H*)-acridine-9-carbaldehyde; acridone-*N*-carbaldehyde; and 1-(2-benzaldehyde)-(1*H*, 3*H*)-quinazoline-2,4-dione	Most of the intermediate products belong to the azaarenes, an established class of air and water pollutants known for their photoenhanced toxicity, mutagenic and carcinogenic activity	Kosjek et al. 2009
O_3, O_3/UV, UV/H_2O_2	1-(2-benzaldehyde)-4-hydro-(1*H*, 3*H*)-quinazoline-2-one; 1-(2-benzaldehyde)-(1*H*, 3*H*)-quinazoline-2,4-dione; 1-(2-benzoic acid)-(1*H*, 3*H*)-quinazoline-2,4-dione; and di-aldehyde	Oxidation products could only be detected in Milli-Q water but not in treated wastewater samples, i.e., O_3-degradation products of carbamazepine as found in *ultra-pure water, could not be observed in* both non-spiked and spiked wastewater samples under the conditions tested	Gebhardt and Schroder, 2007
Photodegradation (UV)	10-hydroxycarbamazepine; hydroxyacridine-9-carboxaldehyde; acridone; acridine; and choloro-10-hydroxycarbamazepine	The direct photodegradation of carbamazepine yields a compound (acridine), the health and environmental impact of which is much higher than the parent compound	Chiron et al. 2006
Photocatalysis (UV/TiO_2)	10, 11- dihydro-carbamazepine-10, 11-epoxide; hydroxycarbamazepine; dihydroxycarbamazepine; acridine; acridine-9-carboxaldehyd; hydroxyacridine-9-carboxaldehyd; and hydroxyacridine-9-carboxalcohol	By-products were persistence and showed other ecological properties	Doll and Frimmel, 2005

AOP	By-products	Summary	Reference
Ozonation	1-(2-benzaldehyde)-4-hydro-(1*H*, 3*H*)-quinazoline-2-one; 1-(2-benzaldehyde)-(1*H*, 3*H*)-quinazoline-2,4-dione; and 1-(2-benzoic acid)-(1*H*, 3*H*)-quinazoline-2,4-dione	All the three by-products were formed by ozonation in waterworks when CBZ is present in the raw water and presence of these products in drinking water cannot be ruled out	McDowell et al. 2005

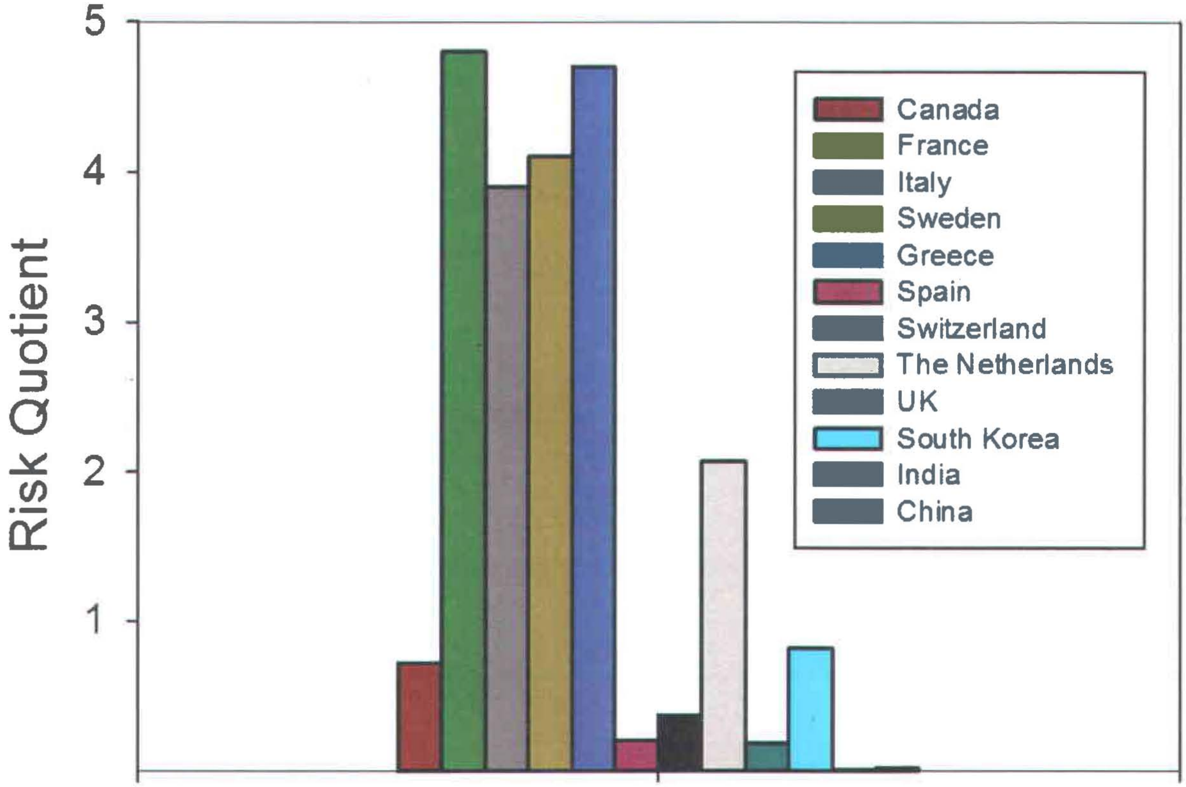


Figure 2. Risk quotient or risk ratio of carbamazepine reported in literature. Aquatic risk is suspected when risk quotient is ≥ 1.

Data from: Canada (Dussault et al. 2008), France (Ferrari et al. 2003), Italy (Ferrari et al. 2003), Sweden (Ferrari et al. 2003), Greece (Ferrari et al. 2003), Spain (Martin et al. 2012), Switzerland (Lienert et al. 2007; Escher et al. 2005), The Netherlands (Brandhof and Montforts, 2010), UK (Jones et al. 2002), South Korea (Kim et al. 2007), India (Malarvizhi et al. 2012; Ramaswamy et al. 2011), and China (Zhao et al. 2010).

CHAPITRE III

DEVENIR DU BPA ET DE LA CBZ DANS LA STATION D'ÉPURATION DES EAUX USÉES

PARTIE 1

OCCURRENCE OF BISPHENOL A IN WASTEWATER AND WASTEWATER SLUDGE OF CUQ TREATMENT PLANT

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PARTIE 2

CARBAMAZEPINE IN MUNICIPAL WASTEWATER AND WASTEWATER SLUDGE: ULTRAFAST QUANTIFICATION BY LASER DIODE THERMAL DESORPTION-ATMOSPHERIC PRESSURE CHEMICAL IONIZATION COUPLED WITH TANDEM MASS SPECTROMETRY

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RÉSUMÉ

La distribution de la carbamazépine (CBZ) dans les eaux usées (EU) et les phases aqueuse et solide des boues d'épuration (BE) a été étudiée. Une méthode d'analyse rapide et fiable, a été développée et validée. Cette méthode ultra-rapide permet une production plus rapide de données, une détection plus rapide et un suivi de la CBZ dans les EU et les BE. La méthode (15 s par échantillon) est basée sur l'utilisation de la désorption thermique induite par diode laser avec une ionisation chimique à pression atmosphérique (LDTD-APCI) couplée à la spectrométrie de masse en tandem (MS/MS). L'optimisation des paramètres d'appareillage et l'application environnementale de la méthode d'analyse sont présentées ici. Les performances de la méthode ont été évaluées en termes de recouvrement au cours de l'extraction, de linéarité, de précision et de limite de détection. Les limites de détection étaient de 12 ng/L dans les EU et 3.4 ng/g dans les BE. Dans les EU, les précisions intra et inter journée étaient de 8% et 11%, tandis qu'elles étaient de 6% et 9% dans les BE. Trois différentes méthodes d'extraction ont été comparées. Il s'agissait de l'extraction par ultrasons, l'extraction par micro-ondes (MAE) et l'extraction accélérée par solvant (ASE). Trois solvants différents ont été utilisés (méthanol, acétone et acétonitrile: acétate d'ethyle) (5:1, v / v) et comparés sur la base des blancs et le recouvrement. Dans l'ensemble, l'extraction accélérée par solvant a montré la meilleure efficacité d'extraction avec du méthanol par rapport aux deux autres méthodes. Par ailleurs, la quantification de la CBZ dans les EU et les échantillons de BE ont révélé la présence du polluant dans toutes les différentes étapes de traitement de la station des eaux.

Mots clés: Carbamazepine; Extraction; LDTD-APCI-MS/MS; Eaux usées; Boues d'épuration

ABSTRACT

In this study, the distribution of the anti-epileptic drug carbamazepine (CBZ) in wastewater (WW) and aqueous and solid phases of wastewater sludge (WWS) was carried out. A rapid and reliable method enabling high-throughput sample analysis for quicker data generation, detection, and monitoring of CBZ in WW and WWS was developed and validated. The ultrafast method (15 s per sample) is based on the laser diode thermal desorption-atmospheric pressure chemical ionization (LDTD-APCI) coupled to tandem mass spectrometry (MS/MS). The optimization of instrumental parameters and method application for environmental analysis are presented. The performance of the novel method was evaluated by estimation of extraction recovery, linearity, precision and detection limit. The method detection limits was 12 ng L^{-1} in WW and 3.4 ng g^{-1} in WWS. The intra- and inter-day precisions were 8% and 11% in WW and 6% and 9% in WWS, respectively. Furthermore, three extraction methods, ultrasonic extraction (USE), microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) with three different solvent condition such as methanol, acetone and acetonitrile:ethyle acetate (5:1, v/v) were compared on the basis of procedural blank and method recovery. Overall, ASE showed the best extraction efficiency with methanol as compared to USE and MAE. Furthermore, the quantification of CBZ in WW and WWS samples showed the presence of contaminant in all stages of the treatment plant.

Keyword: Carbamazepine; Extraction; LDTD-APCI-MS/MS; Wastewater; Wastewater sludge

1. INTRODUCTION

Large volumes of pharmaceuticals are used for the prevention, diagnosis and treatment of diseases in human and animals. The worldwide average per capita consumption of active pharmaceutical ingredients per year is estimated to be about 15 g and in industrialized countries, the value is expected to be in between 50 to 150 g [1]. Due to their extensive use, presence in the aquatic environment and potential for impacts on wildlife and humans, pharmaceutical compounds are becoming an environmental issue [2]. Therefore, study of the fate of these compounds is becoming very important component of assessing the environmental risks associated with them.

Carbamazepine (CBZ) is one such important drug used: (i) as an antieliptic and anticonvulsant; (ii) for the treatment of epilepsy, as well as for various psychotherapeutic applications and; (iii) in combination with other drugs for the treatment of alcohol withdrawal [3]. The physico-chemical and pharmacological properties of CBZ are summarized in Table 1. CBZ has been proposed as an anthropogenic marker in water bodies [6]. Annually, about 1014 tons of CBZ is consumed worldwide (estimated value is in accordance with Intercontinental Marketing Services (IMS) Health data: 942 tons of CBZ were sold in 2007 in 76 major countries which are believed to account for 96% of the global pharmaceutical market) and this yields to more than 30 tons of CBZ which have to be removed from effluents [7]. In Canada, approximately 28 tons of CBZ was sold as prescriptions in 2001 [8].

Following human administration (excreted unchanged and/or as metabolies with feces and urine), CBZ has been detected in wastewater (WW) and wastewater sludge (WWS). Studies in Europe and North America have shown that CBZ is one of the most frequently detected pharmaceuticals in wastewater treatment plants (WWTPs) effluents and in river water [9,10,11]. As WWTPs provide the first and perhaps the most important opportunity for removing CBZ that are destined for discharge into the environment, it is important to characterize the fate of CBZ during the treatment of municipal wastewater. The most important process to study the fate of CBZ in WW and WWS includes to know whether CBZ will primarily enter the aquatic or terrestrial environment or get partitioned from aqueous sewage into sludge and disposed with further application of sludge. Several groups have investigated the elimination of CBZ during sewage treatment and also fate of the compound in different contaminated media including WW and WWS [12,13]. However, to the best of our knowledge there have been no studies conducted on partitioning of CBZ in different compartment of WWTPs which is very important to know the fate of compound and to select different treatment processes for effective degradation of compound.

Furthermore, the measurement problems associated with quantification of these pharmaceutical compounds including CBZ in WW and WWS is to detect the analyte in trace levels (ng L^{-1} or below) and to avoid the impact on the analyte signals caused by matrix components. Identification and quantification of different pharmaceutical compounds including CBZ are usually performed by LC or GC-MS/MS [13,14]. To reduce sample preparation, analysis time and concentration of organic solvents (during quantification), the development of an ultrafast method for the analysis of CBZ in WW and WWS has been carried out using a laser diode thermal desorption (LDTD) coupled to an atmospheric pressure APCI source for tandem mass spectrometry (MS/MS). LDTD-APCI-MS/MS analysis has been recently developed to enhance the high throughput capacity in MS by reducing LC-MS/MS runs of 5 to 30 min to 10 to 30 s in LDTD-APCI-MS/MS run [15].

In this study, we developed a suitable ultrafast method based on LDTD-APCI-MS/MS method for quantification of CBZ in WW and WWS which has never been studied earlier. Furthermore, three extraction methods namely, ultrasonic extraction (USE), microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) were compared for extraction of CBZ from WWS by spiked recovery experiments. Furthermore, the concentration of CBZ was monitored in WW and WWS at various stages of treatment in the WWTP for the Quebec City, Qc, Canada.

2. MATERIALS AND METHODS

2.1. Chemicals

CBZ was obtained from Sigma-Aldrich (St Louis, MO, USA). The internal standard (IS), carbamazepine-d_{10} was purchased from C/D/N Isotopes Inc. (Montreal, Quebec, Canada). HPLC-grade methanol (MeOH), acetonitrile, ethyl acetate, and acetone, were purchased from Fisher Scientific (Ontario, Canada). Sep-Pak Plus C18 environmental cartridges used for solid phase extraction (SPE) clean-up was purchased from Waters (Milford, MA, USA). HPLC grade water was prepared in the laboratory using a Milli-Q/Milli-RO Millipore system (Milford, MA, USA).

2.2. Wastewater treatment plants and sampling

Samples were collected from Quebec Urban Community (CUQ) wastewater treatment plant (Beauport, Quebec city, Quebec, Canada) which receives wastewater originating from domestic zones, industries, commercial enterprises and institutions present in the city. The eastern station of WWTP serves a population of 528,016 (2006 estimate) and has a treatment capacity of 400,000 m^3 day^{-1}. The CUQ treatment plant accomplishes primary and physical-chemical treatment of sewage before discharging the treated water into the Saint-Lawrence River.

Samples of WW and WWS were collected as grab samples during the sampling periods (August 20th, 21st, 22nd, 23rd, 24th, 2011). Fig. 1 represents a schematic of the treatment process and the different sampling locations. Samples were collected in pre-cleaned glass amber bottles with aluminum foil-lined caps. After sample collection, in order to study the partitioning of CBZ in WWS, the liquid fraction of WWS was separated from the solid fraction by centrifugation at 7650 *x* g for 15 min. Later, WWS (primary sludge, secondary sludge, mixed sludge, dewatered sludge) and the solid fraction of WWS (primary sludge solids, secondary sludge solids and mixed sludge solids) were stored at 4±1 ^{0}C in a cold room until preparation for analysis, which generally occurred within 24 h of collection. Furthermore, WW (influent, grit influent, effluent) and liquid fraction of WWS (primary sludge liquid, secondary sludge liquid and mixed sludge liquid) samples were filtered through a 0.45 µm glass-fiber (Fisherbrand G6 filter circles, Fisher Scientific, Ontario, Canada) and immediately stored at 4±1 ^{0}C until analysis.

2.3. Extraction

Different WWS and solid fraction of WWS were frozen using liquid nitrogen prior to lyophilization by the freeze-dry system (Dura Freeze Dryer, Kinetics). Three types of extraction methods, ultrasonic extraction (USE), microwave-assisted extraction (MAE) and accelerated solvent extraction (ASE) were carried out in order to optimize the extraction method for higher recovery of CBZ from WWS. The experiments were carried out by spiking the known concentration of CBZ (100 ng g^{-1}) to WWS samples.

2.3.1. Ultrasonic extraction (USE)

Ultrasonic extraction process was carried out by using a Fisher 100 Model Ultrasonicator. An amount of 0.5 g of lyophilized and homogenized sludge was transferred to a glass tube followed by addition of 20 mL of solvent. The tubes were shaken for 20 min in a mechanical shaker and sonicated for 15 min. The extract was separated by centrifugation at 7650 × g for 15 min and the procedure was repeated three times. The extracts were combined, concentrated to an approximate volume of 1 mL with gentle stream of nitrogen and re-dissolved in 100 mL of HPLC grade water. After the extraction process, the sample was transferred for clean-up procedure.

2.3.2. Microwave-assisted extraction (MAE)

A CEM MARS Microwave Accelerated Reaction System (CEM Corporation, North Carolina, USA) was used. This system allowed up to 14 extraction vessels to be simultaneously irradiated. An amount of 0.5 g of lyophilized and homogenized sludge was transferred to a vessel equipped with Teflon-sealed lip-tight caps and polyetheretherketone (PEEK)-liners followed by addition of 20 mL of solvent. Microwave power was 1200W (100%) and the extraction was performed in a temperature-controlled mode. The extraction temperature was 110 ± 1 ^{0}C and programmed as follows: ramp to 110 ± 1 ^{0}C for 10 min, holding at 110 ± 1 ^{0}C for 10 min. The extract was separated by centrifugation at 7650 × g for 15 min. Furthermore, the extracts were concentrated to an approximate volume of 1 mL with gentle stream of nitrogen and re-dissolved in 100 mL of HPLC grade water. The sample was then subjected to the clean-up procedure.

2.3.3. Accelerated solvent extraction (ASE)

Accelerated solvent extraction was carried out by using an ASE 350 extractor (Dionex, Sunnyvale, CA, USA). Lyophilized and homogenized sludge sample (0.5 g) was extracted in a 34 mL stainless steel vessel. Extraction was performed with 20 mL of solvent at 140 ^{0}C and 1500 psi for a 6 min heat-up followed by a 5 min static extraction. The vessel was then rinsed with 10 mL of solvent and the extract was separated by centrifugation at 7650 × g for 15 min. Furthermore, the extract was concentrated to an approximate volume of 1 mL with gentle stream of nitrogen and r-edissolved in 100 mL of HPLC grade water. The sample was then subjected to the clean-up procedure.

2.4. Clean-up

Solid phase extraction (SPE) method was used for clean-up and pre-concentration of extract obtained from USE, MAE and ASE methods. Sep-Pak Plus C18 environmental cartridges were fitted into the vacuum manifold (Welch, USA) which was connected to a vacuum pump (Welch Rietschle Thomas, USA) to dispense samples through the cartridges. Cartridges were pre-conditioned by passing 7 mL of methanol and 3 mL of HPLC water at a flow rate of 1 mL min^{-1}. WWS and solid fraction of WWS extracts and filtered WW and liquid fraction of WWS (100 mL each) were passed at a flow rate of 5 mL min^{-1}. After pre-concentration, the sorbents were dried by using a vacuum system set at (-15) psi.

The elution was performed by adding 2 x 4 mL of methanol to the cartridge at a flow rate of 1 mL min^{-1} and giving it a wait time of 10 min in order to give enough duration of contact between the solvent and the adsorbed compounds. The extracts were later evaporated to dryness with a gentle stream of nitrogen and reconstituted with methanol to a final volume of 200 µL prior to Laser Diode Thermal Desorption-Atmospheric Pressure Chemical Ionization-Mass Spectrometry/Mass Spectrometry (LDTD-APCI-MS/MS) analysis. The overall scheme of the analytical procedure used for the quantification of CBZ in WW and WWS, solid and liquid fraction of WWS is presented in Fig. 2.

2.5. LDTD-APCI-MS/MS analysis

Quantification of CBZ in WW and WWS was achieved with the LDTD-APCI ionization source (Phytronix Technologies, Quebec, Canada) mounted on a TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples were first spotted (2 µL) into the LazWell 96-well polypropylene plate cavities containing inserts made of proprietary stainless steel alloy and left to dry at room temperature. The designed well shape allows the sample to concentrate in the heating zone while drying. The loaded plate is then transferred to an *X-Y* movable stage of the LDTD housing unit. An infrared (IR) laser diode (980 nm, 20 W, continuous) is then focalized to impact the back of the inserts, thermally desorbing the dried sample, which is vaporized into gas phase. The desorbed gas phase neutral molecules are carried over by a carrier gas (medical grade purified air) into a corona discharge region to undergo APCI and then introduced directly into the mass spectrometer.

Optimization of LDTD-APCI-MS/MS method for analysis of CBZ in WW and WWS was carried out in following steps: (a) LDTD-APCI sample optimization for MS and MS/MS conditions in negative ionization mode (NI) and positive ionization mode (PI) with following conditions: corona discharge voltage (5000 V in PI mode and 5500 V in NI mode); vaporizer temperature (450 ^{0}C); sheath gas pressure (0 arbitrary units); auxiliary gas pressure (0 arbitrary units); capillary temperature (270 ^{o}C); collision pressure (1.5 mTorr) and scan time (0.1 s); (b) optimization of the LDTD/APCI physical parameters using the defined SRM scans.

2.6. Data analysis and method validation

The LDTD-APCI source was controlled by the LazSoft 4.0 software (Phytronix Inc., Quebec, Canada). Quantitative analysis was performed using selected reaction- monitoring mode (SRM). Quantification of CBZ was performed by the internal standard method based on peak areas and relative retention time, using carbamazepine d_{10} as internal standard. A three-point identification approach was adopted with peak areas from the first transition (SRM1) for the quantitative analysis and a second transition (SRM2) along with the ratio of areas obtained from the first to second transitions were used for confirmation with sample tolerance established by the relative standard deviation of the ratio obtained from the standards [16]. Resulting MS/MS peaks were integrated by using the ICIS algorithm of the Xcalibur 1.2 software from Thermo Fisher Scientific (Waltham, MA, USA). Experiments with spiked samples and blank (solvent with IS) (minimum two experiments) were carried out in parallel for each set of extractions, clean-up and quantification studies.

The matrix effects were studied by the evaluation of signal suppression or enhancement. The signal suppression was calculated as the percentage of the decrease or increase in signal intensity in a sample matrix versus in methanol-water (1:9, v/v). Equation (1) was used to calculate the signal suppression:

$$\text{Signal suppression (\%)} = \left(1 - \frac{S_m}{S_s}\right) \times 100 \qquad (1)$$

where, S_m is the slope of the calibration curve of CBZ in the sample extract (WW or WWS) spiked after extraction and S_s is the slope in the solution standard (methanol-water (1:9, v/v)) at the same concentration than the spiked sample. To overcome the effect of matrix, standard addition was used and performed as described in Ellison and Thompson [17]. The recoveries of CBZ in different samples of WW and WWS through the method were evaluated by analyzing five replicates and then applying Equation (2):

$$\text{Recovery (\%)} = \frac{C_m - C_O}{C_s} \times 100 \qquad (2)$$

where, C_m = measured concentration of the CBZ observed in the spiked matrix, C_o = initial concentration of CBZ observed in matrix and C_s = concentration of CBZ spiked in the matrix. In order to measure the recovery, the spiked samples were stirred to homogenize the analytes within the matrix and were left to equilibrate for 24 h before extraction. A five point standard addition was carried out for calibration curve (triplicate injections) ranging from 50 ng L^{-1} and 700 ng L^{-1} for WW and 10 ng g^{-1} and 500 ng g^{-1} for WWS. The area ratio (analyte:internal standard) was plotted against the analyte additions to obtain the calibration curve.

The limits of detection (LOD) were determined using the standard error of the intercept and the slope of the calibration curve. LODs were defined as the concentration of a compound giving a signal-to-noise ratio of 3. The intra-day method repeatability was expressed as the relative standard deviation (in %) was calculated by analysis of the same spiked sample for each type of matrix at 100 ng L^{-1} (three times) on a given working day. Reproducibility (inter-day precision) was calculated by extracting a sample spiked at 100 ng L^{-1} freshly prepared each day for three days.

3. RESULTS AND DISCUSSION

3.1. LDTD-APCI-MS/MS optimization

MS/MS optimization showed a good sensitivity for quantification of CBZ in WW and WWS using APCI mode. The signal found with APCI showed a good intensity in positive ionization mode. Hence, the positive mode was selected for analysis of CBZ in WW and WWS. Once SRMs were fixed in the APCI mode, MS and MS/MS scan by the LDTD source were performed. The optimization of LDTD-APCI physical parameters was then calculated using the defined SRM scans.

The LDTD-APCI physical parameters were optimized for the quantification of CBZ in WW (influent was considered due to presence of higher concentration of CBZ) and WWS (secondary sludge was considered due to low matrix effect) samples. The deposition solvent, laser power, laser pattern and carrier gas flow rate are parameters affecting the thermal desorption and the ionization of the analytes in LDTD [18]. Thus, all parameters mentioned above were optimized for analysis of CBZ in WW and WWS.

The solvent used for analyte deposition should have a surface tension superior to 27 mN/m to prevent the sample droplet to flow outside the designed cavity of the sample well [19]. The peak intensity of CBZ analyzed in WW and WWS for six different solvents used for analyte deposition is presented in Fig. 3. Maximum peak intensity of CBZ was observed with methanol:water (2:1, v/v) with relative standard deviation below 10% in WW and WWS. Therefore, methanol:water (2:1, v/v) was the preferred solvent used for analyte deposition into the well cavity for all type of WW and WWS samples.

The laser power, laser duration and pattern as well as the carrier gas flow rate were optimized with methanol:water (2:1, v/v) as the selected solvent. Laser power was optimized in both WW and WWS by using CBZ and internal standard (carbamazepine d_{10}). Each sample was analyzed in triplicate from 5 to 50% laser power. The maximum peak intensity was observed at 25% and 35% laser power in WW and WWS, respectively.

Carrier gas flow rate was optimized in WW and WWS by using CBZ and internal standard (carbamazepine d_{10}). The peak intensity of CBZ analyzed in WW and WWS for a carrier gas flow rate varied from 1 to 10 L min^{-1} as presented in Fig. 4. Maximum peak intensity of CBZ was observed for a carrier gas flow rate of 3 L min^{-1} in WW and WWS. The results were in agreement with previously published LDTD optimization studies for hormones in WW [20].

Furthermore, other factor that affects the optimization of LDTD method is the laser pattern. Different laser patterns were tried with the optimum laser power obtained in WW and WWS. The laser pattern programming that gave the maximum peak area intensity in WW was 1 s ramp from 0% to 25%, held for 3 s in 25% before shutting off the laser. In WWS, the laser pattern was 2 s ramp from 0% to 35%, held for 2 s at 35% before shutting off the laser. Increasing holding time past 3 s (at 25%) and 2 s (at 35%) in WW and WWS samples respectively, with the same laser pattern did not improve peak area intensities (data not shown) and would have lengthened the analysis time.

3.2. Method validation

The LDTD/APCI-MS/MS method with the optimized physical parameters was applied to spiked WW and WWS samples. For each matrix, three spiked samples (50 ng L^{-1} for WW samples and 100 ng g^{-1} for WWS samples) and three non-spiked samples were extracted and analyzed. Good recoveries of CBZ was observed and ranged from 98% to 113% and 96% to 107% for WW and WWS samples, respectively. The calibration curve observed for quantification of CBZ in WW and WWS showed good linearity with correlation coefficients (R^2) of 0.9997 and 0.994 in WW and WWS, respectively (Table 2).

The repeatability (intra-day precision) and reproducibility (inter-day precision) were observed to be 8% and 11%, respectively in WW (Table 2). In case of WWS, the repeatability and reproducibility was observed as 6% and 9%, respectively. In both WW and WWS, the results of repeatability and reproducibility showed robustness and stability. Method detection limits (MDL) calculated from the calibration curves were 12 ng L^{-1} and 3.4 ng g^{-1} for WW and WWS, respectively (Table 2). The resulting MDL were comparable to several other analytical methods applied to analysis of CBZ in WW and WWS, including GC-MS or LC-MS/MS methods [21,22].

3.3. Comparison of USE, MAE and ASE methods

The experiment was carried out using the comparison of procedure blanks of the three extraction methods, namely USE, MAE and ASE. A series of procedure blanks of the three extraction methods were performed in order to check the contamination due to extraction procedure. The experiments were carried out in triplicate. The procedural blank values (data not reported) obtained were up to two orders of magnitude smaller than the concentration of CBZ in actual samples and thus did not exhibit notable influence to the experimental results. In order to investigate whether the blank values obtained from the three different extraction methods were statistically significant; a STATISTICA 7 of STATSOFT Inc. (Thulsa, USA), ANOVA test was performed at the significance level of 0.05. There was no significant difference in the blank values were observed among the three extraction method. Overall, no contamination to target compound was observed during the whole experimental procedure of extracting the CBZ from different WWS samples.

Furthermore, the three extraction methods, USE, MAE and ASE were compared on the basis of recovery efficiency of CBZ from WWS samples. The recovery experiments were performed using spiked (100 ng g^{-1}) WWS samples. Table 3 represents recoveries of CBZ from spiked WWS samples for different type of solvents with the methods of USE, MAE and ASE. The recoveries obtained from USE with methnol, acetone and acetonitrile:ethyl acetate (5:1, v/v) were 85.4-90.1%, 80.1-86.3% and 77.6-84.2% respectively. The recoveries obtained from MAE with methanol, acetone and acetonitrile:ethyl acetate (5:1, v/v) were 91.1-95.3%, 82.1-87.9% and 70.0-88.2% respectively. The recoveries obtained from ASE with methanol, acetone and acetonitrile:ethyl acetate (5:1, v/v) were 96.9-107.0%, 84.2-93.0% and 82.5-90.8% respectively. Higher extraction recoveries of CBZ from different WWS samples were observed with ASE ($P <$ 0.01) under methanol as solvent. To investigate the statistical significance of the difference in recovery efficiency of the three extraction methods, a statistical software, STATISTICA 7 of STATSOFT Inc. (Thulsa, USA), ANOVA test was used. Statistical significance was defined as a P value <0.05. The statistical significance was determined based on comparison of F-value (F) with critical F-value (Fc) at 95%, 99% and 99.9% confidence. The results showed that the differences in extraction recoveries were significant (Supplementary material, Table. 1S) among ASE and MAE and not significant in some results of USE extraction methods.

Furthermore, the recovery test was also carried out with the internal standard (carbamazepine d_{10}) under three different extraction methods. Higher recovery was also observed (data not reported) with ASE as compared to USE and MAE under methanol as solvent.

Among three type of extraction methods, the order of recovery of CBZ from different WWS samples by using three type of solvents, methanol, acetone and acetonitrile:ethyl acetate was ASE > MAE > USE. The method recoveries observed are in accordance with the values from most of other studies [23,24,25]. Higher recoveries observed in ASE and MAE as compared to USE may be due to higher extraction temperature (140 ^{0}C and 110 ^{o}C, respectively) used. During the extraction process, the rate-limited step was desorption of the analytes from the active spots of the matrix. The higher temperature condition helps to improve the dissolving capability and to minimize the force between the analytes and the active spot of the matrix. Furthermore, the higher temperature lowered the surface tension and viscosity of the solvent, thus making a full contact between the solvent and analytes. Furthermore, the higher recovery of CBZ observed in ASE as compared to MAE was due to the combination of high temperature and pressure conditions. Higher pressure leads to extraction of analytes from the pore water.

An additional comparison of the three extraction methods was performed based on desorption peak. LDTD-APCI-MS/MS desorption peaks obtained from extracting CBZ from secondary sludge with methanol as solvent using USE, MAE and ASE is presented in Fig. 5. It was observed that the desorption peaks of CBZ and internal standard obtained in all the three type of extraction methods were clear without any other interference. The slightly different desorption peaks observed for the same secondary sludge samples may be due to different extraction intensities of the three methods.

3.4. Application of optimization method

The applicability of the method was tested by the determination of CBZ in WW and WWS of CUQ wastewater treatment plants. Results are shown in Table 4. CBZ was detected in all the process streams of the wastewater treatment plant. It was detected at the highest concentration in the influent where a concentration of 420 ng L^{-1} was observed. The higher concentration of CBZ in influent suggests that the sources of CBZ in urban wastewater are very significant. Under the sampling conditions of this study, since no precipitation was recorded in the region served by the municipal sewer system in the days preceding the sampling, the flows mainly consisted of domestic, commercial and industrial discharges. The concentration of CBZ in the aqueous phase of WW declined from 420 ng L^{-1} in influent to 261 ng L^{-1} (38% decline) in effluent, indicating moderate removal of CBZ from the aqueous phase during different steps of treatment. Removal rate of CBZ over the total treatment process observed in this study (38%) was higher than the average removal rate of 1-10% reported for WWTP [7]. Further investigation should be conducted to determine the specific mechanisms for removal of CBZ within the WWTP.

To investigate if the concentration of CBZ observed in different samples of WWTP were statistically significant, a STATISTICA 7 of STATSOFT Inc. (Thulsa, USA), ANOVA test was performed at the significance level of 0.05. The results (Suplementary material, Table 2S) showed that the CBZ concentrations were significantly different ($P < 0.05$) among the different samples and highly significantly different in effluent ($P < 0.01$).

CBZ was detected in all types of sludge and sludge solids (Table 4). Among the four types of sludge (primary sludge, secondary sludge, mixed sludge and dewatered sludge), the highest concentration of CBZ was found in primary sludge and lowest in secondary sludge. However, the concentration detected in the solid fraction of sludge was found to very low as most of CBZ was removed with the liquid fraction. The higher concentration of CBZ detected in liquid fraction of sludge as compared to their solid counterpart was mainly due to low $log\ K_{ow}$ value (2.45) (Table 1) of CBZ. The results point towards the liquid portion or wastewater requiring more treatment for degradation of CBZ and also in selection of different treatment methods.

The estimation of daily mass flows of CBZ is presented in Table 5. The results presented can be considered as preliminary estimates of mass balance which is a reasonable estimation of quantity of CBZ distributed in various streams of WWTP. Approximately 146 g d^{-1} of CBZ was received by WWTP. Following treatment, approximately 62% of CBZ was discharged in the effluent into receiving surface water (Saint-Lawrence River), in this case CBZ is present in the effluent in environmentally important concentrations that may cause physiological effects in aquatic life. Finally, there is a possibility of ingestion by aquatic organisms, such as invertebrates resulting in potential aquatic toxicity. Ferrari et al. [26] studied the toxic effects of CBZ on bacteria, algae, microcrustaceans and fish and observed that CBZ had a limited acute ecotoxicity on the tested organism. According to the results of the present European legislation on the classification and labeling of chemicals (92/32/EEC), Jos et al. [27] classified CBZ as "R52/53 Harmful to aquatic organisms and may cause long term adverse effects in the aquatic environment.

With respect to the analyzed solid residues, grit residues, secondary and mixed sludge solids did not represent an important output for CBZ. In contrast, 1.2% of CBZ was found in dewatered sludge, which was subsequently incinerated. The dewatered sludge from the CUQ WWTP is incinerated at approximately 900 ^{0}C, which mineralizes the organic components. Hence, it is presumed that CBZ would be absent from ash residues. The reduction in sludge volume by incineration produces secondary environmental pollution and is cost intensive. Hence, if incineration was discontinued for beneficial end use of sludge, such as land application (agriculture) and land spreading, the presence of CBZ might raise question in sludge reuse with further possibility of contamination of groundwater aquifers.

4. CONCLUSIONS

The study on optimization of LDTD-APCI-MS/MS method, extraction methods for CBZ and fate of CBZ in WW and WWS lead to following conclusions:

1. Different optimization and operation parameters for LDTD-APCI-MS/MS is suitable for the rapid detection and quantification of CBZ in WW and WWS and help to overcome the traditional use of liquid chromatography which composes use of expensive organic solvents, cost of maintenance of chromatography pumps and replacing chromatography columns.
2. The different optimum conditions for analysis of CBZ in WW and WWS included: (a) optimal solvent used for analyte deposition to the sample well cavities as methanol:water (2:1, v/v); (b) desorption laser power as 25% for wastewater and 35% for wastewater sludge and; (c) carrier gas flow rate as 3 L min^{-1} .
3. Due to the simplicity of this system, generic methods can be developed and applied for the high-throughput analysis of wide variety of organic compounds in WW and WWS without using the solvents (no mobile phase) during quantification respecting the green chemistry principles.
4. For extraction of CBZ from WWS samples, among the three extraction methods, accelerated solvent extraction with methanol as solvent offered the best extraction efficiency (96.9-107.0%) as compared to ultrasonic and microwave-assisted extraction.
5. Quantity of CBZ present in effluent (261 ng L^{-1}) and dewatered sludge (15 ng g^{-1}), questioning the release of effluent to river and reuse and recycle of sludge, thus requiring the efficient removal of CBZ from WW and WWS streams.

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Table 1. Physico-chemical and pharmacological properties of CBZ

Molecular formula, CAS No. and molecular weight	$C_{15}H_{12}N_2O$, 298-46-4 and 236.27 g mol^{-1}
Water solubility[a]	17.7 mg L^{-1} (20 ^{O}C)
Log K_{ow} (octanol-water partitioning)[a]	2.45
Henry's Law Constant[a]	1.09 X 10^{-5} Pa m^3 mol^{-1} (25 ^{O}C)
p*K*a	Neutral
Melting Point	189-193 ^{o}C
Usage	Analgesic, anticonvulsant, antimanic agent
Elimination half-life[b]	25-65 h
Appearance	White, light yellowish powder
Toxicity	Mild ingestion cause vomiting, drowsiness, ataxia, slurred speech, nystagmus, dystonic reactions, and hallucinations. Severe intoxications may produce coma, seizures, respiratory depression and hypotension
Affected organisms	Human and aquatic organisms

[a][4]; [b][5]

Table 2. Linearity (R2), repeatability, reproducibility and method detection limit (MDL) of the LDTD-APCI-MS/MS method for CBZ in positive ionization (PI) mode

	R^2	Intra-day precision (% RSD)	Inter-day precision (% RSD)	MDL
WW	0.9997	8	11	12 ng L^{-1}
WWS	0.994	6	9	3.4 ng g^{-1}

Table 3. Mean recoveries (%) and R.S.D. (%) of CBZ by USE, MAE and ASE method with different solvents

	Methanol						Acetone						Acetonitrile:ethyle acetate (5:1, v/v)					
	USE		**MAE**		**ASE**		**USE**		**MAE**		**ASE**		**USE**		**MAE**		**ASE**	
	Reco	RSD	Reco	RSD	Reco	RSD	Reco	RSD	Reco	RSD	Reco	RSD	Reco	RSD	Reco	RSD	Reco	RSD
P.S	89.17	2.9	93.77	5.3	97.19	2.5	82.8	2.5	85.7	1.7	92.6	5.9	80.1	3.5	76.19	5.1	82.51	2.9
S.S	90.03	4.8	95.3	2.9	107	1.8	86.3	1.1	87.93	4.2	92.9	1.0	83.6	1.3	72.51	2.6	86.03	4.0
M.S	89.95	5.6	93.97	4.0	100.1	0.86	83.2	3.7	85.91	7.1	90.7	7.2	80.95	4.7	75.9	2.2	90.71	4.6
D.S	88.26	2.1	91.08	4.6	99.83	1.93	80.1	4.2	82.1	2.3	84.1	2.5	77.58	8.1	70.01	4.7	83.90	2.9
P.S.S	84.98	5.3	92.51	2.7	96.92	1.25	82.53	3.7	82.8	5.2	86.2	1.9	79.1	2.4	88.19	6.1	88.15	2.2
S.S.S	86.0	1.4	94.03	4.9	103	2.0	83.8	1.9	84	1.9	88.0	4.1	84.15	1.3	79.05	4.0	90.08	1.5
M.S.S	85.41	3.7	93.10	3.5	98.25	1.21	83.97	1.0	86.1	4.5	86.9	3.3	82.75	1.0	83.91	1.9	90	7.3

Recoveries are the average of the three determinations at spiked concentrations of 100 ng g^{-1}. P.S:primary sludge;S.S:secondary sludge;M.S:mixed sludge; D.S: dewatered sludge;P.S.S:primary sludge solids;S.S.S:secondary sludge solids;M.S.S:mixed sludge solids

Table 4. Mean concentrations of CBZ (*mean* ± SD, *n* = 3) in WW and WWS of Quebec Urban Community (CUQ) wastewater treatment plant

Sample	**Concentration (ng L^{-1})**	**Sample**	**Concentration (ng g^{-1})**
Influent	420 ± 17	Primary sludge	94 ± 12
Grit liquid	327 ± 9	Secondary sludge	56 ± 9
Primary sludge liquid	73 ± 3	Mixed sludge	81 ± 4
Secondary sludge liquid	29 ± 12	Dewatered sludge	15 ± 7
Mixed sludge liquid	46 ± 9	Grit residue	69 ± 11
Effluent	261±7	Primary sludge solid	21 ± 3
		Secondary sludge solid	13 ± 5
		Mixed sludge solid	18 ± 9

Table 5. Estimation of daily mass flows of CBZ in wastewater and sludge of the CUQ treatment plant and quantities of CBZ in these streams expressed relative to those observed in the influent (in %)

Compartments	Stream flow	Mass flows (g d^{-1}) (residual quantities relative to influent, in %)
Influent	346951 m^3d^{-1}	146
Effluent	346951 m^3d^{-1}	91 (62%)
Grit residues	2.8 tons d^{-1}	0.2 (~ 0%)
Primary sludge	63.8 tons d^{-1}	6.0 (4%)
Secondary sludge	24.71 tons d^{-1}	1.4 (1%)
Mixed sludge	47.93 tons d^{-1}	3.9 (3%)
Dewatered sludge	76 tons d^{-1}	1.2 (1%)

The mass balance of CBZ along the different units of WWTP was carried out by using the equation: m = Q × S where m is the mass flow (g d^{-1}), Q is stream flow (m^3 d^{-1} or tons d^{-1}) and S is the concentration of CBZ (ng L^{-1} or ng g^{-1}). Value of Q was calculated by averaging the stream flow in each unit during the sampling periods (August 20th, 21st, 22nd, 23rd, 24th, 2011)

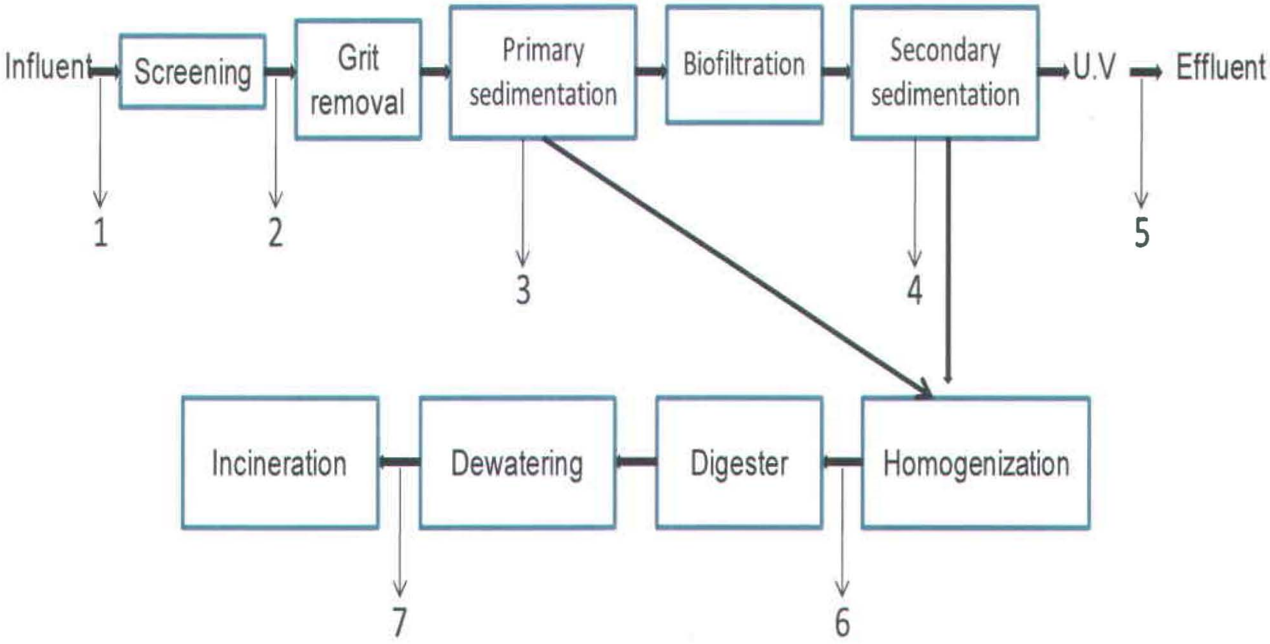

Figure 1. Schematic of the treatment steps of WWTP located in Quebec (Quebec), Canada with different sampling points (1: Influent; 2: Grit influent; 3: Primary sludge;4: Secondary sludge; 5: Effluent; 6: Mixed sludge;7: Dewatered sludge)

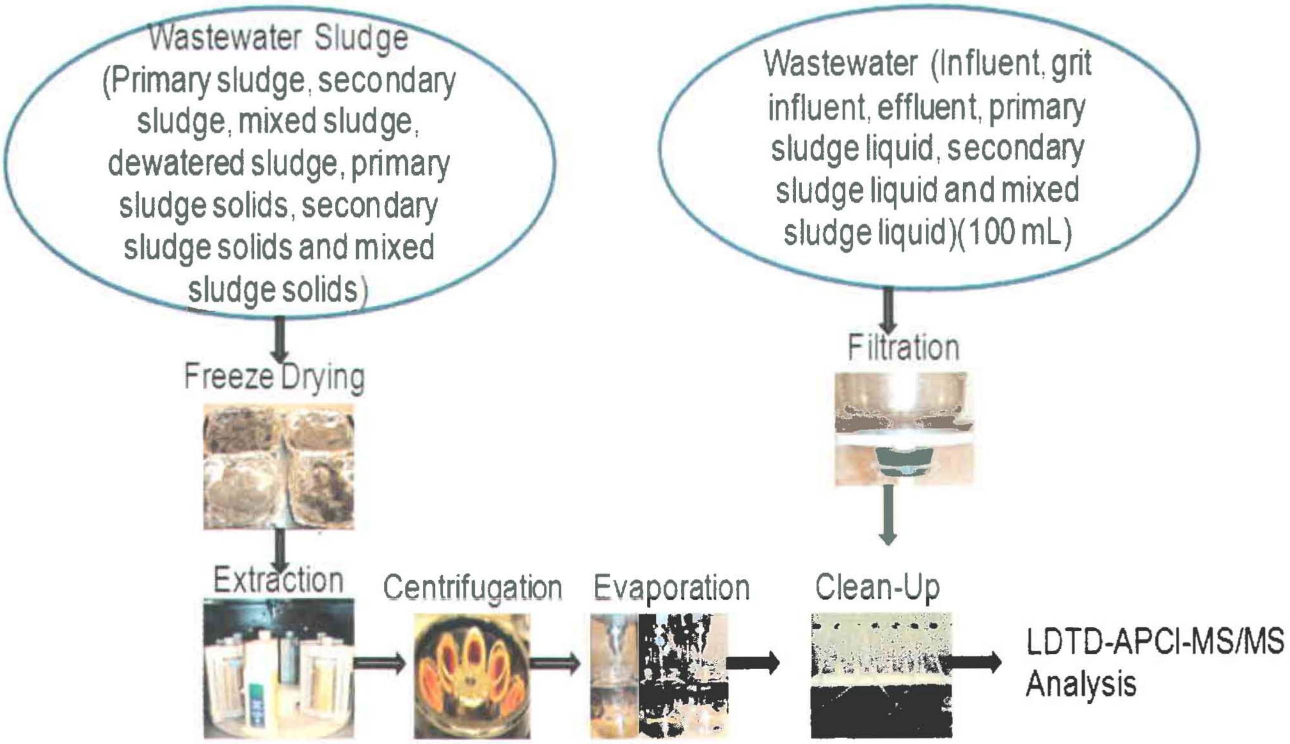

Figure 2. Sample processing and chemical analysis scheme used for analysis of CBZ in WW and WWS samples

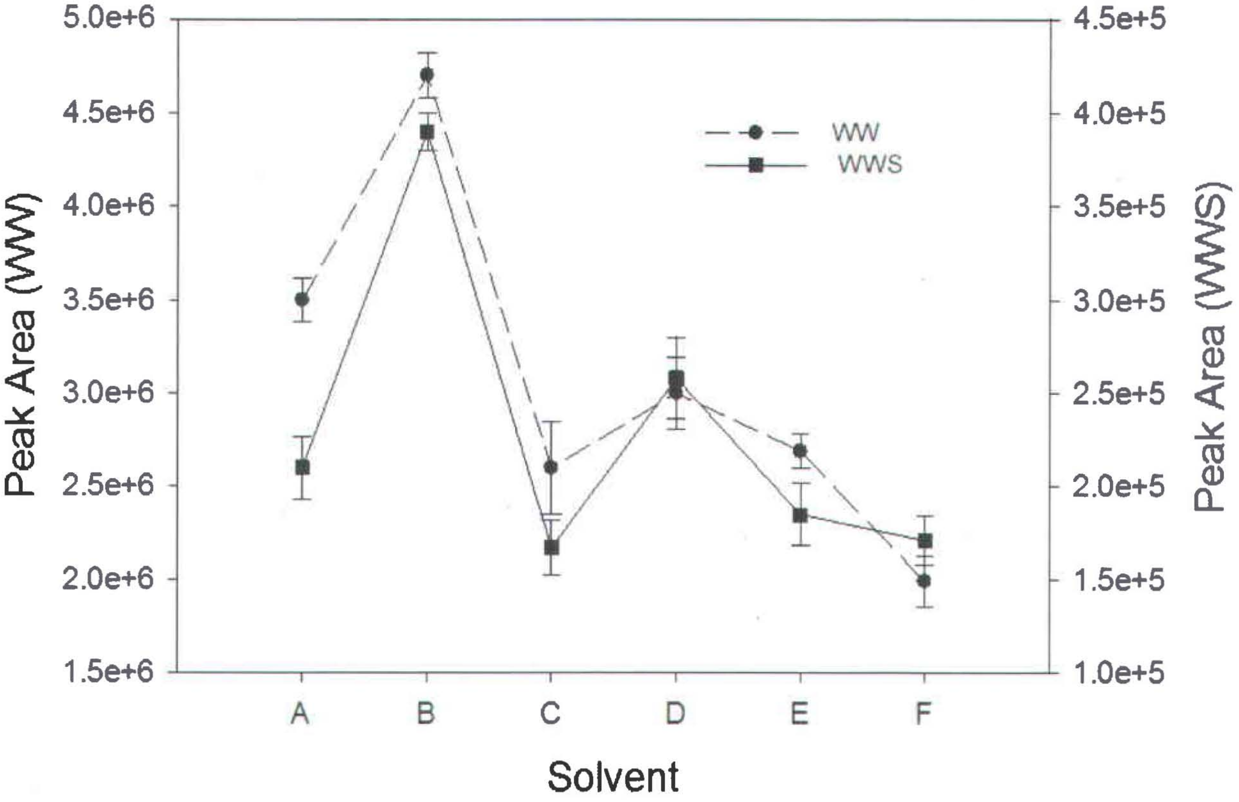

Figure3. LDTD-APCI optimization of different solvents used for analyte deposition in plate well. The results are the mean of triplicate spikes and the error bar lengths represent the relative standard deviations.

(A, methanol; B, methanol:water (2:1, v/v); C, acetonitrile; D, acetonitrile:water (2:1, v/v); E, methanol:acetonitrile (2:1, v/v); F, ethyl acetate. WW: Influent, WWS: Secondary sludge)

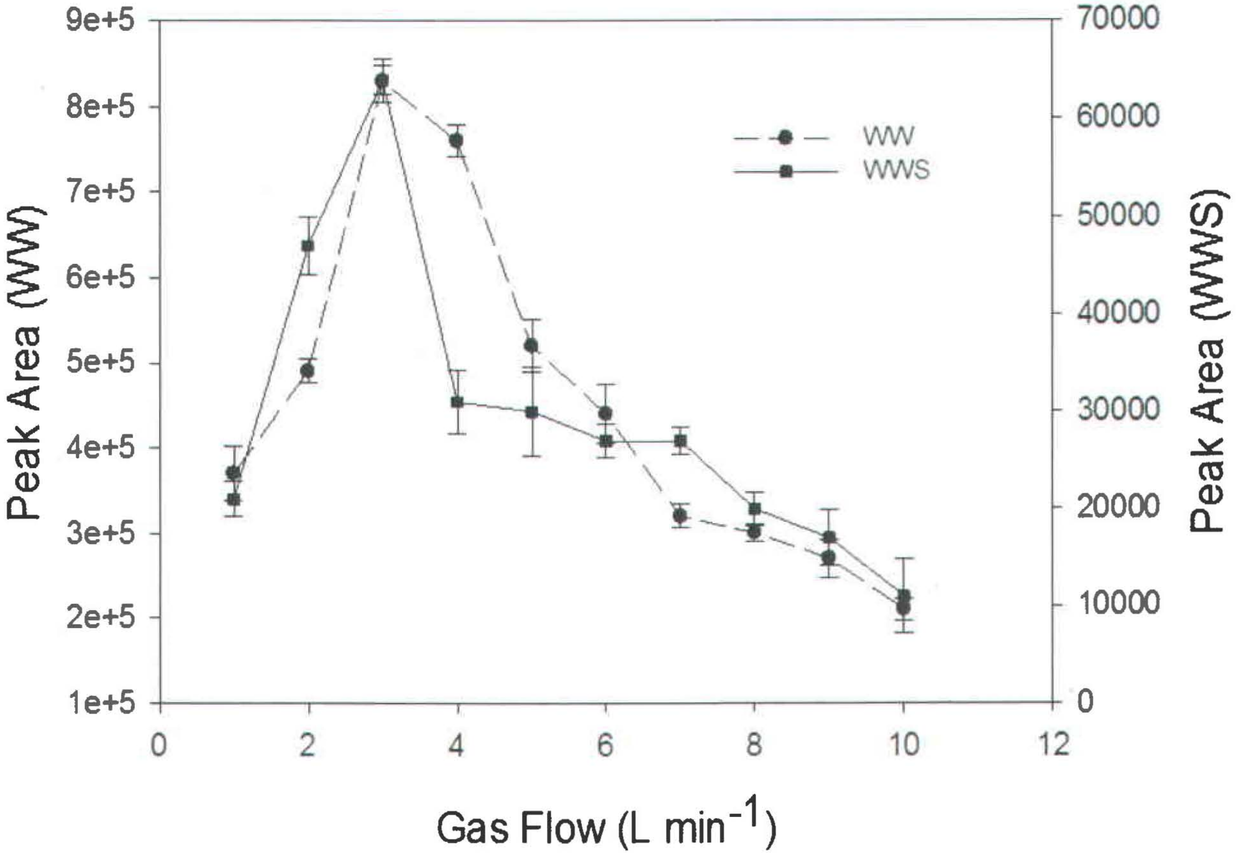

Figure 4. Gas flow (L min-1) optimization for CBZ analysis in WW and WWS samples. The results are the mean of triplicate spikes and the error bar lengths represent the relative standard deviations.

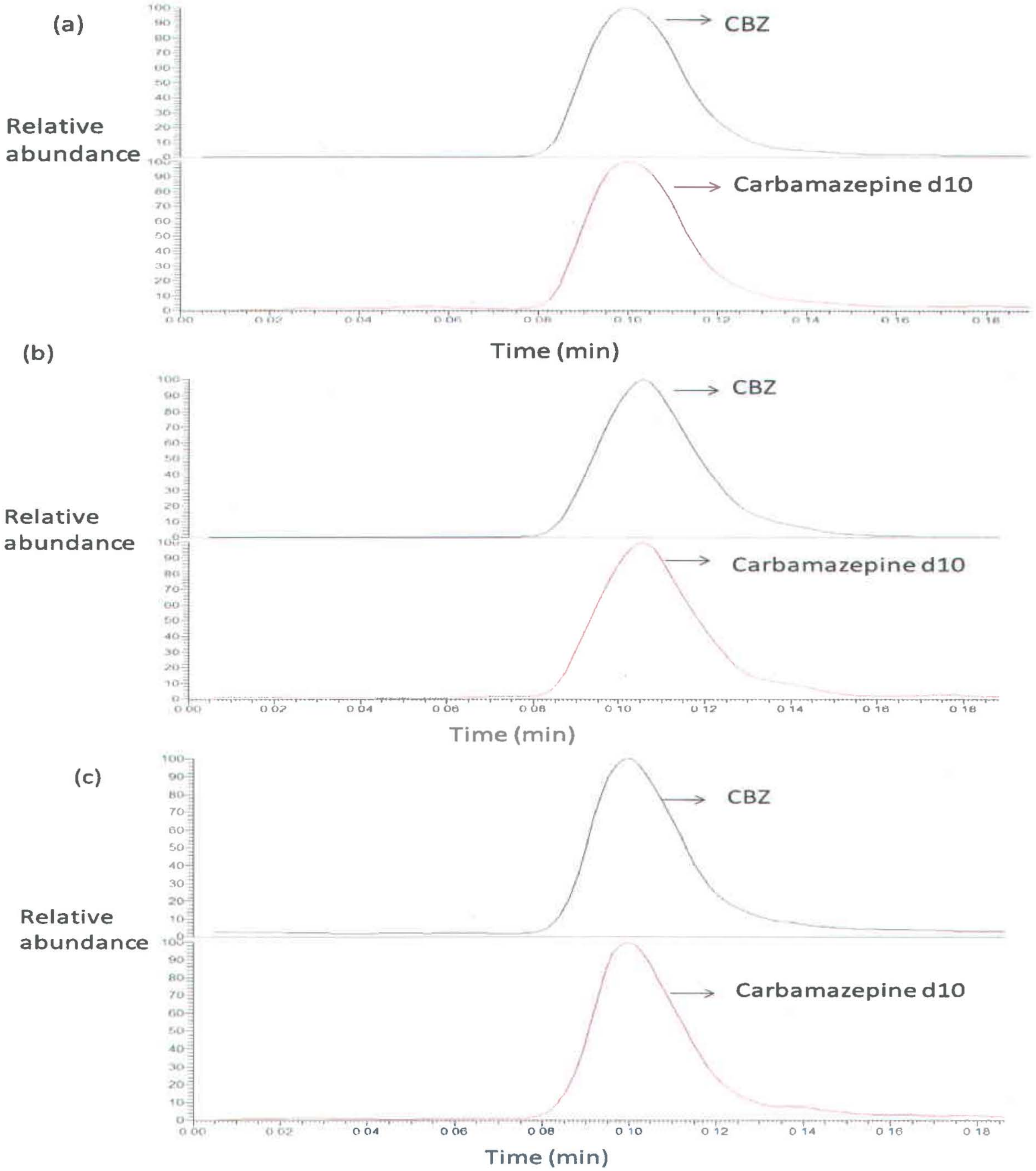

Figure 5. Desorption peak of CBZ in secondary sludge extracted by USE, MAE and ASE under methanol as solvent: (a) USE; (b) MAE; and (c) ASE

CHAPITRE IV

PRÉTRAITEMENTS UTILISÉS POUR LA DÉGRADATION DU BPA

PARTIE 1

DEGRADATION OF ENDOCRINE DISRUPTING BISPHENOL A DURING PRE-TREATMENT AND BIOTRANSFORMATION OF WASTEWATER SLUDGE

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RÉSUMÉ

L'effet de méthodes de prétraitement diverses comme l'hydrolyse alcaline (AH), l'hydrolyse thermique (TH), l'hydrolyse alcaline thermique (TAH), l'oxydation thermique (TO) et l'oxydation alcaline thermique (TAO), sur la solubilisation et la dégradation simultanée de bisphénol A (BPA), un perturbateurs endocriniens dans des boues d'épuration (WWS) a été examiné. Les résultats ont montré que parmi les prétraitements de AH, TH et TAH, le TAH a significativement amélioré la solubilisation des WWS (41,6 % des matières en suspension (SS), 70,7 % des matières en suspension volatiles (VSS) et 48,5 % de la demande chimique d'oxygène (COD)) avec une dégradation très élevée de BPA (38,4 %). Les plus faibles teneurs de SS, VSS et de DCO soluble ont été observées avec les prétraitements de TO et de TAO en comparaison avec le prétraitement TAH. Cependant, la dégradation la plus élevée de BPA (75,0 % et 78,9 %) a été observée avec les prétraitements de TO et TAO due au processus d'oxydation. Les effets de la rhéologie et du zêta potentiel sur la dégradation de BPA dans les boues brutes et les différentes boues prétraitées ont été aussi analysés. Les résultats ont montré que la diminution de la viscosité et de la taille des particules, ainsi que l'augmentation du zêta potentiel provient d'une forte dégradation de BPA. La dégradation de BPA par les laccases produites par *Sinorhizobium meliloti* dans les boues brutes et les boues prétraitées a été aussi déterminée. Une forte activité des laccases (230,9 U L^{-1}) a été observée dans les boues prétraitées par TAH résultant de la dégradation la plus élevée de BPA (21,9 %) suggérant une dégradation biologique concomitante de BPA.

Mots-clés : Bisphénol A; Boues d'épuration des eaux usées; Prétraitement; Rhéologie; Potentiel Zêta; Activité des laccases

ABSTRACT

The effect of various pre-treatment methods, including alkaline hydrolysis (AH), thermal hydrolysis (TH), thermal alkaline hydrolysis (TAH), thermal oxidation (TO) and thermal alkaline oxidation (TAO), on solubilization and simultaneous degradation of bisphenol A (BPA), an endocrine disruptor, in wastewater sludge (WWS) were investigated. The results showed that among AH, TH and TAH pre-treatments, TAH significantly improved the solubilization of WWS (41.6% suspended solid (SS), 70.7% volatile suspended solid (VSS) and 48.5% chemical oxygen demand (COD)) with higher degradation of BPA (38.4%). SS, VSS and COD solubilization were observed to be lower in TO and TAO pre-treatment as compared to TAH pre-treatment. However, higher degradation of BPA (75.0% and 78.9%) was observed in TO and TAO pre-treatment due to the presence of oxidation process. The effects of rheology and zeta potential on degradation of BPA in raw sludge and different pre-treated sludges were also investigated. The results showed that decrease in viscosity and particle size and increase in zeta potential resulted in higher degradation of BPA. BPA degradation by laccases produced by *Sinorhizobium meliloti* in raw and pre-treated sludge was also determined. Higher activity of laccases (230.9 U L^{-1}) was observed in TAH pre-treated sludge resulting in high degradation of BPA (21.9%) suggesting concomitant biological degradation of BPA.

Keywords: Bisphenol A; Wastewater sludge; Pre-treatment; Rheology; Zeta potential; Laccase activity

1. INTRODUCTION

Endocrine disrupting compounds (EDCs) have caused various adverse health effects which have been reported in recent years. The EDCs are defined as "exogenous substances or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations" [1]. Bisphenol A (BPA) has been identified as EDC by the U.S Environmental Protection Agency (EPA), World Wide Fund for Nature (WWF) and is declared as a social, environmental and global issue [2].

An effective treatment and disposal of sludge has been one of the major concerns in wastewater treatment processes to degrade organic pollutants including BPA. Many authors extensively investigated and reviewed sludge treatment and disposal, pointing to the challenges involved and future possibilities of reuse [3]. However, the presence of toxic organic compounds in wastewater sludge (WWS) pose problems to sludge recycling making its final disposal a challenge. Further, pre-treatment of WWS is adopted to solubilize and/or to reduce the size (in case of polymers) of organic compounds, in order to make them more easily biodegradable [4].

Numerous research findings reporting effect of WWS treatment/pre-treatment on EDCs have been compiled by Scrimshaw and Lester [5]. In fact, the pre-treatment of WWS and subsequent bioconversion by microorganisms is a very promising approach to dispose and reuse these residues. It allows sludge volume reduction, destruction of organic compounds, improvement of sludge quality and potential gains from value-added product sales. There are several kinds of pre-treatment methods studied so far which involve chemical treatment, mechanical treatment, oxidative treatment, biological hydrolysis or combination of any two of these methods [6].

Out of the existing pre-treatment methods, alkaline hydrolysis (AH), thermal hydrolysis (TH) and thermal alkaline hydrolysis (TAH) are known to increase biodegradability, convert biorecalcitrant molecules to readily biodegradable by-products and/or detoxify the medium by enhancing the mineralization of organic matter and degradation of recalcitrant compounds during biological treatment [7]. Sludge hydrolysis is a technique to break the major part of the sludge solid fraction into soluble and less complex molecules, and thus has effects on the viscosity and the filterability of sludge [8], which contribute significantly to removal of pathogens and organic compounds [9]. Advanced oxidation processes (AOPs) have been proposed as an attractive alternative method for the treatment of potential EDCs present in water, wastewater and WWS, among others. Thermal oxidation (TO) and thermal alkaline oxidation (TAO) are oxidative treatment processes, characterized by the generation of hydroxyl radicals which oxidize and mineralize organic molecules present in WWS. However, majority of research on EDCs, including BPA so far has covered their treatability in aqueous solutions. To the best of our knowledge, no studies have been reported on the degradation of BPA in WWS by AH, TH, TAH, TO and TAO pre-treatment processes and moreover, the effect of rheological changes of WWS during these pre-treatments on removal of BPA.

The indigenous microbial flora of WWS can produce panoply of enzymes, such as fungal or bacterial peroxidases, tyrosinases and laccases, which form a group of phenol-oxidising enzymes that degrade or detoxify organic pollutants [10]. An enzyme-catalyzed polymerization and precipitation process has been widely studied as a new method for the treatment of aqueous phenols [11], with the bulk of the work reported using laccases and peroxidases. Laccases produced by *Sinorhizobium meliloti* [12], possesses the potential to degrade or detoxify organic compounds, especially with phenolic groups present in WWS. However, no specific studies have been reported on degradation of BPA in raw sludge and different pre-treated sludges by the activity of laccase produced by *S. meliloti.*

Therefore, the present study constituted following objectives: i) effect of different pre-treatment methods including AH, TH, TAH, TO and TAO on the solubilization of WWS and simultaneous elimination of BPA; ii) effect of rheology and zeta potential changes on BPA removal/degradation during different pre-treatment of WWS; iii) cell growth of *S. meliloti* in different pre-treated sludges and its correlation with BPA; iv) effect of laccases produced by *S. meliloti* for degradation of BPA in WWS.

2. MATERIALS AND METHODS

2.1. Reagents

BPA (98% purity assay) was obtained from Sigma-Aldrich (Ontario, Canada). HPLC-grade methanol (MeOH), dichloromethane (DCM), acetone and chloroform, used for cleaning and extraction purposes, were purchased from Fisher Scientific (Ontario, Canada). Sep-Pak Plus C18 environmental cartridges used for solid phase extraction (SPE) clean-up was purchased from Waters (Milford, MA, USA). Acetic acid, hydrogen peroxide, sodium hydroxide and sulphuric acid were supplied by Fisher scientific (Ontario, Canada) and were of analytical grade. HPLC grade water was prepared in the laboratory using a Milli-Q/Milli-RO Millipore system (Milford, MA, USA).

2.2. Sample collection and analysis

Secondary sludge used in the study was collected from Quebec Urban Community (CUQ) wastewater treatment plant (Beauport, Quebec city, Quebec, Canada) which receives wastewater originating from domestic zones, industries, commercial enterprises and institutions present in the city. Sampling was performed on September 23, 2009 between 8:30 to 10:00 a.m. There was no rain or snow precipitation on the day of sampling. Samples were collected in pre-cleaned amber glass bottles with aluminum foil-lined caps and stored under dark conditions at 4±1 ^{0}C. The sludge suspended solids (SS) was concentrated from 1.5% (w/v) to higher SS concentrations by gravity settling and centrifugation of the settled sludge at 1600 × *g* for 3 min in a Sorvall RC 5C plus Macrocentrifuge (rotor SA-600). The supernatant was discarded in order to obtain a concentration of 30 g L^{-1} SS.

2.3. Pre-treatment of wastewater sludge

Different pre-treatments, such as AH, TH, TAH, TO and TAO were conducted with optimum SS concentration (30 g L^{-1}) [13]. The concentration of BPA was measured in raw sludge (secondary sludge) before pre-treatment and after each pre-treatment. In each case, a spiked BPA sample (0.12 mg) was treated to compare the results with the unspiked sample.

2.3.1. Alkaline hydrolysis (AH)

Alkaline hydrolysis was adjusted by adjusting the pH of sludge to 10.0±0.1 by using 4N NaOH. Approximately 0.062g of NaOH solution was added per gram of SS to obtain the required pH. Later, the sludge was placed in incubator shaker (Infors HT Multitron Standard, Switzerland) at 30±1 ^{0}C and 200 rpm for 24 h.

2.3.2. Thermal hydrolysis (TH) and Thermal alkaline hydrolysis (TAH)

Thermal hydrolysis was conducted by adjusting the pH of sludge to 7.0±0.1 by addition of either 4N NaOH or 4N H_2SO_4. The digestion process was performed by using a Multiwave-microwave sample preparation system (Paar Physica, Perkin Elmer) with infrared temperature control and hydraulic pressure control with feedback function capable of reducing microwave power near the limit of operating pressure. The sludge was transferred to PTFE digestion vessels, 100 mL capacity and the microwave digestion process was conducted at microwave power of 1000 W, operating pressure of 3000 kPa and temperature 140±1 ^{0}C for 30 min.

Thermal alkaline hydrolysis was conducted by adjusting the pH of sludge to 10.0±0.1 by adding 4N NaOH solution followed by microwave digestion. The microwave digestion for TAH pre-treatment followed the same procedure as in TH pre-treatment.

2.3.3. Thermal oxidation (TO) and Thermal alkaline oxidation (TAO)

Thermal oxidation was conducted by adjusting the pH of sludge to 7.0±0.1 and the digestion process was carried out by microwave digestion at 140±1 ^{0}C for 30 min. After digestion, pH of sludge was adjusted to 3.0±0.1 by adding 2N H_2SO_4. Later, 0.01 mL of H_2O_2 (30% v/v) per gram of SS was added to sludge.

Thermal alkaline oxidation was carried out by adjusting the pH of sludge to 10.0±0.1 by adding 4N NaOH solution and then the same method was followed as for TO pre-treatment. For convenience, AH, TH and TAH pre-treatments have been grouped together and the other category, based on oxidation has been enumerated for TO and TAO pre-treatments.

2.4. Analytical methods

The samples were subject to different analysis including BPA within two to three days to avoid degradation or transformation of native samples. Analysis of pH, ammonical nitrogen, phosphorus, total solids (TS), suspended solids (SS), volatile suspended solids (VSS), total and soluble chemical oxygen demand (TCOD and SCOD), total and soluble organic carbon (TOC and SOC) were carried out as per the Standard Methods [14]. SS_0, VSS_0 and $SCOD_0$ referred to as the parameters of WWS before pre-treatment, while SS_S, VSS_S and $SCOD_S$ referred to the parameters after pre-treatment. The soluble increment of SS, VSS and SCOD were calculated as follows:

$$S_{SS} = \frac{(SS_0 - SS_S)}{SS_0} \times 100\% \quad (1)$$

$$S_{VSS} = \frac{(VSS_0 - VSS_S)}{VSS_0} \times 100\% \quad (2)$$

$$SCOD_{increment} = \frac{(SCOD_S - SCOD_0)}{TCOD} \times 100\% \quad (3)$$

2.5. BPA analysis

2.5.1. Sample preparation

Sludge was frozen using liquid nitrogen prior to lyophilization by the freeze-dry system (Dura Freeze Dryer, Kinetics). About 0.5 grams of lyophilized and homogenized sample was transferred to a glass tube and 20 mL of dichloromethane-methanol (7:3, v/v) was added to the tube. The extraction was carried out by microwave assisted extraction method (MARS microwave extractor, CEM Corporation, North Carolina, USA). This system allowed simultaneous irradiation of 14 extraction vessels. Microwave power was 1200W (100%) and the extraction was performed in a temperature-controlled mode. The extraction temperature was 110±1 ^{0}C and programmed as follows: ramp to 110±1 ^{0}C for 10 min, holding at 110±1 ^{0}C for 10 min. The extract was separated by centrifugation at 7650 × g for 15 min and the procedure was repeated three times. The extracts were combined, concentrated to an approximate volume of 1 mL with gentle stream of nitrogen and redissolved in 100 mL of HPLC grade water.

2.5.2. Clean-up

Solid phase extraction (SPE) method was used for clean-up and pre-concentration of extract. Sep-Pak Plus C18 environmental cartridges were fitted into the vacuum manifold (Welch, USA) which was connected to a vacuum pump (Welch Rietschle Thomas, USA) to dispense samples through the cartridges. Cartridges were pre-conditioned by passing 7 mL of methanol and 3 mL of HPLC water at a flow rate of 1 mL min^{-1}. Sludge extracts (100 mL) were passed at a flow rate of 5 mL min^{-1}. After pre-concentration, the sorbents were dried by using a vacuum system set at (-15) psi.

The elution was performed by adding 2 x 4 mL of methanol/dichloromethane (3:1, v/v) mixture to the cartridge at a flow rate of 1mL min^{-1} and giving it a wait time of 10 min in order to give enough duration of contact between the solvent and the adsorbed compounds. The extracts were later evaporated to dryness with a gentle stream of nitrogen and reconstituted with methanol to a final volume of 1 mL prior to Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) analysis. The overall scheme of the analytical procedure used for the determination of BPA in WWS is presented in Fig. 1.

2.5.3. LC-MS/MS analysis

LC-MS/MS analyses were performed on a TSQ Quantum Access (Thermo Scientific, Mississauga, Ontario) with a Finnigan surveyor LC pump equipped with a 120-vial capacity sample management system. The analytes were separated on a 3 µM, 100 × 2.1 mm Hypersil Gold C18 reverse phase column (Thermo Scientific, Peterborough). The sample injection volume was set at 10 µL.

A binary mobile phase gradient with water [A] and methanol [B] was used for analyte separation at a flow rate of 200 µL min^{-1}. The gradient was performed as isocratic 5% A and 95% B for 6 min. The identification of BPA was done in a full-scan mode by matching the retention time and mass spectrum with true standards. Quantitative LC-MS/MS analysis was carried out in negative ionization (NI) condition and in selective reactions monitoring (SRM) mode using BPA d_{16} as internal standard.

2.6. Rheological study

2.6.1. Viscosity

Viscosity of raw sludges and different pre-treated sludges were measured by using a rotational viscometer Brookefield DVII PRO+ (Brookfield Engineering Laboratories, Inc., Stoughton, MA, USA) equipped with Rheocalc32 software. The viscosity data acquisition and analysis was carried out using Rheocalc V2.6 software, (B.E.A.V.I.S. – Brookfield Engineering Advanced Viscometer Instruction Set). All measurements were performed at 25±1 °C, 36.69 s^{-1} shear rate and viscosity was referred to as "apparent viscosity".

2.6.2. Particle size analysis

Particle size analysis was carried out by using Fritsch Laser particle sizer analysette 22, which is based on LASER diffraction principles. The stirrer and recirculation pump speed were also kept moderate at 250 and 500 rpm, respectively to minimize the damage of sludge particles. For analysis, each sample was diluted approximately 400-fold in tap water and analyzed in triplicate. This method is based on the principles of Fraunhoffer diffraction and Mie scattering. The results were then averaged to produce the particle size distribution, and the readings were recorded as particle volume percent in 51 discrete particle ranges between 0.1 to 1000 µm. Floc disruption at D_{43} (volume mean diameter) analogous to the particle size expressed as diameter 43% size distribution cutoff points was chosen as average particle size as it showed the volume mean diameter.

2.7. Zeta potential

Surface charge (zeta potential) measurement of flocs formed before and after pre-treatment of sludge was carried out by using a zetaphoremeter (Zetaphoremeter IV and Zetacompact Z8000, CAD Instrumentation, Les Essarts le Roi, France) and Smoluckowski equation [15]. Raw and pre-treated sludge sample of 10 µL was diluted 100 times with distilled water before passing through the zetaphoremeter.

2.8. Rhizobial growth

The fast-growing *S. meliloti* strain A_2 (Agriculture and Agri-food Canada, Sainte-Foy, Quebec, Canada) was used throughout this study. Cultures were maintained at 4±1 ^{0}C on yeast mannitol agar (YMA) slants [16]. Cell production was carried out in different liquid media: the standard medium yeast mannitol broth (YMB), raw sludge, alkaline hydrolysis sludge, thermal hydrolysis sludge, thermal alkaline hydrolysis sludge, thermal oxidation sludge, thermal alkaline oxidation sludge and each sludge sample with spiked BPA. All sludge samples were sterilized at 121± 1°C for 30 min prior to inoculation. The YMB medium contained the following constituents in grams per liter: K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; yeast extract, 1; and mannitol, 10.

The strain A_2 was grown at 30 ^{0}C for 72 h on a rotary shaker at 200 rpm and the cell counts were determined on YMA plate supplemented with Congo red (0.25%) after a serial dilution on saline solution (NaCl, 0.85% (w/v)). The samples were plated in triplicates. For enumeration, 30–300 colonies were counted per plate. The results were expressed in colony forming units per mL (CFU/mL). Colonies were counted after incubation of plates for 0 h, 6 h, 9 h, 12 h, 24 h, 36 h and 48 h at 30 ^{0}C.

2.9. Laccase activity

Laccase activity was measured with 2,2-azino bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) in 0.1 M phosphate-citrate buffer (pH 4). Oxidation of ABTS was determined by the increase in A420 (ε420 = 36 (mM cm) $^{-1}$) [17]. One unit of laccase activity was defined as the amount of enzyme required to oxidize 1 µmol of ABTS per min.

2.10. Statistical analysis

All data presented in Table 1 and Figs. 2, 4, 6, 7 and 8 are representative of at least three independent experiments with all samples measured in triplicates within each experiment. Repetition of statistical results was carried out using Statistical 6.0 software for Windows by employing the Student's t-test.

3. RESULTS AND DISCUSSION

3.1. Effects of pre-treatment on solubilization of WWS and BPA removal

Table 1 shows the characteristics of raw sludge (non pre-treated) and pre-treated sludge by AH, TH, TAH, TO and TAO methods. The SOC value observed in raw sludge was 0.15 g L^{-1} and a higher value of 0.32 g L^{-1} was observed in TAH pre-treatment process. The lower organic carbon solubilization observed in TO and TAO as compared to TAH pre-treatment process was due to formation of aliphatic acids in the medium during reaction of hydroxyl radical with BPA [18].

The pre-treatment efficiency was analyzed in terms of SS, VSS and organic matter (COD) solubilization and simultaneous BPA concentration reduction. Pre-treatment of WWS was adopted to improve the solubilization of sludge and to reduce the organic compounds [19]. The solubilization of SS, VSS and COD in WWS and simultaneous BPA degradation during different types of pre-treatment methods is presented in Fig. 2. As seen in Fig. 2, higher SS, VSS and COD solubilization was observed during thermal pre-treatment processes as compared to alkaline pre-treatment process. The concentration of BPA observed in raw sludge was 5.2 μg g^{-1} and the concentration decreased to 4.3, 3.8, 3.2, 1.3 and 1.1 μg g^{-1} in AH, TH, TAH, TO and TAO pre-treated sludge, respectively. LC-MS/MS chromatogram of BPA degradation during different pre-treatment processes is presented in Fig. 1s (supplementary material). Higher degradation of BPA was observed in thermal pre-treated sludge as compared to alkaline pre-treated sludge. Higher temperature (60-180 °C) during thermal pre-treatment processes leads to more lysis or disintegration of sludge cells [3]. Heating of sludge causes hydration of proteins, polysaccharides, lipids and other intracellular macromolecules secreted from disrupted sludge cells [20]. With the improvement of sludge's solubilization during thermal pre-treatment, organic substances will be transferred from solid to aqueous phase, which resulted in the increase of soluble protein and soluble carbohydrate concentration in aqueous phase and decrease of SS, VSS and COD in solid phase.

A comparison between five pre-treatment processes, such as AH, TH, TAH, TO and TAO, were carried out to observe the significance of alkaline conditions in thermal process (TAH and TAO pre-treatment) for SS, VSS and COD solubilization and simultaneous degradation of BPA. It was observed that TAH pre-treatment was more efficient (41.6% SS, 70.7% VSS and 48.5% COD) for solubilization of sludge and simultaneous degradation of BPA (38.4%) as compared to AH and TH pre-treatment process. The SS, VSS and COD solubilization observed in AH and TH pre-treatment was 22.3%, 27.6% and 23.1% and 33.3%, 50.5% and 37%, resulting in 16.6% and 26.7% of BPA degradation from raw sludge, respectively. TAO pre-treatment process also showed higher SS, VSS and COD (40.6%, 69.2% and 46.2%, respectively) solubilization and higher degradation of BPA (78.9%) as compared to AH, TH and TO pre-treatment processes. The SS, VSS and COD solubilization observed in TO pre-treatment was 35.6%, 62.9% and 41.6%, resulting 75% of BPA degradation from raw sludge. However, when a comparison was done in between TAH and TO pre-treatment, higher SS, VSS and COD solubilization was observed in TAH pre-treatment with lower degradation of BPA. The higher degradation of BPA during TO pre-treatment process as compared to TAH pre-treatment process was due to formation of hydroxyl radicals (discussed later). Tanaka et al. [21] compared three different methods of pre-treatment, NaOH addition (chemical), heating (thermal) and heating with NaOH addition (thermochemical) in terms of VSS solubilization of waste activated sludge from domestic, commercial and industrial wastewater. They observed that the thermochemical pre-treatment gave the best result in terms of VSS solubilization (40-50%) when sludge was heated at 130 ^{0}C for 5 min with the dose of 0.3 g NaOH/g VSS. Sodium hydroxide at relatively low dosage level is effective in solubilizing WWS at ambient temperature [22]. There are two distinct phases in hydrolysis under temperature and alkaline condition, initial rapid hydrolysis phase where solubilization started with the hydrolysis of carbohydrate followed by aminoacids and proteins and finally by fats and lipids and the subsequent phase where most of the organic matter are solubilized. Alkaline treatment becomes especially effective in terms of sludge solubilization and organic matter removal when combined with thermal hydrolysis [23].

Among AH, TH, TAH, TO and TAO pre-treatment process, the order of efficiency of pre-treatment method for solubilization and BPA degradation was TAH>TAO>TO>TH>AH and TAO>TO>TAH>TH>AH, respectively. Higher sludge solids and organic matter solubilization in TAH pre-treatment process leads to higher BPA degradation followed by TH and AH pre-treatment process. However, no significant difference of SS, VSS and COD solubilization between TAH and TAO pre-treatment process was observed. It was assumed that in TAO pre-treatment process, higher COD solubilization will be observed as compared to TAH pre-treatment. However, the formation of some aliphatic acids, such as oxalic, formic and acetic acid during reaction of hydroxyl radical with BPA (discussed later) act as recalcitrant towards the oxidation action leading to poor COD solubilization [18]. Valo et al. [24] studied TAH pre-treatment effect on the solubilization of WWS under the following conditions: 17.1 g TS L^{-1}, 3.65 g L^{-1} KOH, 170 °C for 60 min and observed that the pre-treatment process can solubilize upto 83% of COD. Similarly, Barnabe [13] has also confirmed the 70-80% solubilization of COD on TAH pre-treatment of WWS. Chiu et al. [25] studied the solubilization of WWS (1%, w/v) pre-treated with the combination of ultrasonic and alkaline (NaOH, 40 meq/L) and observed 50 to 60% of SS solubilization.

However, despite higher solubilization of solids and organic matter, TAH pre-treatment process was found to be less efficient for degradation of BPA as compared to TO and TAO pre-treatment process. This change may be attributed to, the possibility as to why advanced oxidation processes such as ultrasonication, ozonation, Fenton's oxidation and photocatalysis were preferred for degradation of potential EDCs including BPA, rather than the hydrolysis process [26]. In these advanced oxidation processes, chemical reaction plays an important role rather than physical process. These processes require the addition of catalysts and oxidants to the solution which lead to the formation of free hydroxyl radicals responsible for the degradation of EDCs [27].

Higher removal of BPA in TO and TAO pre-treatment was due to the presence of hydrogen peroxide oxidant. Thus, TO and TAO pre-treatment processes were characterized by the generation of hydroxyl radicals, which is the strongest known oxidant [13]. The hydroxyl radical oxidized and mineralized BPA into CO_2 and inorganic ions during TO and TAO pre-treatment of WWS. The possible pathway of degradation of BPA during TO and TAO pre-treatment processes is presented in Fig. 3 based on M/Z ratios obtained during LC-MS/MS analysis. It was found that during TO and TAO pre-treatment, three intermediates, such as hydroquinone (6.4 min retention time), 4-hydroxyacetophenone (11.9 min retention time) and 3-hydroxybisphenol A (13 min retention time) were formed when hydroxyl radical reacted with BPA. Aromatic ring cleavage takes place due to continuous reaction of these intermediates with hydroxyl radicals leading to formation of light-weight hydrocarbons, such as formic acid, propionic acid and formaldehyde among others [18]. Further, mineralization of the solution gives rise to the formation of CO_2 and H_2O. Torres et al. [18] studied the effect of ultrasonic pre-treatment process on degradation of BPA in aqueous solution and observed 80% of removal after 30 min of reaction.

3.2. Effects of rheology on BPA degradation during different pre-treatments of WWS

The pre-treatment processes will typically influence the rheological behavior by modifying overall sludge properties, including structure, strength and size of sludge flocs and sludge composition. Fig. 4 presents the effect of viscosity and particle size on removal of BPA from raw sludge during different pre-treatment processes. The viscosity observed in raw sludge was 123.8 mPa.s and the value decreased to 56.4 mPa.s, 42.8 mPa.s, 26.3 mPa.s, 69.4 mPa.s and 58.1 mPa.s after AH, TH, TAH, TO and TAO pre-treatments, respectively. The decrease in viscosity from raw sludge to pre-treated sludge was mainly due to increase in percentage of solubilization of SS, VSS and COD. WWS pre-treatments rupture suspended solids (microbial cells), liberate the nutrients, partially solubilize the suspended solids, increase the soluble chemical oxygen demand, decrease viscosity and improve the overall WWS biodegradability [13]. However, higher viscosity was observed in TO and TAO pre-treated sludge as compared to AH, TH and TAH pre-treated sludge. The increase in viscosity during TO and TAO pre-treatment process was mainly attributed to decrease in pH (discussed later).

Among the three pre-treatment processes, AH, TH and TAH, lower viscosity was observed in TAH pre-treatment. The lower viscosity during TAH pre-treatment was mainly due to higher solid solubilization. Structural units of suspension in WWS are typically larger in size and knitted close to each other at higher concentration of solids. As particle concentration increased, a network of particles is formed with the number of interactions increasing rapidly with the increase in volume concentration of particles leading to increase in resistance to flow of sludge [28]. Therefore, higher solid solubilization resulted in lower resistance to flow leading to decrease in viscosity.

The order of viscosity and BPA degradation for AH, TH and TAH pre-treatment processes were AH>TH>TAH and TAH>TH>AH, respectively. These results indicated that increase in solubilization of WWS during pre-treatment resulted in decrease in viscosity and hence higher degradation of BPA. Decrease in viscosity improved mass transfer rate leading to higher reaction rate and easy transportation of BPA mainly its partitioning causing higher degradation of BPA within the medium.

The increase in viscosity during TO and TAO pre-treatment as compared to AH, TH and TAH pre-treatment process was due to operating conditions, i.e. acidification (lower pH) and oxidation by free hydroxyl radicals. The reaction conditions will lead to changes in inter-particle interactions between sludge flocs and their components. The decrease in pH will result in a decrease in electrostatic repulsions between flocs (which are mostly negatively charged) causing higher resistance to flow leading to higher viscosity [29]. In between TO and TAO pre-treatment, the order of viscosity and BPA degradation were TO>TAO and TAO>TO, respectively. Higher viscosity observed in TO pre-treated sludge as compared to TAO pre-treated sludge was due to lower pH. However, higher removal of BPA was observed in TO and TAO pre-treated sludge at higher viscosity compared to AH, TH and TAH pre-treated sludge, was mainly attributed to the presence of oxidation process.

As seen in Fig. 4, the particle size of raw sludge decreased after AH, TH and TAH pre-treatment and increased after TO and TAO pre-treatment. The schematic presentation of increase and decrease in particle size during different pre-treatment process due to change in pH is presented in Fig. 5. Our previous studies have demonstrated that pre-treatment can result in a change in particle size (larger volume formation of fine particles) [30]. Among the AH, TH and TAH pre-treatment, the order of larger formation of fine particles was TAH>TH>AH. The lower particle size (26.3 μm and 42.7 μm) observed in TAH and TH pre-treated sludge, respectively was due to the cell wall degradation. Thermal pre-treatment at 60-180 °C disintegrated the cells and liberated intracellular substances [3]. During experimentation, it was observed that in TH and TAH pre-treated sludge, many minute cavities appeared on the surface of sludge particle (picture not shown), caused by the disruption of microbial cells leading to liberation of intracellular substances into the bulk liquid. Higher temperature used in both the pre-treatment processes breaks flocs and releases intracellular material. This results in increase of soluble protein concentration in aqueous phase leading to lower particle size. As discussed earlier, alkaline condition in thermal process (TAH pre-treatment) was more efficient in sludge cell lysis or disintegration as compared to TH pre-treatment. This might be the reason for lower particle size observed in TAH pre-treated sludge. The scavenging of organic compounds is largely influenced by the particle size distribution [31]. The decrease in particle size in TAH pre-treated sludge caused higher interaction (due to more fine particles, increasing the reaction rate) of BPA with OH^- ions, converting it to corresponding salt and other forms. Thus, lower concentration of BPA was observed in TAH pre-treated sludge as compared to AH and TH pre-treated sludge. While, AH pre-treated sludge comprised OH^- ions, but the reaction rate was not thermally enhanced and in TH, there was no reacting hydroxyl species which can react with BPA. In order to verify this hypothesis, we used the Arrhenius equation (Equation 4-5).

$$K = Ae^{-\frac{E_A}{RT}} \quad (4)$$

$$\ln K = \ln A - \frac{E_A}{RT} \quad (5)$$

Where, K = rate constant, E_A = activation energy, R = gas constant, T = temperature. Higher temperature and pressure used during TH and TAH pre-treatment process lead to generation of some radical species. With higher temperature, activation energy decreases resulting in higher rate of reaction between BPA and these radical species causing higher degradation of BPA in TH and TAH pre-treated sludge as compared to AH pre-treated sludge. Further, higher degradation of BPA in TAH pre-treated sludge as compared to TH pre-treated sludge was due to further reaction of OH^- ions with BPA.

The increase in particle size after TO and TAO pre-treatment was due to decrease in pH (pH 3). Neyens et al. [23] observed that particle size can be modified by the presence of acids, i.e. supracolloidal particles (1 – 100 µm range) decrease with acidic pH. Since repulsive electrostatic interactions created from the surface charge of sludge particles are minimized at pH in the range of 2.6 – 3.6, leading to proximity of small particles as also seen in Fig. 6. In between TO and TAO pre-treatment, lower particle size was observed in TAO pre-treatment resulting in higher removal of BPA. Thus, decrease in particle size increased the reduction of BPA by enhancing the reaction kinetics between the substrate and reactant (depending on the pre-treatment). However, higher removal of BPA was observed in TO and TAO pretreated sludge at higher particle size as compared to AH, TH and TAH pre-treated sludge. This ambiguity was mainly due to the oxidation of BPA by hydroxyl radicals in TO and TAO pre-treatment.

3.3. Effect of zeta potential on BPA degradation during different pre-treatments of WWS

The zeta potential obtained in raw and pre-treated sludges at different pH is presented in Fig. 6. The zeta potential values of –44.1, –57.2, –56.8, –71.1, –28.9 and –35.98 mV were reported for raw, AH, TH, TAH, TO and TAO pre-treated sludge, respectively. Among the five pre-treatment processes, lower zeta potential was observed in TO pre-treatment with pH 2.6 and highest in TAH pre-treatment with pH 10.09. Higher zeta potential was observed in AH, TH and TAH pretreatment as compared to TO and TAO pre-treatment. Lower zeta potential in TO and TAO pre-treatment was due to addition of acid (lower pH) which resulted in neutralization of charge within the medium. Meanwhile, higher zeta potential in AH and TAH was due to addition of alkali (higher pH) that results in net increase in negative charge.

Among AH, TH and TAH pre-treatment, TAH revealed higher zeta potential as compared to AH and TH pre-treatment. However, no significant difference of zeta potential was observed in between AH and TH pre-treated sludge even though the pH is higher in AH pre-treatment. The results may be due to smaller floc size during TH pre-treatment (Fig.5). The particles come together and flocculation takes place (formation of large particle size) at low zeta potential and vice versa. Based on DLVO (Derjaguin, Landau, Verwey, and Overbeek) theory, a higher (negative) value of zeta potential is expected to result in larger electrostatic repulsion within the flocs, leading to a higher sludge-shear sensitivity and, consequently, smaller floc sizes [32]. The higher zeta potential in TAH pre-treatment resulted in formation of larger number of sludge flocs per unit volume of WWS. As the size of the flocs decreased with increasing zeta potential, overall availability of sorbed BPA molecules to react with OH^- ion increased resulting in higher degradation of BPA during TAH pre-treatment.

Increase in zeta potential leads to decrease in viscosity as the particle size decreases. It can be explained well by using Henry equation which established a relationship between zeta potential, electrophoretic mobility and viscosity.

$$U_E = \frac{2\,\varepsilon\, z\, f(Ka)}{3\eta} \qquad (6)$$

Where, z = zeta potential, η = viscosity, U_E = electrophoretic mobility, ε = dielectric constant and f(Ka) = Henry's function. However, all the three phenomenon, increase in zeta potential, decrease in viscosity and particle size occur simultaneously within the media leading to increased reaction rate and partitioning of BPA causing higher removal in TAH pre-treated sludge followed by TH and AH. In between TO and TAO pre-treatment, higher zeta potential was observed in TAO pre-treatment resulting in higher removal of BPA as per the phenomenon discussed earlier.

3.4. S. meliloti growth profile

The cell growth of *S. meliloti* in raw sludge, different pre-treated sludges and each sludge with spiked BPA was observed. Fig. 7 presents the viable cell counts of *S. meliloti* growth in raw sludge and different pre-treated sludges (unspiked). Spiked BPA sample (0.12 mg) was taken in order to compare the cell growth of *S. meliloti* with unspiked sample (data not reported). No difference was observed in cell growth of *S. meliloti* in spiked and unspiked samples. However, the generation time of viable cells in spiked sample was observed 12 h later than the unspiked ones.

It was observed that in AH, TH, TAH, TO and TAO pre-treated sludge, the growth of *S. meliloti* in terms of viable cells enhanced. The increase in viable cell count in pre-treated sludge as compared to raw sludge was probably due to higher SS, VSS and COD solubilization of raw sludge. As *S. meliloti* is an aerobic bacteria, the increase in solubilization during different pre-treatment processes leads to higher oxygen diffusion and consequently increased cell count. The concentration of suspended solids in sludge affected the growth of *S. meliloti* in secondary sludge from municipal wastewater treatment plant (Quebec, Canada) [33]. Other factors, such as decrease in viscosity and particle size also increased the oxygen transfer rate (OTR) within the medium leading to higher cell multiplication.

Among the three types of pre-treated sludges, AH, TH and TAH, high count of viable cells (4.4×10^{10} CFU mL^{-1}) was observed in TAH pre-treated sludge at 24 h. The higher cell count of *S. meliloti* in TAH pre-treated sludge was due to higher SS, VSS and COD solubilization as compared to other pre-treated sludges. The order of sludge solids solubilization and cell count of *S. meliloti* was TAH>TH>AH and TAH>TH>AH, respectively. Possible reason of higher cell count in TAH pre-treated sludge was due to lower viscosity as compared to AH and TH pre-treated sludge. Higher solids solubilization and decreased viscosity during TAH pre-treatment resulted in higher oxygen or substrate mass transfer within the medium leading to higher cell count [34]. In between AH and TH pre-treated sludge, higher solids solubilization and lower viscosity was observed in TH pre-treated sludge causing higher cell count. Higher cell count (1.4×10^{10} CFU mL^{-1}) in TH pre-treated sludge was observed in 24 h. In between TO and TAO pre-treated sludge, higher solids solubilization and lower viscosity was observed in TAO pre-treated sludge again leading to higher cell count.

3.5. Laccase activity and BPA degradation

Fig. 8 a and b, presents the laccase activity profile and simultaneous BPA degradation in raw and different pre-treated sludges. It was evident that *S. meliloti* was capable of producing laccase enzyme in raw and pre-treated sludge. In AH pre-treated sludge, lower activity of laccase was observed as compared to other pre-treated sludges. The results were in concordance with less viable cell count of *S. meliloti* in AH pre-treated sludge. Rosconi et al. [12] carried out a comparative study between *Rhizobium* sp. to identify the laccase strain and they observed higher identity (99%) was established with *S. meliloti.* They also observed the higher production of laccase with higher cell count of *S. meliloti.*

Among TH, TAH, TO and TAO pre-treated sludge, higher activity of laccase was observed in TAH pre-treated sludge. This trend might be due to higher viable cell count of *S. meliloti* in TAH pre-treated sludge. The laccase activity maximum was observed at 24 h in TAH and TH pre-treated sludge and 12 h of incubation in TO and TAO pre-treated sludge, respectively. As seen in Fig 6a, laccase activity started increasing from 6 h to 12 h of fermentation time in raw sludge and TH, TAH, TO and TAO pre-treated sludge. However, in between 12 h to 24 h, increase in activity of laccase was observed in TAH and TH pre-treated sludge and decreased activity was observed in TO and TAO pre-treated sludge. The results were in agreement with the increase in cell count of *S. meliloti* upto 24 h in TAH and TH pre-treated sludge and decrease after 12 h in TO and TAO pre-treated sludge.

As seen in Fig. 8a and b, degradation of BPA was observed in raw sludge and TH, TAH, TO and TAO pre-treated sludge with increasing laccase activity except AH pre-treated sludge where laccase activity was lower. Thus, laccase was capable of degrading BPA in these media. Laccases have multiple copper atoms in their active sites and utilize molecular oxygen as the oxidant for a variety of phenols, to form corresponding reactive quinines [35]. Laccases produced by *S. meliloti* [12] possess the potential to degrade or detoxify organic compounds, especially with phenolic groups present in WWS simultaneously during bioconversion of WWS into value added products. A proposed pathway of degradation of BPA and its by-products, formed during TO and TAO pre-treatment of WWS by laccase activity is presented in Figs. 9 a, b and c. As seen in the Figures, oxidation by $OH^{\bullet}$ radicals during TO and TAO pre-treatment degraded BPA in raw sludge, characterized by production of by-products which are further degraded by activity of laccase. Hence, the contribution of two mechanisms such as oxidation degradation by radicals followed by biodegradation by laccase enzyme enhanced the degradation of BPA from raw sludge.

Degradation of BPA with increasing laccase activity during TH, TAH, TO and TAO might be due to solubilization of organic compounds which resulted in higher activity of laccase to degrade BPA. Alkaline and oxidative treatments of lignocellulosic materials can induce swelling in particulate organics, making the cellular substances more susceptible to enzymatic attack during saccharification [36].

The concentration of BPA observed at 0 h of incubation in raw, AH, TH, TAH, TO and TAO pre-treated sludge were 5.2, 4.3, 3.8, 3.2, 1.3 and 1.1 $\mu g\ g^{-1}$, respectively. The concentration of BPA observed after 48 h of laccase activity in raw sludge, AH, TH, TAH, TO and TAO pre-treated sludge were 5.1, 4.2, 3.3, 2.5, 1.0 and 0.7 µg g^{-1}, respectively. Significant concentration of BPA (0.7 µg g^{-1}) was degraded due to laccase activity in TAH pre-treated sludge.

Among AH, TH, TAH, TO and TAO pre-treatment, it was observed that TAH pre-treatment was more efficient (21.9%) in degradation of BPA in raw sludge due to relatively higher laccase activity induced by high viable cell count. Another reason might be lower particle size (larger formation of fine particles) and higher zeta potential observed in TAH pre-treated sludge. The larger formation of fine particles in TAH pre-treatment resulted in larger number of small particles per unit volume of the medium so that BPA was adsorbed on these large surface flocs. Further, laccase produced by *S. meliloti* have enhanced access to BPA and finally favor biodegradation of the compound.

The concentration of BPA observed in raw sludge was 5.2 µg g^{-1} and the concentration reduced to 4.2, 3.3, 2.5, 1.0 and 0.7 µg g^{-1} in AH, TH, TAH, TO and TAO pre-treated sludge, respectively after combined application of pre-treatment process and laccase activity. The pre-treatment processes AH, TH, TAH, TO and TAO for BPA degradation became more efficient (19.2%, 40.3%, 51.9%, 80.8% and 86.7%, respectively) when a combined application of different pre-treatment processes and laccase activity were taken into account.

Further investigation is required to identify the different by-products formation during these pre-treatment processes and classify these by-products on the basis of toxicity as compared to the parent compound. Thus, future work is under progress in our laboratory to identify these by-products, their toxic effects and to understand whether there is any residual H_2O_2 still present in the pre-treated sludge during TO and TAO pre-treatment process where higher degradation of BPA was observed.

4. CONCLUSIONS

The degradation of BPA in raw sludge by AH, TH, TAH, TO and TAO pre-treatment and laccase activity were encouraging and led to the following conclusions:

1. Among AH, TH, TAH, TO and TAO pre-treatment, the higher SS, VSS and COD solubilization was observed in TAH pre-treatment, however, BPA degradation was higher in TAO pre-treatment due to the contribution of oxidation by $OH^{\bullet}$ radicals and thermal alkaline treatment.
2. AH, TH, TAH, TO and TAO pre-treatment caused a decrease in apparent viscosity of sludge. Among AH, TH and TAH pre-treatment, decreased viscosity and particle size and increased

zeta potential was observed in TAH pre-treatment causing higher removal (38.4%) of BPA from raw sludge.

3. Among TO and TAO pre-treatment, decreased viscosity and particle size and increased zeta potential was observed in TAO pre-treatment causing higher removal (78.9%) of BPA from raw sludge.
4. The viable cell count of *S. meliloti* increased in TH, TAH, TO and TAO pre-treated sludge and higher growth (4.4 × 10^{10} CFU mL^{-1}) was observed in TAH pre-treatment due to higher percentage of SS, VSS and COD solubilization as compared to other pre-treatment process.
5. Higher laccase activity was observed in TAH pre-treated sludge leading to higher biodegradation of BPA (22.9%) as compared to TH, TO and TAO pre-treated sludge.
6. During AH, TH and TAH pre-treatment processes, BPA removal may be attributed to four different parameters, namely solubilization, lower viscosity, lower particle size and higher zeta potential. In TO and TAO pre-treatment process, BPA removal was also attributed to these four parameters along with intervention by $OH^{\cdot}$ radicals.
7. During further biotransformation of the pre-treated sludges, BPA removal was enhanced by the action of laccase enzymes.

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ABBREVIATIONS

AH, Alkaline hydrolysis; BPA, Bisphenol-A; D_{43}, Volume mean diameter; LC-MS/MS, Liquid chromatography-mass spectrometry/mass spectrometry; log K_{ow}, Octanol–water partition coefficient; SS, Suspended solids; SPE, Solid phase extraction; SCOD, Soluble chemical oxygen demand; TH, Thermal hydrolysis; TAH, Thermal alkaline hydrolysis; TO, Thermal oxidation; TAO, Thermal alkaline oxidation; TS, Total solids; TCOD, Total chemical oxygen demand; VSS, Volatile suspended solids; WWS, Wastewater sludge

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Table 1. Changes in physical-chemical parameters before and after pre-treatment of WWS

Parameters	Raw sludge	Alkaline hydrolyzed sludge	Thermal hydrolyzed sludge	Thermal alkaline hydrolyzed sludge	Thermal oxidative sludge	Thermal alkaline oxidative sludge
pH	6.5	9.8	7.3	10.0	2.6	3.4
TS (g L^{-1})	33.1±0.6	33.0±0.5	32.8±0.6	31.7±1.2	32.5±0.9	32.5±0.7
SS (g L^{-1})	30±0.31	23.3±0.8	20.0±0.7	17.5±0.5	19.3±0.7	17.8±1.0
VSS (g L^{-1})	14.9±0.08	10.8±0.1	7.3±0.2	4.4±0.5	5.6±0.08	4.6±0.09
TCOD (g L^{-1})	17.3±1.5	17.2±3.2	17.1±1.8	16.8±2.8	16.9±3.3	16.6±4.0
SCOD (g L^{-1})	3.0±0.5	7.0±0.9	9.4±2.8	11.4±1.2	10.2±3.0	11.0±2.6
Ammonical nitrogen (g L^{-1})	0.1±0.03	0.2±0.01	0.4±0.06	0.7±0.02	0.3±0.07	0.4±0.05
Phosphorus (g L^{-1})	0.3±0.07	0.3±0.04	0.3±0.03	0.4±0.07	0.2±0.02	0.3±0.06
TOC (g L^{-1})	282±10	282±7	283±11	282±14	278±4	282±6
SOC (g L^{-1})	0.15±0.08	0.2±0.01	0.23±0.01	0.32±0.08	0.26±0.07	0.29±0.05

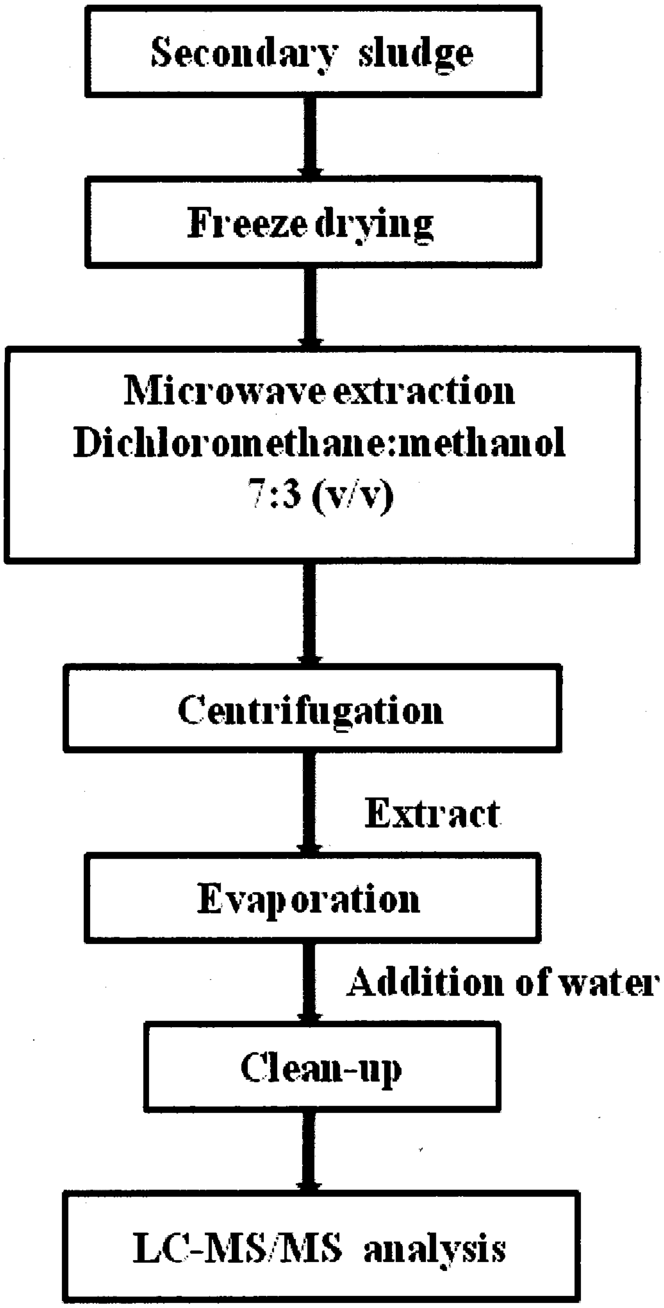

Figure 1. Schematic diagram of analytical procedure used for analysis of BPA in wastewater sludge

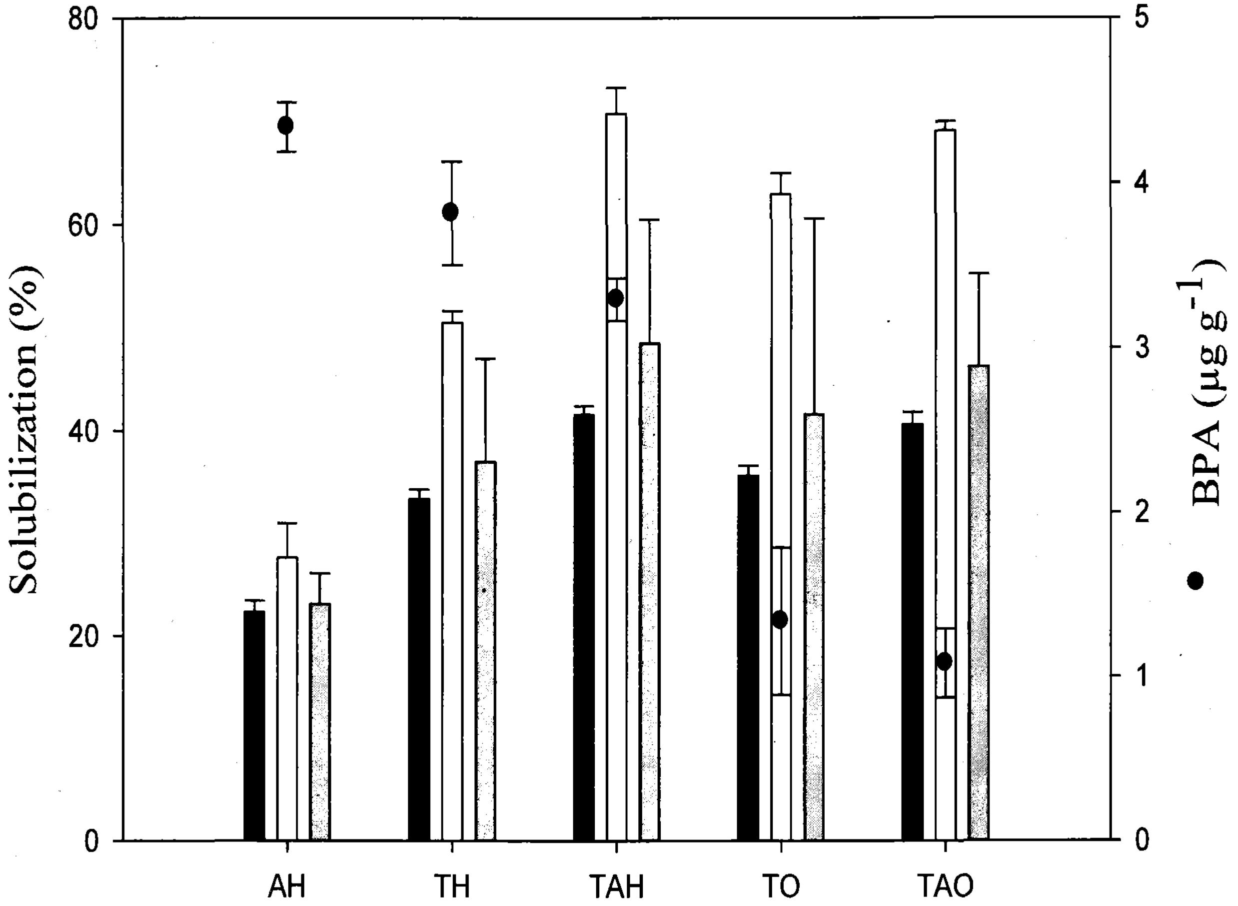

Figure 2. The solubilization of SS, VSS and COD in raw sludge after pre-treatment and simultaneous BPA degradation. SS solubilization, VSS solubilization, COD solubilization

■ □ ▨

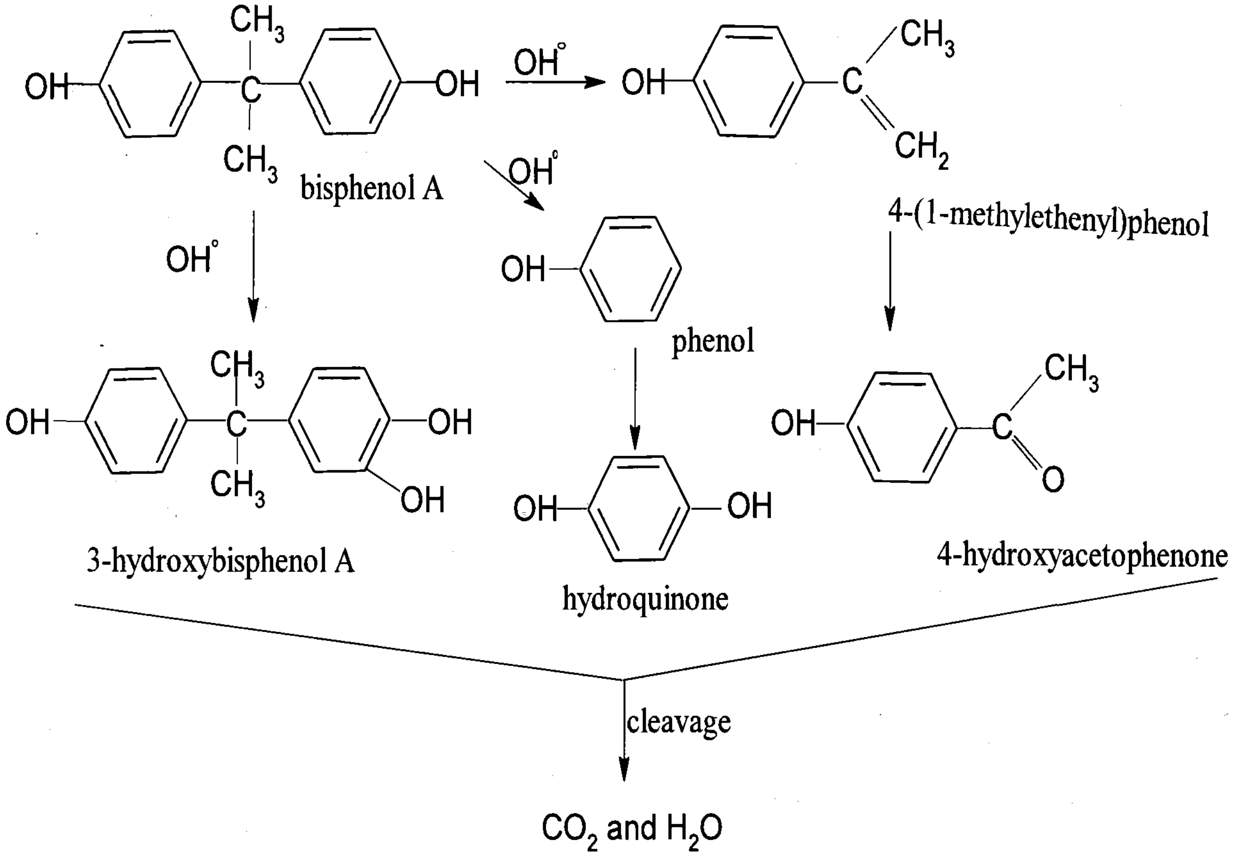

Figure 3. Different proposed degradation pathways of BPA during thermal oxidation and thermal alkaline oxidation treatment of wastewater sludge

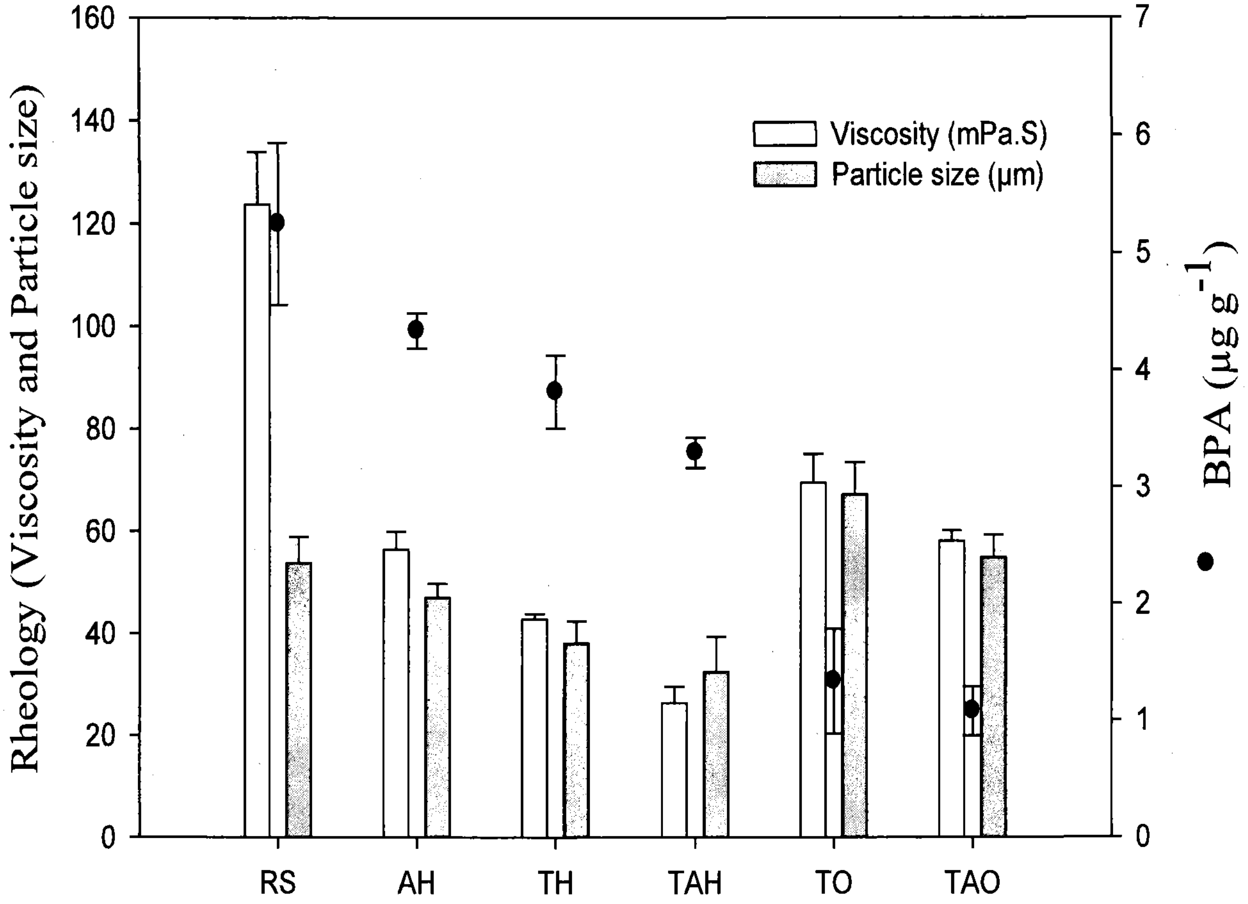

Figure 4. Effect of viscosity and particle size on removal of BPA in raw sludge and different pre-treated sludges

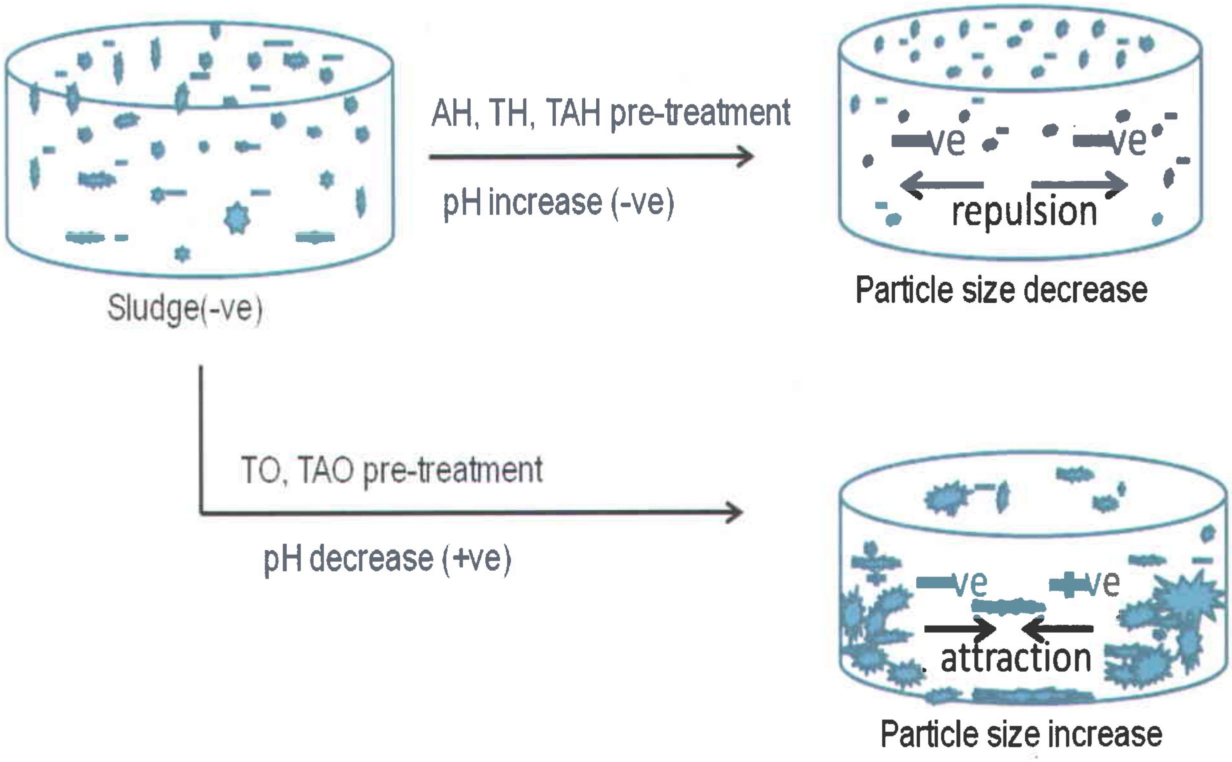

Figure 5. Schematic presentation of change in particle size during different pre-treatment processes

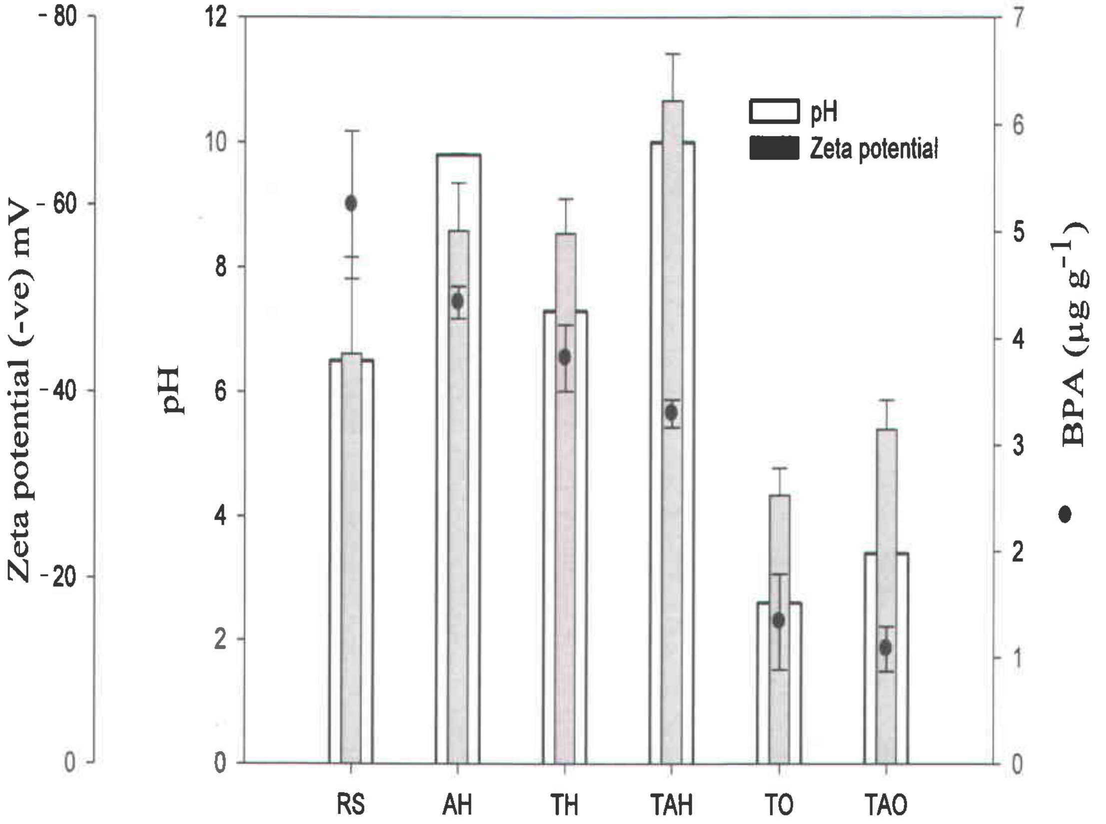

Figure 6. Correlation of zeta potential values of raw and pre-treated sludges with degradation of BPA

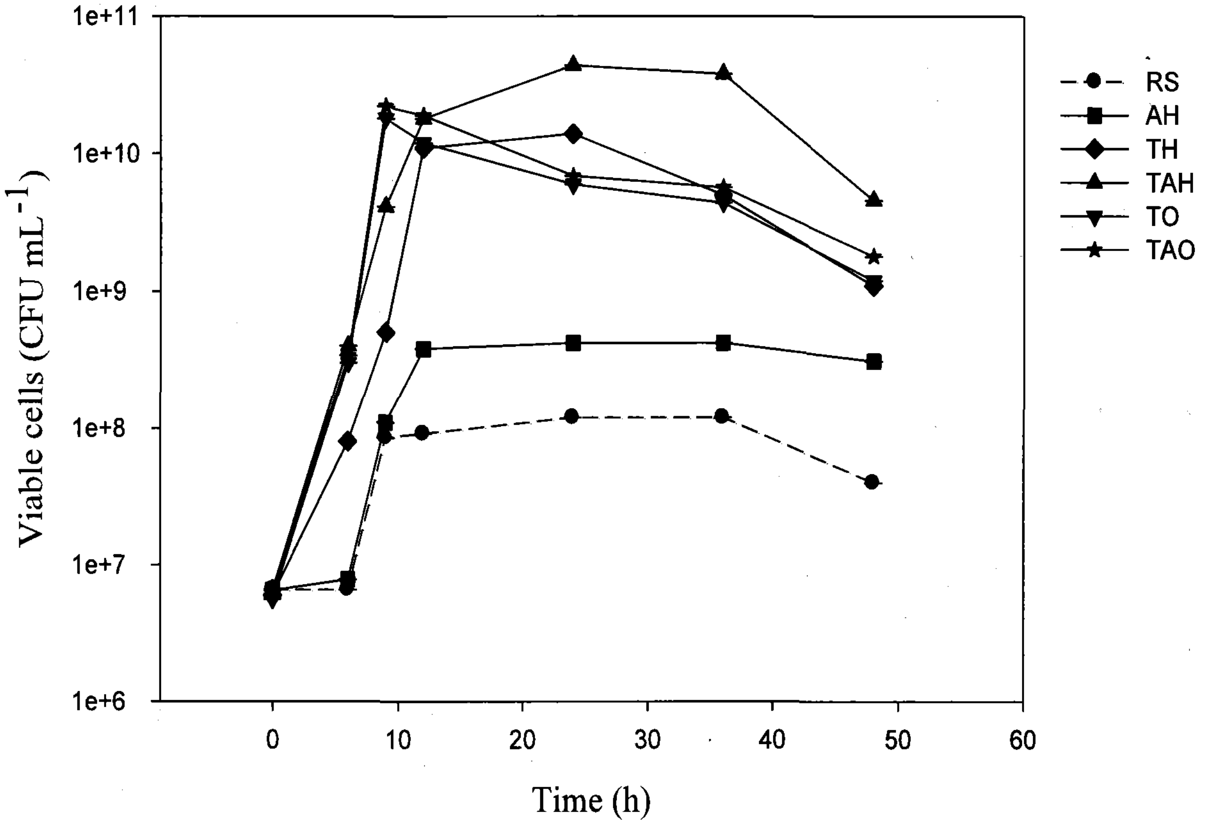

Figure 7. S. meliloti growth curves in raw sludge and different pre-treated sludges

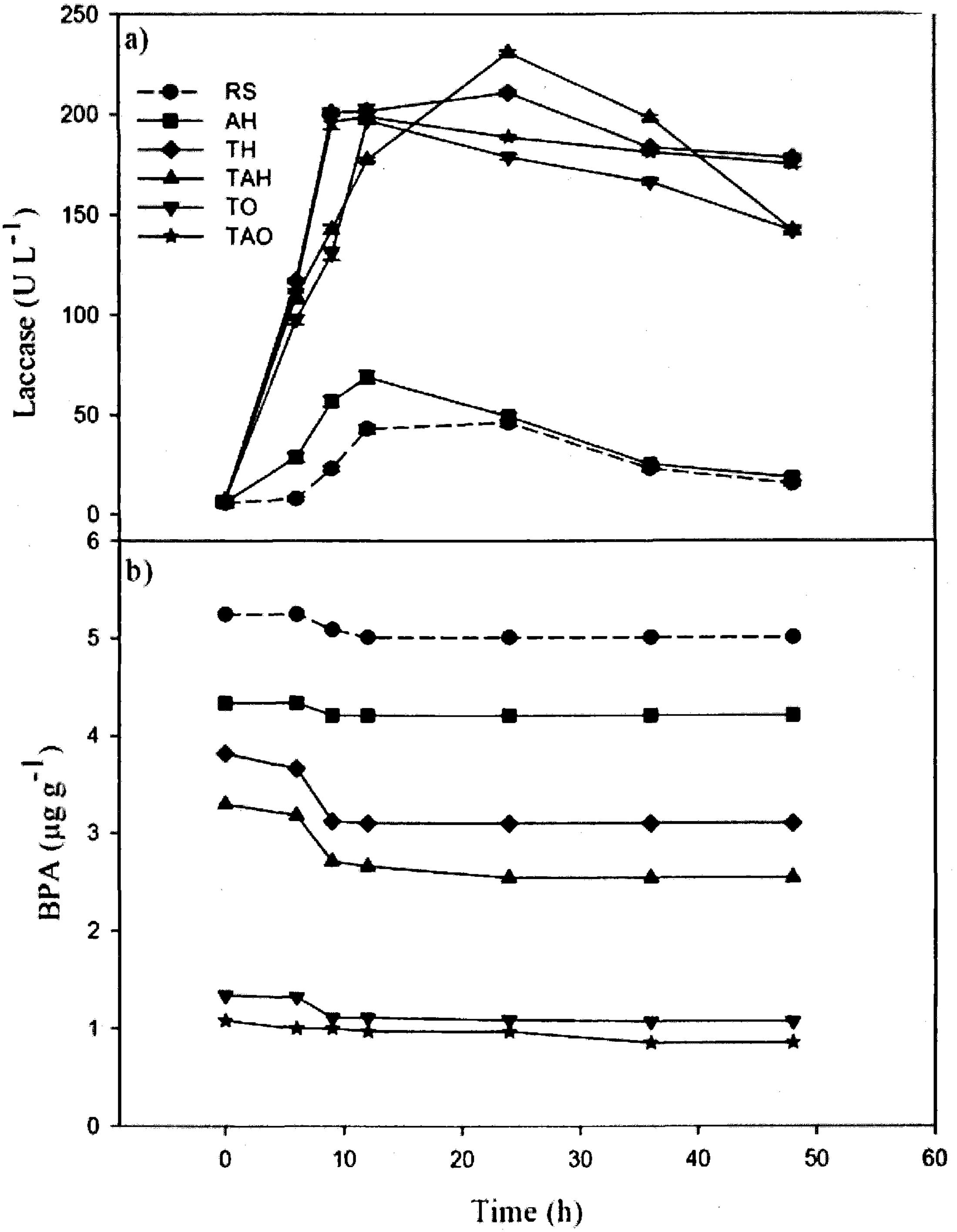

Figure 8. BPA degradation in raw sludge and different pre-treated sludge due to laccase activity: (a) laccase activity and (b) BPA degradation

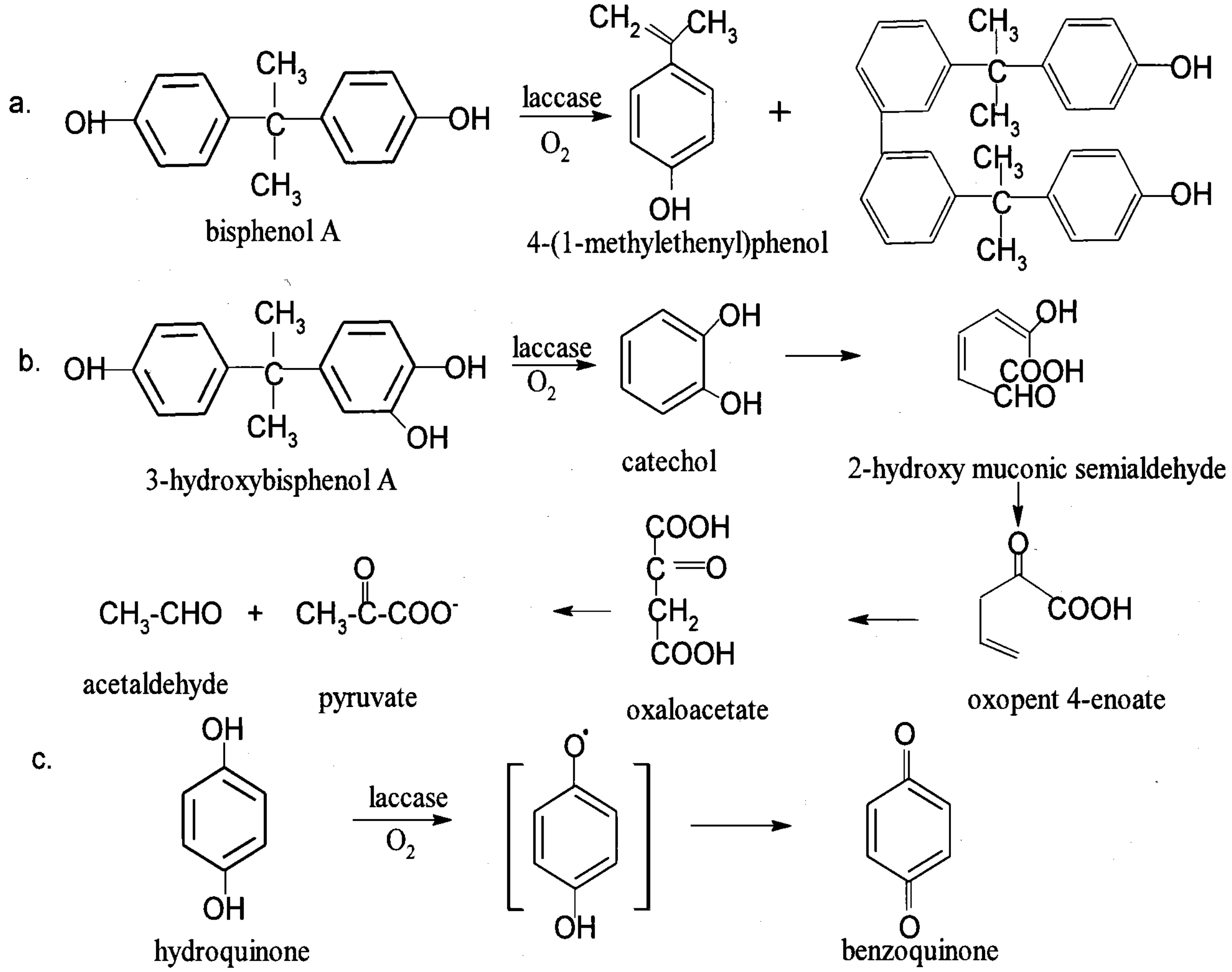

Figure 9. Proposed pathways of BPA and its by-products degradation formed during TO and TAO pre-treatment by laccase activity; a) BPA degradation, b) 3-hydroxybisphenol A degradation, c) hydroquinone degradation [37, 38]

PARTIE 2

CONCOMITANT DEGRADATION OF BISPHENOL A DURING ULTRASONICATION AND FENTON OXIDATION AND PRODUCTION OF BIOFERTILIZER FROM WASTEWATER SLUDGE

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RÉSUME

La dégradation du bisphénol A (BPA), un perturbateur endocrinien, des boues d'épuration des eaux usées (BE) a attiré un grand intérêt récemment. Dans l'étude présente, les effets des différentes méthodes de prétraitement, comprenant l'ultrasonication (US), l'oxydation de Fenton (FO) et le ferro-sonication (FS) ont été évalués en termes d'augmentation de la solubilisation de BE et la dégradation simultanée de BPA. Parmi les prétraitements de US, FO et FS, les teneurs les plus élevées en matières en suspension (SS), en matières en suspension volatiles (VSS), en demande chimique en oxygène (COD) et en carbone organique soluble (SOC) (respectivement 39,7 %; 51,2 %; 64,5 % et 17,6 %) ont été observées durant le processus du prétraitement ferro-sonication effectué pendant 180 minutes, et résultant de la dégradation la plus élevée de BPA (82,7 %). De plus, l'effet des paramètres rhéologiques (la viscosité et la taille des particules) et le potentiel zêta sur la dégradation de BPA dans les boues brutes et les différentes boues prétraitées a été aussi examiné. Les résultats ont montré une diminution dans la viscosité et la taille de particule et une augmentation du potentiel zêta qui résultent à la dégradation la plus élevée de BPA. La dégradation de BPA par les laccases produit par *Sinorhizobium meliloti* dans les boues brutes et les boues prétraitées a été aussi déterminée. L'activité la plus élevée de laccases (207,9 U L^{-1}) a été observée dans la boue prétraitée par ferro-sonication (180 min de temps), résultant de la dégradation la plus élevée de BPA (0,083 µg g^{-1}), suggérant la dégradation biologique concomitante de BPA.

Mots-clés : Bisphénol A; Prétraitement; Rhéologie; Potentiel zêta; Activité de laccase

ABSTRACT

Degradation of bisphenol A (BPA), an endocrine disruptor, from wastewater sludge (WWS) has attracted great interest recently. In the present study, the effects of different pre-treatment methods, including ultrasonication (US), Fenton's oxidation (FO) and ferro- sonication (FS) was assessed in terms of increase in solubilization of WWS and simultaneous degradation of BPA. Among US, FO and FS pre-treatment, higher suspended solids (SS), volatile suspended solids (VSS), chemical oxygen demand (COD) and soluble organic carbon (SOC) solubilization (39.7%, 51.2%, 64.5% and 17.6%, respectively) was observed during a ferro-sonication pre-treatment process carried out for 180 min, resulting in higher degradation of BPA (82.7%). In addition, the effect of rheological parameters (viscosity and particle size) and zeta potential on the degradation of BPA in raw and different pre-treated sludges were also investigated. The results showed that a decrease in viscosity and particle size and an increase in zeta potential resulted in higher degradation of BPA. BPA degradation by laccases produced by *Sinorhizobium meliloti* in raw and pre-treated sludge was also determined. Higher activity of laccases (207.9 U L^{-1}) was observed in ferro-sonicated pre-treated sludge (180 min ultrasonic time), resulting in higher removal of BPA (0.083 µg g^{-1}), suggesting concomitant biological degradation of BPA.

Keywords: Bisphenol A; Pre-treatment; Rheology; Zeta potential; Laccase activity

1. INTRODUCTION

Over the past decade, a number of reports have addressed bisphenol A (BPA) as a potential endocrine disrupting compound (EDC). BPA is manufactured in high quantities, 90% or more being used for production of powder paints, adhesives, building materials, compact disks, thermal paper and paper coatings, among others. Due to the daily use of these products, high concentrations of BPA are observed in wastewater (WW) and in wastewater sludge (WWS) (0.004-1.36 mg kg^{-1}) [1, 2].

Advanced treatment technologies are required to effectively eliminate organic pollutants, including BPA, from WWS [3]. Several authors have reported the successful application of ultrasonication and Fenton's oxidation pre-treatment process for the degradation of BPA in aqueous solution and WW [4, 5]. However, most of the studies, even in WW, are only fortification-based (spiked BPA), rather than considering the real-time concentration of BPA, so that the results are often overestimated.

Ultrasonic treatment is considered as an advanced oxidation processes (AOP) that generates $OH^{\bullet}$ radicals through the acoustic cavitation [6].

$$H_2O \rightarrow H^{\bullet} + HO^{\bullet} \qquad (1)$$

Cavitational collapse produces intense local heating and high pressure on liquid-gas interface, turbulence and high shearing phenomena in the liquid phase, resulting in dissolution of the organic compounds which may penetrate into the bubbles. Fenton's oxidation has been exploited in laboratory and wastewater treatment plants (WWTPs) for oxidation and degradation of organic substances [7]. In Fenton's oxidation process, hydrogen peroxide is used as an oxidizing agent and iron(II) sulphate heptahydratate as a catalyzing agent at acidic pH (pH=3).

$$Fe^{3+} + e^{-} \rightarrow Fe^{2+} \qquad (2)$$
$$H_2O_2 + Fe^{2+} \rightarrow OH^{-} + HO^{\bullet} + Fe^{3+} \qquad (3)$$

However, the majority of research on EDCs, including BPA, so far has covered their treatability in aqueous solutions. To the best of our knowledge, no studies have been reported on the degradation of BPA in WWS by ultrasonication, Fenton's oxidation or combination of both (ferro-sonication) pre-treatment processes. Moreover, the effects of rheological changes of WWS during these pre-treatments on removal of BPA have also not been invetigated. Rheology consists of two important parameters, apparent viscosity and particle size, which will impact the removal of BPA from WWS in several ways, such as: i) the decreased viscosity will increase oxygen transfer during microbial fermentation to produce value-added products and aid in biodegradation; ii) the decreased particle size during pre-treatment will enhance BPA reaction rate through formation of various active sites and thus degradation; and iii) decreased particle size will improve access by microbial enzymes during value-addition and thus biodegradation.

Value addition of WWS by utilizing it as a raw material for commercially viable products such as biopesticides, biofertilizers, and enzymes is a novel sustainable approach of sludge management [8]. However, the presence of toxic organic compounds including BPA in WWS may jeopardize sludge recycling, making its final disposal a challenge. Hence, selecting microorganisms cultivable in WWS to obtain value-added products (VAPs) and concomitantly produce enzymes to degrade organic pollutants including BPA becomes more interesting. *Sinorhizobium meliloti* produce laccases [9], and possesses the potential to degrade organic compounds, especially with phenolic groups present in WWS. Laccase is a blue oxidase enzyme capable of oxidizing phenols and aromatic amines by reducing molecular oxygen to water by a multicopper system. The molecular reaction models of Yaropolov et al. [10] can be simplified to the following equation:

$$2\mathrm{Cu}^{2+} + \mathrm{benzendiol} \rightarrow 2\mathrm{Cu}^{+} + \mathrm{quinone} + 2\mathrm{H}^{+} \tag{4}$$

$$2\mathrm{Cu}^{+} + {}^{1}\!/_{2}\ \mathrm{O}_2 + 2\mathrm{H}^{+} \rightarrow 2\mathrm{Cu}^{2+} + \mathrm{H}_2\mathrm{O} \tag{5}$$

However, no specific studies have been reported on degradation of BPA in raw sludge and different physico-chemical pre-treated sludges by the activity of laccase produced by *S. meliloti.*

The present study was conducted in WWS with real concentrations of BPA in order to examine the following parameters: i) the effect of ultrasonication, Fenton's oxidation and ferro-sonication pre-treatment process on the solubilization of WWS and simultaneous elimination of BPA; ii) the effect of rheology and zeta potential changes on BPA removal/degradation during different pre-treatments of WWS; and iii) the effect of laccases produced by *S. meliloti* on degradation of BPA in physico-chemical pre-treated WWS.

2. MATERIALS AND METHODS

2.1. Chemicals

BPA (98% purity assay) was obtained from Sigma-Aldrich (Ontario, Canada). HPLC-grade methanol (MeOH), dichloromethane (DCM), acetone and chloroform, used for cleaning and extraction purposes, were purchased from Fisher Scientific (Ontario, Canada). Sep-Pak Plus

C18 environmental cartridges used for solid phase extraction (SPE) clean-up was purchased from Waters (Milford, MA, USA). Acetic acid, hydrogen peroxide, sodium hydroxide and sulfuric acid were supplied by Fisher scientific (Ontario, Canada) and were of analytical grade. HPLC grade water was prepared in the laboratory using a Milli-Q/Milli-RO Millipore system (Milford, MA, USA).

2.2. Wastewater sludge

Secondary sludge used in the study was collected from Quebec Urban Community (CUQ) wastewater treatment plant (Beauport, Quebec city, Quebec, Canada). Samples were collected in pre-cleaned amber glass bottles with aluminum foil-lined caps and stored under dark conditions at 4±1 ^{0}C. The sludge suspended solids (SS) were concentrated from 1.5% (w/v) to higher SS concentrations by gravity settling and centrifugation of the settled sludge at 1600 × *g* for 3 min in a Sorvall RC 5C plus Macrocentrifuge (rotor SA-600). The supernatant was discarded in order to obtain 30 g L^{-1} SS.

2.3. Pre-treatment of wastewater sludge

Different pre-treatments, such as ultrasonication, Fenton's oxidation and ferro- sonication were conducted with optimum SS concentration (30 g L^{-1}) [11, 12]. The concentration of BPA was measured in raw sludge (secondary sludge) before and after each pre-treatment. In each case, a spiked BPA sample (0.12 mg) was treated to compare the results with the unspiked sample (real WWTP samples).

2.3.1. Ultrasonic pre-treatment

The ultrasonication was carried out using an ultrasonic homogenizer (Autotune 750W, Cole-Parmer Instruments, Vernon Hills, Illinois, US). The ultrasonication equipment was operated at a frequency of 20 kHz by using a titanium probe with a tip diameter of 12 mm. The ultrasonication was carried out with an ultrasonic intensity of 2 W cm^{-2} for 40 min and 15 W cm^{-2} for 120 min and 180 min. The ultrasonic intensity was defined as the actual power density dissipated by the apparatus divided by the area of the transducer. For each sonication experiment, 400 mL of WWS was filled in a 1 L beaker and the ultrasonic probe was dipped 2 cm into the sludge. Batch experiments were carried out without temperature regulation (no cooling). The temperature of the medium was increased from 20 °C to 38 °C, 52 °C and 74 °C, respectively, when ultrasonic experiment was conducted for 40 min, 120 min and 180 min. Further, the ultrasonication pre-treatment carried out for 40 min, 120 min and 180 min was referred to as US1, US2 and US3 respectively.

2.3.2. Fenton's oxidation pre-treatment

WWS sample of 400 mL was acidified to pH 3 by using 10 N H_2SO_4 and placed in a 1 L flask. Fenton's oxidation process was conducted at pH 3 to avoid iron hydroxide precipitation. To initiate Fenton's oxidation reaction, H_2O_2 and $FeSO_4$ solution were added to the sludge samples according to the following operating conditions: 0.01 mL H_2O_2/g SS (14.63 mg H_2O_2/g SS); $[H_2O_2]_0/[Fe^{2+}]_0 = 150$. SS was used as the basis for Fenton conditions, and the ultimate objective was to enhance the biodegradability at specific SS with simultaneous BPA removal. Fenton's oxidation was carried out at ambient temperature for 1 h using a shaker at 150 rpm. Henceforth, Fenton's oxidation pre-treatment will be referred to as FO.

2.3.3. Ferro-sonication pre-treatment

This pre-treatment was carried out by addition of $FeSO_4$ solution in WWS followed by ultrasonic pre-treatment. Four hundred milliliters of WWS was acidified to pH 3 by using 10 N H_2SO_4 and placed in a 1 L flask. The same amount of $FeSO_4$ solution as in Fenton's oxidation was added to the WWS followed by ultrasonic pre-treatment for 120 and 180 min as described earlier. Ferro-sonication pre-treatment with 120 min and 180 min will be henceforth referred to as FS1 and FS2, respectively.

2.4. Analysis

2.4.1. General

Analysis of pH, total solids (TS), suspended solids (SS), volatile suspended solids (VSS), total and soluble chemical oxygen demand (TCOD and SCOD), total and soluble organic carbon (TOC and SOC) were carried out as per the Standard Methods [13]. The SS, VSS, SCOD and SOC solubility increment were calculated as follows:

$$S_{SS} = \frac{(SS_0 - SS_S)}{SS_0} \times 100\% \qquad (6)$$

$$S_{VSS} = \frac{(VSS_0 - VSS_S)}{VSS_0} \times 100\% \qquad (7)$$

$$SCOD_{increment} = \frac{(SCOD_S - SCOD_0)}{TCOD} \times 100\% \qquad (8)$$

$$\mathrm{SOC_{increment}} = \frac{(\mathrm{SOC_S} - \mathrm{SOC_0})}{\mathrm{TOC}} \times 100\% \qquad (9)$$

Where, SS_0, VSS_0, $SCOD_0$ and SOC_0 are referred to as the parameters of WWS before pre-treatment, and SS_S, VSS_S, $SCOD_S$ and SOC_S are referred to as the parameters after pre-treatment.

2.4.2. Viscosity

The viscosity of raw sludges and different pre-treated sludges were measured by using a rotational viscometer (Brookefield DVII PRO+, Brookfield Engineering Laboratories, Inc., Stoughton, MA, USA) equipped with Rheocalc32 software. The viscosity data acquisition and analysis was carried out using Rheocalc V2.6 software, (B.E.A.V.I.S. – Brookfield Engineering Advanced Viscometer Instruction Set). All measurements were performed at 25±1 °C, 36.7 s^{-1} shear rate, and the viscosity was referred to as "apparent viscosity".

2.4.3. Particle size

Particle size analysis was carried out by using Fritsch Laser particle sizer analysette 22, which is based on LASER diffraction principles. The stirrer and recirculation pump speed were also kept moderate at 250 and 500 rpm, respectively to minimize the damage of sludge particles. For analysis, each sample was diluted approximately 400-fold in tap water and analyzed in triplicate. This method is based on the principles of Fraunhoffer diffraction and Mie scattering. The results were then averaged to produce the particle size distribution, and the readings were recorded as particle volume percent in 51 discrete particle ranges between 0.1 to 1000 µm. Floc disruption at D_{43} (volume mean diameter), analogous to the particle size expressed as the diameter that is 43% of the size distribution cutoff points, was chosen as an average particle size, as it showed the volume mean diameter.

2.4.4. Zeta potential

Surface charge (zeta potential) measurement of flocs formed before and after pre-treatment of sludge was carried out by using a zetaphoremeter (Zetaphoremeter IV and Zetacompact Z8000, CAD Instrumentation, Les Essarts le Roi, France) and the Smoluckowski equation [14]. Raw and pre-treated sludge samples of 10 µL were diluted 100 times with distilled water before passing it through the zetaphoremeter.

2.5. BPA analysis

2.5.1. Sample preparation and clean-up

Sludge samples (raw and pre-treated) were frozen using liquid nitrogen prior to lyophilization by the freeze-dry system (Dura Freeze Dryer, Kinetics). About 0.5 grams of lyophilized and homogenized sample were transferred to a glass tube and 20 mL of dichloromethane-methanol (7:3, v/v) was added to the tube. The extraction was carried out by a microwave-assisted extraction method (MARS microwave extractor, CEM Corporation, North Carolina, USA). Sep-Pak Plus C18 environmental cartridges were pre-conditioned by passing 7 mL of methanol and 3 mL of HPLC water at a flow rate of 1 mL min^{-1}. Sludge extracts (100 mL) were passed at a flow rate of 5 mL min^{-1}. After pre-concentration, the sorbents were dried by using a vacuum system set at (- 103) kPa.

The elution was performed by adding 2 x 4 mL of methanol/dichloromethane (3:1, v/v) mixture to the cartridge at a flow rate of 1 mL min^{-1} and giving it a wait time of 10 min in order to give enough duration of contact between the solvent and the adsorbed compounds. The extracts were later evaporated to dryness with a gentle stream of nitrogen and reconstituted with methanol to a final volume of 1 mL prior to Liquid Chromatography-Mass Spectrometry/Mass Spectrometry (LC-MS/MS) analysis.

2.5.2. LC-MS/MS analysis

LC-MS/MS analyses were performed on a TSQ Quantum Access (Thermo Scientific, Mississauga, Ontario) with a Finnigan surveyor LC pump equipped with a 120-vial capacity sample management system. The analytes were separated on a 3 µM, 100 × 2.1 mm Hypersil Gold C18 reverse phase column (Thermo Scientific, Peterborough). The sample injection volume was set at 10 µL.

A binary mobile phase gradient with water [A] and methanol [B] was used for analyte separation at a flow rate of 200 µL min^{-1}. The gradient was performed as isocratic 5% A and 95% B for 6 min. The identification of BPA was done in a full-scan mode by matching the retention time and mass spectrum with true standards. Quantitative LC-MS/MS analysis was carried out in negative ionization (NI) condition and in selective reactions monitoring (SRM) mode using BPA d_{16} as internal standard.

2.6. Rhizobial growth

The fast-growing *S. meliloti* strain A_2 (Agriculture and Agri-food Canada, Sainte-Foy, Quebec, Canada) was used throughout this study. Cultures were maintained at 4±1 ^{0}C on yeast mannitol agar (YMA) slants [15]. Cell production was carried out in different liquid media: the standard medium yeast mannitol broth (YMB), raw sludge, US sludge, FS sludge, FUS sludge and each sludge with spiked BPA. The pH of each sludge was re-adjusted to 7. The YMB medium contained the following constituents in grams per liter: K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; yeast extract, 1; and mannitol, 10.

The strain A_2 was grown at 30±1 ^{0}C for 48 h on a rotary shaker at 200 rpm. Samples were withdrawn at regular intervals and the cell counts were determined on YMA plates supplemented with Congo red (0.25% w/v) after a serial dilution using saline solution (NaCl, 0.85% w/v). The samples were plated in triplicates. For enumeration, 30–300 colonies were counted per plate. The results were expressed in colony forming units per mL (CFU mL^{-1}). Colonies were counted after incubation of plates for 0 h, 6 h, 9 h, 12 h, 24 h, 36 h and 48 h at 30 ^{0}C.

2.7. Laccase activity

Laccase activity was measured with 2,2-azino bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) in 0.1 M phosphate-citrate buffer (pH 4). Oxidation of ABTS was determined by the increase in A420 ($\varepsilon420 = 36\ (mM\ cm)^{-1}$) [16]. One unit of laccase activity was defined as the amount of enzyme required to oxidize 1 µmol of ABTS per min.

3. RESULTS AND DISCUSSION

3.1. Solubilization of WWS and BPA removal

Characteristics of raw sludge and US, FO and FS pre-treated sludge are presented in Table 1. The pH of raw sludge was observed to be 5.8 and the value increased to 6.0, 6.2 and 6.5 during US1, US2 and US3 pre-treatment processes, respectively. Comparing FO and FS pre-treatment, the higher pH (3.7) was observed in FS2 pre-treated sludge followed by FO and FS1.

The effectiveness of US1, US2, US3, FO, FS1 and FS2 processes was analyzed in terms of SS, VSS and organic matter (SCOD and SOC) solubilization and simultaneous degradation/elimination of BPA from raw sludge. Fig. 1 presents the solubilization of SS, VSS, COD and SOC in raw sludge and simultaneous degradation of BPA during different pre-treatment methods. As seen in Fig. 1, higher SS, VSS, COD and SOC solubilization was observed during Fenton's oxidation and ferro-sonication pre-treatment processes as compared to ultrasonic pre-treatment processes. The concentration of BPA observed in raw sludge was 2.85 μg g^{-1} and the concentration decreased to 2.49, 1.84, 0.96, 0.58, 0.73 and 0.51 μg g^{-1} in US1, US2, US3, FO, FS1 and FS2 pre-treated sludge respectively. LC-MS/MS chromatograms of BPA degradation during different pre-treatment processes are presented in Fig. 2. Higher degradation of BPA was observed in Fenton's oxidation and ferro-sonication processes as compared to the ultrasonic processes. The increased SS, VSS, COD and SOC solubilization during FO and FS pre-treatment processes as compared to US pre-treatment processes was due to acid hydrolysis and partial oxidation (hydroxyl radicals) of cells and organic matter leading to enhanced lysis or disintegration of sludge cells. With the improvement of the sludge's solubilization during these pre-treatment processes, organic substances are transferred from the solid to the aqueous phase, which results in an increase in soluble protein and soluble carbohydrate concentration in the aqueous phase and a decrease of SS, VSS, COD and SOC in the solid phase. Torres et al. [6] compared ultrasonication and Fenton's oxidation pre-treatment processes on the basis of organic matter solubilization and observed that 75% of COD and 20% of TOC were removed using the Fenton process after 180 min, whereas, at the same time only 40% of COD and 5% of TOC were removed using ultrasonication.

The order of efficiency of pre-treatment methods for solubilization and BPA degradation was FS2>FO>FS1>US3>US2>US1 and FS2>FO>FS1>US3>US2>US1, respectively. The results show that an increase in solubilization of solids and organic matter during FO and FS pre-treatment processes results in higher degradation of BPA from raw sludge as compared to US pre-treatment processes. Pre-treatment of WWS has been reported to improve the solubilization of sludge and to reduce organic compounds [17]. The other reason for higher degradation of BPA during FO and FS pre-treatment processes as compared to US pre-treatment process was due to the addition of $FeSO_4$, which enhances the formation of $OH^{\bullet}$ radicals in solution (Equation 3). The hydroxyl radicals generated during Fenton and ferro-sonication pre-treatment processes have an oxidizing potential of 2.8 V compared to the normal hydrogen electrode (NHE), and are capable of oxidizing a wide range of organic compounds including BPA in WW and WWS [18].

A comparison between the six pre-treatment processes, such as US1, US2, US3, FO, FS1 and FS2 was carried out to understand the importance of ferro-sonication (FS1 and FS2) pre-treatment processes for SS, VSS, COD and SOC solubilization and simultaneous BPA degradation. It was observed that the FS2 pre-treatment process was more efficient (39.7% SS, 51.2% VSS, 64.5% SCOD and 17.6% SOC) for solubilization of sludge and simultaneous degradation of BPA (82.7%) as compared to other pre-treatment processes. The higher solids and organic matter solubilization and BPA degradation during FS2 pre-treatment process were due to the operational conditions, including: i) a decrease in pH (pH 3), which leads to acid hydrolysis, ii) an increase in the temperature within the medium (from 20 °C to 74 °C as observed) during ultrasonication, resulting in thermal acid hydrolysis, iii) a higher formation of H_2O_2 (higher ultrasonic time of 180 min) due to bubble collapse and mixing, and iv) a higher efficiency for the production of $OH^{\cdot}$ radicals due to the addition of $FeSO_4$. The faster degradation of BPA with sono-Fenton as compared to Fenton is believed to be due to the higher efficiency for the production of $OH^{\cdot}$ radicals as well as due to the ultrasonic physical and chemical processes [19]. Ultrasonication can be a suitable method to improve the Fenton degradation of BPA [4]. However, in between FO and FS1, higher SS, VSS, COD and SOC solubilization (37% SS, 48% VSS, 58.5% COD and 14.6% SOC) was observed in the FO pre-treatment process, leading to a higher degradation (79.4%) of BPA. This can be explained by the fact that during FS1 pre-treatment process the concentration of H_2O_2 produced during ultrasonication (120 min) was not enough to react with all of the iron ions released during the Fenton reaction to form the reactive $OH^{\cdot}$ radicals that lead to BPA degradation.

Among US1, US2 and US3 pre-treatment process, the order of efficiency of pre-treatment process for solids and organic matter solubilization and BPA degradation was US3>US2>US1 and US3>US2>US1, respectively. Higher sludge solids and organic matter solubilization (30.66% SS, 41.6% VSS, 45.18% COD and 11.7% SOC) in US3 pre-treatment process conducted at ultrasonic intensity of 15 W cm^{-2} for 180 min leads to higher degradation (66.3%) of BPA followed by US2 and US1 pre-treatment process. The results show that an increase in ultrasonic intensity induces an increase in the BPA degradation rate due to higher sludge solids and organic matter solubilization. As the ultrasonic intensity increases, the number of $OH^{\cdot}$ radicals in the medium increases, leading to higher oxidation of BPA and, consequencely, an increase in organic matter (SCOD and SOC) solubilization. Guo and Feng [5] observed that 33.2%, 44.9%, 51.1% and 55.0% of BPA was degraded within 120 min at an ultrasonic intensity of 20 W cm^{-2}, 40 W cm^{-2}, 60 W cm^{-2} and 80 W cm^{-2}, respectively. Similarly, Bougrier et al. [20] has also confirmed that COD, organic matter and nitrogen solubilization increased with increasing ultrasonic intensity. US1 was conducted with an ultrasonication intensity of 2 W cm^{-2} for 40 min. The ultrasonic intensity and time in US1 process was considered based on earlier optimal studies for higher solubilization of COD and biodegradability in WWS [11]. However, in this study, with a sonication intensity of 2 W cm^{-2} for 40 min, a lower removal of BPA (12.6%)

from raw sludge was observed. Hence, ultrasonication intensity and time was increased to 15 W cm^{-2} for 120 and 180 min in US2 and US3 pre-treatment process. Thus, moderate ultrasonication intensity with an increase in biodegradability and simultaneous BPA removal is desired, which was achieved by increasing the ultrasonication intensity to 15 W cm^{-2}.

Higher sludge solids and organic matter solubilization observed in US3 pre-treated sludge was also due to the high energy input (114640.0 kJ/kg TS). The energy input during US1 and US2 pre-treatment processes was determined as 4818.7 and 78016.5 kJ/kg TS, respectively. Ultrasonication supplied energy referred to as specific supplied energy (E) was determined by using the Equation 10:

$$E = \frac{P \times t}{V \times TS} \tag{10}$$

Where, P = ultrasonic power (W), t = ultrasonication time (s), V = sample volume (L) and TS = total solid (g L^{-1}). Higher energy input causes more acoustic cavitation in the medium, which then causes sludge floc disintegration and cell breakage, leading to release of intracellular materials to the aqueous phase. The cavitation threshold depends on the medium's viscosity, density, purity, temperature, pressure, and ultrasonic frequency, among others [12]. Fig. 3 shows a comparative study on the effect of specific energy on the solubilization of COD during ultrasonic pre-treatment of WAS. It was observed that with increasing specific energy, the SCOD increases. Higher power supplied could lead to higher solubilization levels [20]. Salsabil et al. [21] observed lower COD solubilization as compared to this study, which can be explained by the sludge composition: the mineral matter represented an important part (20%) and the organic matter are mainly particulate (97.9%). It is well known that protein and carbohydrate are two predominant organic matter media in WWS. Thus, SCOD was mainly composed of soluble proteins and carbohydrates. Hence, at higher specific energy, solubilization started with the hydrolysis of carbohydrate followed by aminoacids and proteins and, finally, fats and lipids and the subsequent phase where most of the organic matter was solubilized. Therefore, higher ultrasonic intensity and energy input in US3 pre-treatment process resulting in higher solubilization of sludge solids and organic matter, which led to higher degradation of BPA, followed by US2 and US1 pre-treatment processes.

3.2. Rheological changes and correlation with BPA removal from raw sludge

The pre-treatment processes will typically influence the rheological behavior by modifying overall sludge properties, including structure, strength and size of sludge flocs and sludge composition. The effect of pre-treatment methods on the change in viscosity and the particle sizes of raw sludge with the simultaneous effect on BPA removal are shown in Fig. 4. The viscosity observed in raw sludge was 126.9 mPa.s and the value decreased to 80.8, 57.6, 53.1, 65.1, 77.8 and 58.2 mPa.s after US1, US2, US3, FO, FS1 and FS2 pre-treatments, respectively. The decrease in viscosity from raw sludge to pre-treated sludge was due to an increase in the percentage of solubilization of SS, VSS, SCOD and SOC. WWS pre-treatments rupture suspended solids (microbial cells), liberate nutrients, partially solubilize suspended solids, increase soluble chemical oxygen demand, decrease viscosity and improve the overall WWS biodegradability [22].

WWS suspensions are invariably non-Newtonian fluids, with the shear stress (τ) being non-linearly related to the shear rate (γ), as shown in Fig. 5. The treated sludge curves were lower than the untreated ones, which shows that viscosity (τ/γ), for a given shear rate decreases with treatments. For example, for a shear rate of 46.2 s^{-1}, the viscosity of raw sludge was found to be 20 mPa.s and the value decreased to 7.6, 3.4, 1.5, 6.5, 7.2 and 4.3 mPa.s in US1, US2, US3, FO, FS1 and FS2 pre-treated sludge, respectively, showing the shear-thinning behavior (pseudoplastic) of sludges. The most commonly used Equations to represent the non-Newtonian behavior of sludge suspensions are Ostwald or power law Eq. (11), or Sisko Eq. (12);

$$\tau = K\gamma^{m} \qquad (11)$$

$$\tau = \eta_{\infty}\gamma + K\gamma^{m} \qquad (12)$$

In this model, K is a constant, linked to the structure; higher the value of K, the higher the viscosity. The structure index m is linked to viscosity dependence to shear rate; m equals to 1 for Newtonian fluids. As can be seen from the Eq. 11 and 12, the shear thinning of the suspension occurs when $m < 1$. The presence of yield stress interferes with mass and heat transfer as noted by Magnin and Piau [23], and it further increases with an increase in solid volume fraction. Thus, the presence of yield stress will also influence the transport of BPA. This kind of yield stress effect on organic contaminant transport has been demonstrated in soil clay barriers and packed beds [24]. Therefore, a decrease in viscosity during different pre-treatment processes improves the mass transfer rate, leading to higher reaction rates and easier transportation of BPA, mainly its partitioning (adsorption-desorption profiles) causing degradation of pollutant within the medium.

The order of viscosity and BPA degradation for US1, US2 and US3 pre-treatment processes were US1>US2>US3 and US3>US2>US1, respectively. The lower viscosity observed in US3 pre-treated sludge was due to higher sludge solids and organic matter solubilization. Structural units of suspension in WWS are typically larger in size and knitted close to each other at higher concentration of solids. As particle concentration increases, a network of particles forms (larger particle size), with the number of interactions increasing rapidly with the increase in volume concentration of particles leading to an increase in resistance to the flow of sludge. Therefore, higher solid solubilization results in lower resistance to flow, leading to a decrease in viscosity. The other reason for lower viscosity observed in US3 pre-treatment followed by US2 and US1 pre-treatment process was due to different ultrasonication time and energy input. The high energy input produced floc structure break-up and thus reduced floc strength by cleaving the interactions and binding between flocs. The increase in ultrasonication time resulted in an increase in bulk liquid temperature, which can facilitate floc stability, as net strength between particles weakened due to aggressive motion of particles at high temperature leads to low resistance to flow, causing lower viscosity.

The lower viscosity observed in the US3 pre-treatment process resulted in higher degradation of BPA (66.3%) from raw sludge, followed by the US2 and US1 pre-treatment process. Lower viscosity increased mass transfer rate of BPA causing higher reaction rate and its partitioning, leading to degradation within the medium. In order to verify this hypothesis, we used an Equation showing the relationship between liquid phase mass transfer coefficient (k_L) with viscosity (μ) [25].

$$\frac{k_L a}{D^{0.5}} = 90L^{0.74}\mu^{-0.5} \qquad (13)$$

From the above Equation, it is clear that k_L decreases with an increase in viscosity as a result of the increasing resistance to mass transfer. The negative sign on the exponent of μ indicates a decrease in the k_L value with increasing liquid phase viscosity. The apparent viscosity value influences the global coefficient of mass transfer in an inverse relation [26].

The increase in viscosity during FO, FS1 and FS2 pre-treatment as compared to US2 and US3 pre-treatment process was due to operating conditions, i.e. acidification (lower pH) and oxidation by free hydroxyl radicals. The reaction conditions will lead to changes in inter-particle interactions between sludge flocs and their components. The decrease in pH will result in a decrease in electrostatic repulsions between flocs (which are mostly negatively charged), causing higher resistance to flow and leading to higher viscosity [27]. The order of viscosity and BPA degradation for FO, FS1 and FS2 pre-treatment process were FS1>FO>FS2 and FS2>F0>FS1, respectively. The higher viscosity observed in FS1 pre-treatment was due to lower pH as compared to FO and FS2 pre-treatment process. Lower viscosity in the FS2 pre-treatment leads to higher degradation of BPA as discussed earlier, followed by FO and FS1 pre-treatment process.

As seen in Fig. 4, the particle size of raw sludge decreased after ultrasonic pre-treatment and increased after Fenton and ferro-sonication pre-treatment. The particle size of 55.47 μm was observed in RS and the value decreased to 47.38 μm, 29.73 μm and 22.17 μm in US1, US2 and US3 pre-treated sludge and increased to 61.8 μm, 72 μm and 57 μm in FO, FS1 and FS2 pre-treated sludge, respectively. Our previous study has demonstrated that pre-treatment can result in a change in particle size (formation of larger volume fine particles) [11]. Pham et al. [12] also observed that ultrasonication resulted in a decrease in particle size, while Fenton's oxidation increased the particle size as compared to raw sludge. In order to verify this, the same volume of raw sludge and different pre-treated sludges were transfered to glass tubes and allowed to settle for 3 h as shown in Fig. 6a. It was observed that Fenton and ferro-sonicated sludge settled rapidly due to higher particle size as compared to ultrasonicated sludge. The order of settlement was FS1>FO>FS2>RS>US1>US2>US3.

Among US1, US2 and US3 pre-treatment, the order of formation of larger volume fine particles and BPA degradation was US3>US2>US1 and US3>US2>US1, respectively. The lower particle sizes observed in the US3 pre-treated sludge were due to higher i) ultrasonication time, ii) ultrasonic intensity, iii) untrasonic energy input, and iv) temperature of medium as compared to US1 and US2 pre-treatment process. All these factors simultaneously lead to reduction in the floc size, breaks the flocs and releases intracellular materials. This results in an increase of soluble protein concentration in the aqueous phase, leading to lower particle size.

The decrease in particle size during US3 pre-treated sludge causes higher interaction (due to more fine particles and by increasing the reaction rate) of BPA with OH$^{\bullet}$ radical formed within the medium, leading to higher degradation of BPA as compared to US1 and US2 pre-treatment. In order to verify this hypothesis, we used the Arrhenius equation (Equation 14-15).

$$\mathrm{K} = \mathrm{A}e^{-\frac{\mathrm{E_A}}{\mathrm{RT}}} \quad (14)$$

$$\ln \mathrm{K} = \ln \mathrm{A} - \frac{\mathrm{E_A}}{\mathrm{RT}} \quad (15)$$

Where, K = rate constant, E_A = activation energy, R = gas constant, T = temperature. With higher temperature, the activation energy decreases, resulting in higher rates of reaction between BPA and $OH^{\bullet}$ radicals formed in the medium, enhancing degradation of BPA. The scavenging of organic compounds is largely influenced by the particle size distribution [28].

The increase in particle size in FO, FS1 and FS2 pre-treated sludge was due to a decrease in pH (pH 3). A schematic presentation of increase in particle size during Fenton and ferro-sonication pre-treatment due to change in pH is presented in Fig. 6b. Neyens et al. [29] observed that particle size can be modified by the presence of acids, i.e. supracolloidal particles (1–100 µm range) decrease with acidic pH. The other reason for an increase in particle size was due to the formation of ferric hydroxide and ferric hydroxo complexes during reaction between ferrous ions with hydroxide ions. Ferric hydroxide and ferric hydroxo complexes are known to possess high capacity of coagulation and flocculation. However, comparing FO, FS1 and FS2 pre-treatment, a lower particle size was observed in the FS2 pre-treated sludge, resulting in higher removal of BPA. The lower particle size in FS2 pre-treated sludge was due to higher pH as compared to FO and FS1 pre-treated sludge. Thus, a decrease in particle size increased the reduction of BPA by enhancing the reaction kinetics between the substrate and reactant (depending on the pre-treatment).

3.3. Effect of zeta potential on BPA degradation during pre-treatment of WWS

Fig. 7 presents the zeta potential obtained in RS and different pre-treated sludges at different pH. The zeta potential of -37.51 mV was observed in raw sludge and the value increased to -43.17 mV, -49.41 mV and -58.01 mV in US1, US2 and US3 pre-treated sludge and decreased to -31.27 mV, -26.91 mV and -34.18 mV in FO, FS1 and FS2 pre-treated sludge, respectively. Due to different SS concentrations and pH values, variations in zeta potential values were observed in different pre-treated sludges. The lower zeta potential observed in FO, FS1 and FS2 pre-treated sludge as compared to raw sludge was due to a lower pH.

Among US1, US2 and US3 pre-treatment, the order of higher zeta potential and BPA degradation was US3>US2>US1 and US3>US2>US1, respectively. The higher zeta potential in US3 pre-treated sludge resulted in the formation of larger numbers of sludge flocs per unit volume of WWS. Based on DLVO (Derjaguin, Landau, Verwey, and Overbeek) theory, a higher (negative) value of zeta potential is expected to result in greater electrostatic repulsion within the flocs, leading to a higher sludge-shear sensitivity and, consequently, smaller floc sizes [30]. As the size of the flocs decreased with increasing zeta potential, the overall availability of sorbed BPA molecules to react with $OH^{\bullet}$ radicals increased, resulting in higher degradation of BPA during US3 pre-treatment followed by US2 and US1 pre-treatment. Comparing FO, FS1 and FS2 pre-treatment, the highest zeta potential was observed in FS2 pre-treated sludge, resulting in higher degradation of BPA from WWS followed by FO and FS1.

An increase in zeta potential can also lead to a decrease in viscosity as the particle size decreases. This can be explained using the Henry equation (Eq. 16), which establishes a relationship between zeta potential, electrophoretic mobility and viscosity.

$$U_E = \frac{2\,\varepsilon\, z\, f(Ka)}{3\eta} \qquad (16)$$

Where, z = zeta potential, η = viscosity, U_E = electrophoretic mobility, ε = dielectric constant and f(Ka) = Henry's function. However, the three phenomena, namely, an increase in zeta potential, a decrease in viscosity and a decrease in particle size occurred simultaneously within the media, leading to increased reaction rate and partitioning of BPA, thus causing its higher removal from raw sludge.

3.4. Formation of intermediates during degradation of BPA in WWS

To identify different by-products formed during BPA degradation by ultrasonication, Fenton's oxidation and ferro-sonication treatment of WWS, the experiments were carried out in samples with; i) BPA spiked in milli-Q water, ii) BPA spiked in WWS, and iii) real WWS sample (without spiking). A comparison was done in each case to identify the intermediates. The study of BPA intermediates was achieved in LC-MS/MS experiments that were run in both positive and negative electrospray modes.

Similar by-products were observed in all the three advanced oxidation processes (AOPs), namely, 3-hydroxybisphenol A, hydroquinone and 4-hydroxyacetophenone, due to the reaction of $OH^{\bullet}$ radicals with BPA. A proposed region of attack on BPA by $OH^{\bullet}$ radicals and the possible pathway of degradation of BPA during ultrasonication, Fenton's oxidation, and the ferro-sonication pre-treatment processes is presented in Figs. 8 a and b. Torres et al. [6] also observed the same intermediates during degradation of BPA by ultrasound and Fenton's oxidation in deionized and natural waters. Continuous reaction with radicals will induce aromatic ring cleavage, forming lightweight hydrocarbons, such as formaldehyde, propionic and formic acids. Further, mineralization of the solution gives rise to the formation of CO_2 and H_2O.

3.5. Biofertilizer production, simultaneous production of laccase and BPA degradation

The viable cell counts of *S. meliloti* growth in raw sludge and different pre-treated sludges is presented in Fig. 9a. It was observed that the growth of *S. meliloti* in terms of viable cells enhanced in US1, US2, US3, FO, FS1 and FS2 pre-treated sludges as compared to raw sludge. The increase in viable cell count in pre-treated sludges as compared to raw sludge was probably due to higher sludge solids and organic matter solubilization of raw sludge. As *S. meliloti* is an aerobic bacteria, the increase in solubilization during different pre-treatment processes leads to higher oxygen diffusion and consequently increased cell count. The concentration of suspended solids in sludge affected the growth of *S. meliloti* in secondary sludge from a municipal wastewater treatment plant (Quebec, Canada) [31]. Other factors, such as increase in zeta potential and a decrease in viscosity and particle size also increased the oxygen transfer rate (OTR) within the medium, leading to higher cell multiplication.

Among US1, US2, US3, FO, FS1 and FS2 pre-treatment, a high viable cell count (4.4×10^9 CFU mL^{-1}) of *S. meliloti* was observed in FS2 pre-treated sludge, followed by FO pre-treated sludge (1.7×10^9 CFU mL^{-1}) in 24h. Fig. 9b represents the difference in *S. meliloti* cell growth in raw, FO and FS2 pre-treated sludge at 24 h of incubation. Comparing US1, US2 and US3 pre-treatments, a high count of viable cells (8.6×10^8 CFU mL^{-1}) was observed in the US3 pre-treated sludge at 12 h. It was observed that cell count of *S. meliloti* increased with higher sludge solids and organic matter solubilization. The order of sludge solids and organic matter solubilization and viable cell count of *S. meliloti* was FS2>FO>FS1>US3>US2>US1 and FS2>FO>FS1>US3>US2>US1, respectively, after 24 h of incubation. Higher solids solubilization resulted in higher oxygen or substrate mass transfer within the medium, leading to a higher cell count [32].

Figs. 10 a and b present the laccase activity profile and simultaneous BPA degradation in raw and different pre-treated sludges. It is evident that *S. meliloti* is capable of producing laccase enzyme in raw and pre-treated sludge. As shown in Fig. 10a, an increased activity of laccase was observed in raw and pre-treated sludges with increasing viable cell count of *S. meliloti.* Rosconi et al. [9] carried out a comparative study between *Rhizobium* sp. to identify the laccase strain and they observed higher identity (99%) was established with *S. meliloti.* They also observed the higher production of laccase with higher cell count of *S. meliloti.*

Among US1, US2, US3, FO, FS1 and FS2 pre-treated sludge, higher activity of laccase (207.9 U L^{-1}) was observed in the FS2 pre-treated sludge. This trend might be due to the higher viable cell count of *S. meliloti* in FS2 pre-treated sludge. As seen in Fig 10a, laccase activity started increasing from 0 h to 24 h of fermentation time in raw sludge and US1, US2, FO, FS1 and FS2 pre-treated sludge. However, comparing the results for 12 h to 24 h, a decrease in the activity of laccase was observed in US3 pre-treated sludge. The results were in agreement with an increase in the cell count of *S. meliloti* for up to 24 h in US1, US2, FO, FS1 and FS2 pre-treated sludges and a decrease after 12 h in US3 pre-treated sludge.

As seen in Fig. 10 a and b, degradation of BPA was observed in raw sludge and US1, US2, US3, FO, FS1 and FS2 pre-treated sludge with increasing laccase activity. Thus, laccase was capable of degrading BPA in these media. Laccase is a blue oxidase capable of oxidizing phenols and aromatic amines by reducing molecular oxygen to water by a multicopper system. The catalytic center consists of three types of copper with different functions: type 1 (blue copper) catalyzes the electron transfer, type 2 activates molecular oxygen, and type 3 is a copper dimer, which is responsible for oxygen uptake [33].

The concentration of BPA observed at 0 h of incubation in raw, US1, US2, US3, FO, FS1 and FS2 pre-treated sludge were 2.85, 2.49, 1.84, 0.969, 0.581, 0.73 and 0.513 μg g^{-1}, respectively. The concentration of BPA observed after 48 h of laccase activity in raw, US1, US2, US3, FO, FS1 and FS2 pre-treated sludge were 2.83, 2.415, 1.77, 0.89, 0.5, 0.65 and 0.43 μg g^{-1}, respectively. It was observed that FS2 pre-treatment was more efficient (16.18%) in the degradation of BPA in raw sludge due to relatively higher laccase activity, which was induced by high viable cell count.

The concentration of BPA observed in raw sludge was 2.85 μg g^{-1} and the concentration was reduced to 2.415, 1.77, 0.89, 0.5, 0.65 and 0.43 μg g^{-1} in US1, US2, US3, FO, FS1 and FS2 pre-treated sludges, respectively, after combined application of pre-treatment process and laccase activity. The pre-treatment processes, US1, US2, US3, FO, FS1 and FS2 for BPA degradation became more efficient (15.26%, 37.89%, 68.77%, 82.45%, 77.19% and 85%, respectively) when a combined application of different pre-treatment processes and laccase activity were taken into account. The European Chemicals Bureau has recently completed a comprehensive risk assessment for BPA and has predicted no effect concentrations (PNEC) for fresh waters is 1.5 μg L^{-1}. Hence, US3, FO, FS1 and FS2 pre-treated sludge are applicable for land application (agriculture) and recycling, as the organic matter disposed for land application are continuously transferred to river and ground water due to rainfall and runoff. At this juncture, the economical costs in utilizing these pre-treatment technologies need to be weighed against environmental costs.

4. CONCLUSIONS

The study on the degradation of BPA in raw sludge by ultrasonication, Fenton's oxidation and ferro-sonication pre-treatment processes and laccase activity led to the following conclusions:

1. Among ultrasonication, Fenton's oxidation and ferro-sonication pre-treatment, higher SS, VSS, COD and SOC solubilization was observed in ferro-sonication pre-treatment carried out for 180 min resulting in higher degradation of BPA.
2. US1, US2, US3, FO, FS1 and FS2 pre-treatment caused a decrease in the apparent viscosity of the sludge. Among US1, US2 and US3 pre-treatment, decreased viscosity and particle size and increased zeta potential was observed in US3 pre-treatment, causing higher removal (66.3%) of BPA from raw sludge.
3. Among FO, FS1 and FS2 pre-treatment, decreased viscosity and particle size and increased zeta potential was observed in FS2 pre-treatment, causing higher removal (82.7%) of BPA from raw sludge.
4. The viable cell count of *S. meliloti* increased in US1, US2, US3, FO, FS1 and FS2 pre-treated sludges, and a higher growth (4.4×10^9 CFU mL^{-1}) was observed in FS2 pre-treatment due to the higher percentage of SS, VSS, COD and SOC solubilization as compared to other pre-treatment process.
5. Higher laccase activity was observed in FS2 pre-treated sludge leading to higher removal of BPA (0.083 $\mu g\ g^{-1}$) as compared to other pre-treated sludge.
6. Ultrasonication, Fenton's oxidation and ferro-sonication pre-treatment of wastewater sludge for the degradation of BPA can be attributed to four different parameters, namely solubilization, lower viscosity, lower particle size and higher zeta potential, which leads to higher oxidation potential.
7. During further biotransformation of the pre-treated sludges, BPA removal was enhanced by the intervention of laccase enzymes.

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Table 5. Changes in physical-chemical parameters before and after pre-treatment of wastewater sludge

	Raw sludge	Ultrasonicated Sludge1	Ultrasonicated Sludge 2	Ultrasonicated Sludge 3	Fenton's oxidized Sludge	Ferro-sonicated Sludge 1	Ferro-sonicated Sludge 2
pH	5.8	6.0	6.2	6.5	3.4	3.1	3.7
TS (g L^{-1})	33.2±0.6	32.8±0.5	33.0±0.7	33.1±0.5	32.5±0.8	32.3±0.3	33.0±0.1
SS (g L^{-1})	30±0.3	25.9±0.5	22.7±0.3	20.8±0.1	18.9±0.6	20.2±0.7	18.1±0.2
VSS (g L^{-1})	12.5±0.2	10.3±0.09	9.0±0.4	7.3±0.06	6.5±0.5	6.9±0.09	6.1±0.5
TCOD (g L^{-1})	13.3±6	13.1±3	12.9±4	13.3±1	12.8±3	12.9±7	13.0±2
SCOD (g L^{-1})	1.2±0.4	3.1±1.3	4.9±1.9	7.3±0.07	9.1±2.4	8.3±1.7	9.9±0.05
TOC (g L^{-1})	290.0±6	289.5±11	290.0±3	287.4±10	288.6±5	290.9±5	289±7
SOC (g L^{-1})	0.3±0.1	12.9±2.9	28.9±4.1	34.2±3	42.7±4	37.9±7	51.3±0.7

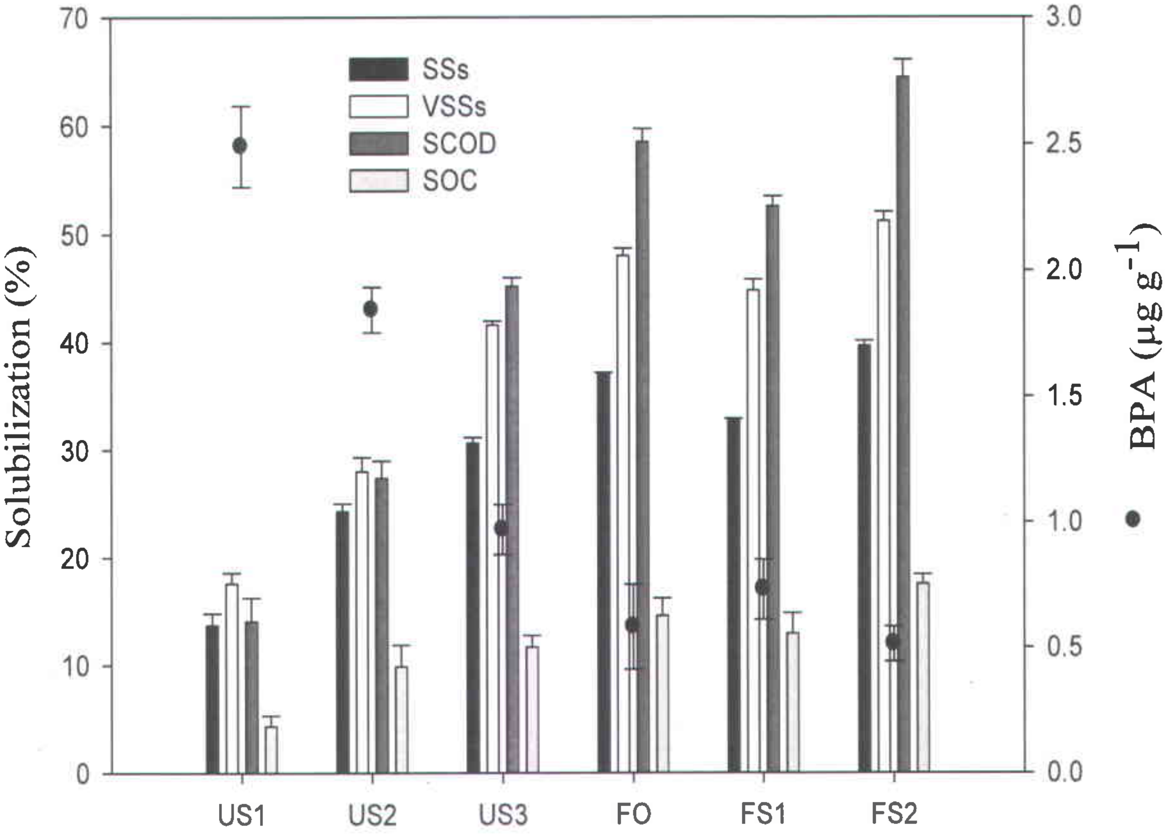

Figure 1. Correlation of BPA removal with solubilization of raw sludge during different pre-treatment. US1, Ultrasonicated sludge 1; US2, Ultrasonicated sludge 2; US3, Ultrasonicated sludge 3; FO, Fenton's oxidized sludge; FS1, Ferro-sonicated sludge 1

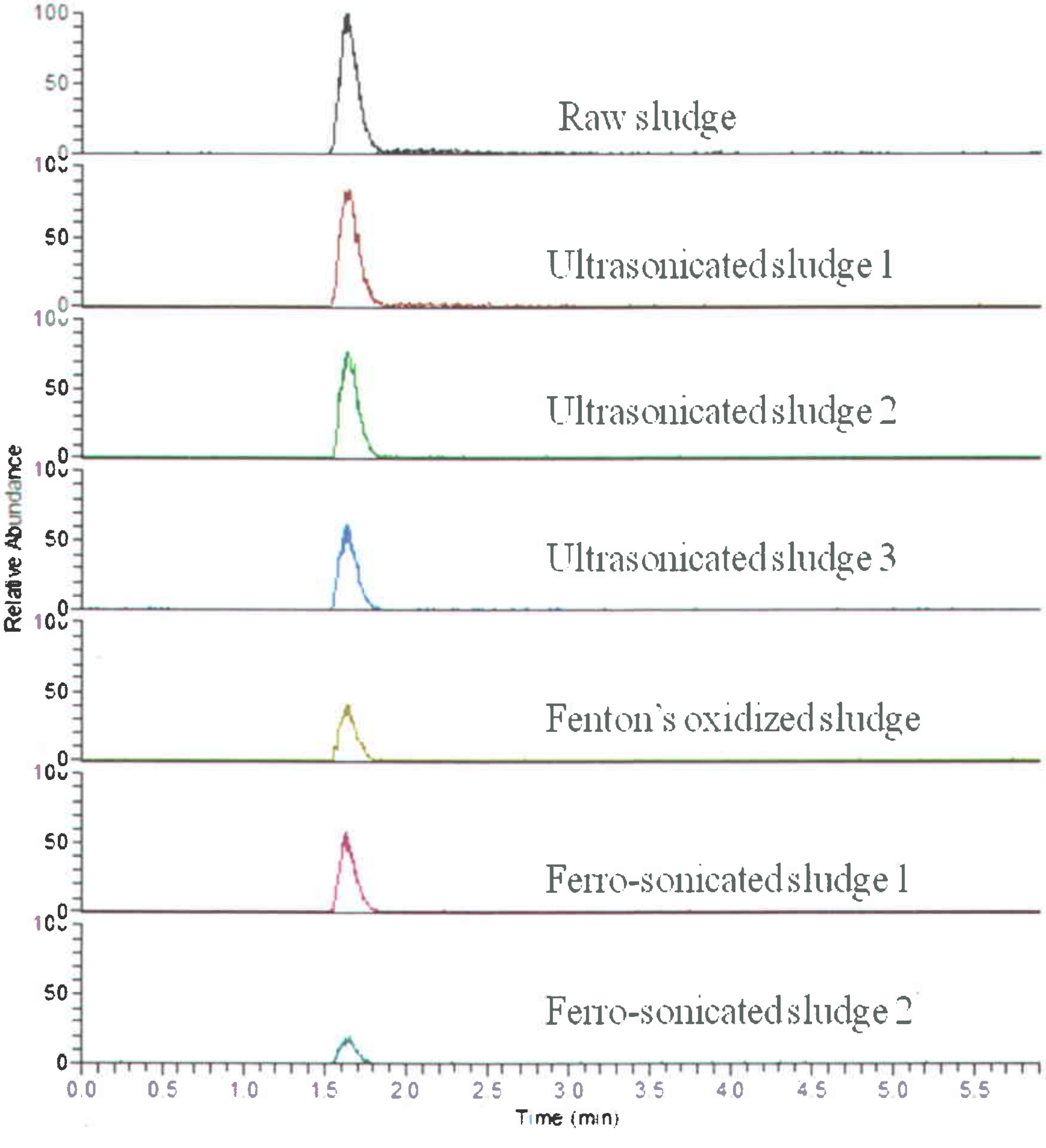

Figure 2. **LC-MS/MS chromatograms of BPA before and after pre-treatment. (Note: decreased area in chromatogram during different pre-treatments of raw sludge resulting in lower concentration of BPA)**

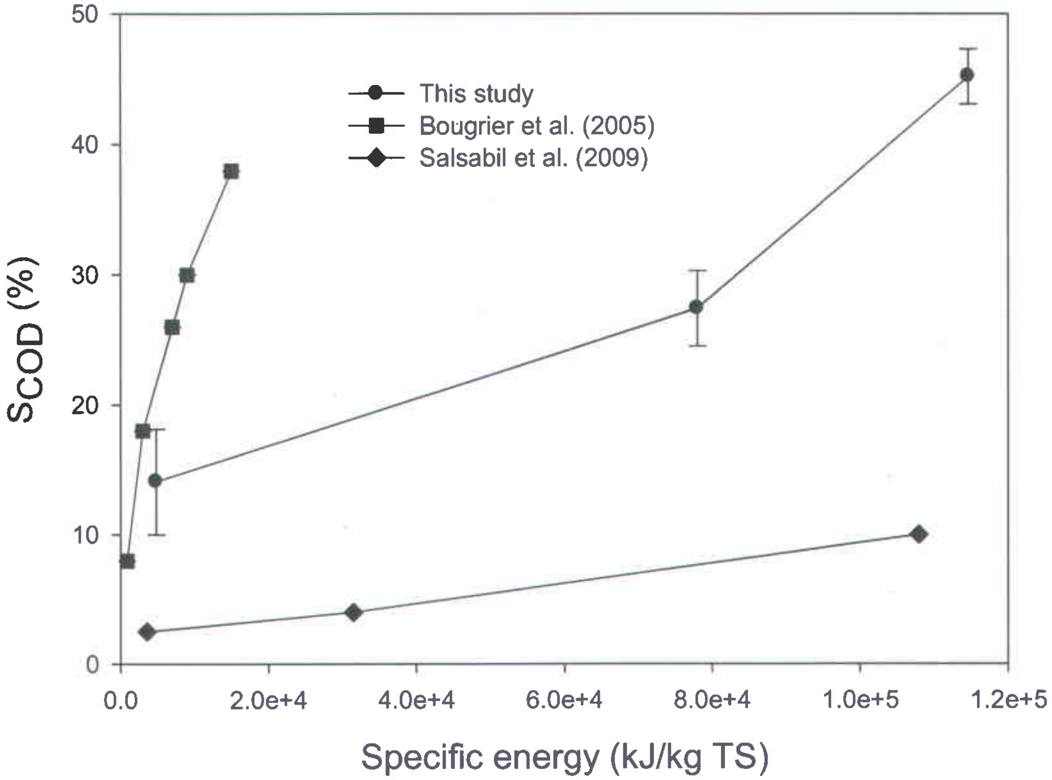

Figure 3. Comparison of COD solubilization with different specific energy during ultrasonication pre-treatment of WWS

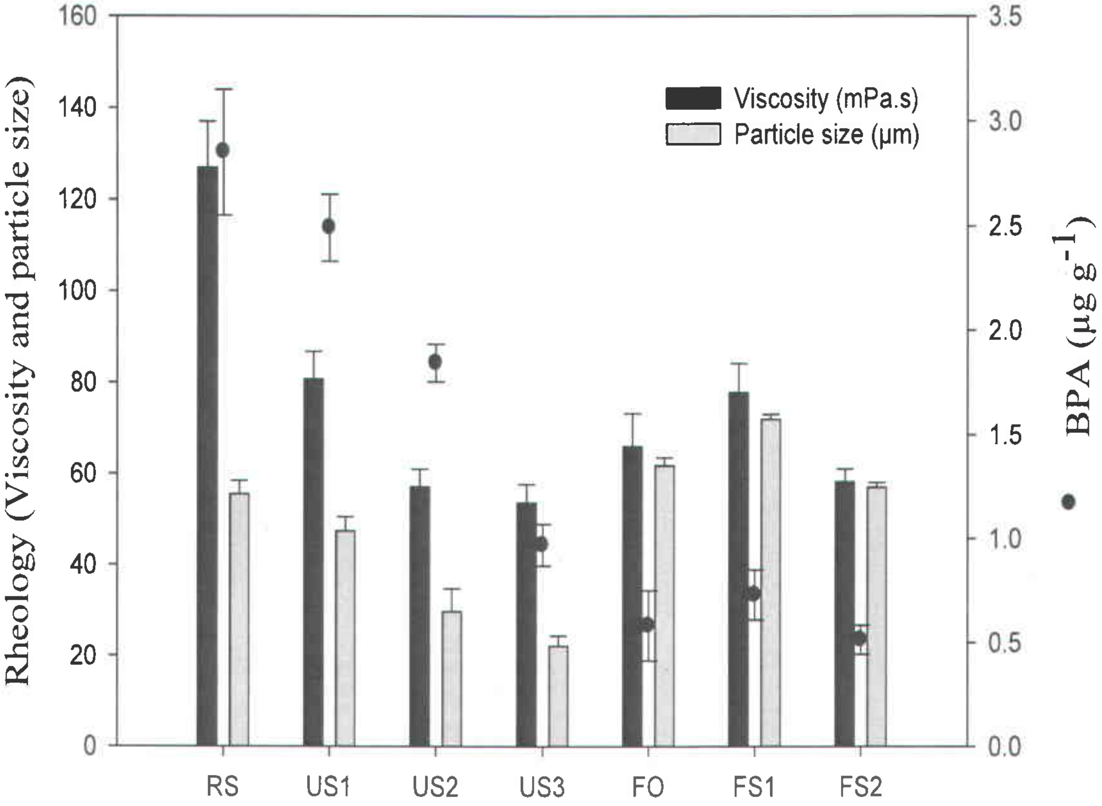

Figure 4. Correlation of viscosity and particle size on removal of BPA from raw sludge during different pre-treatment processes

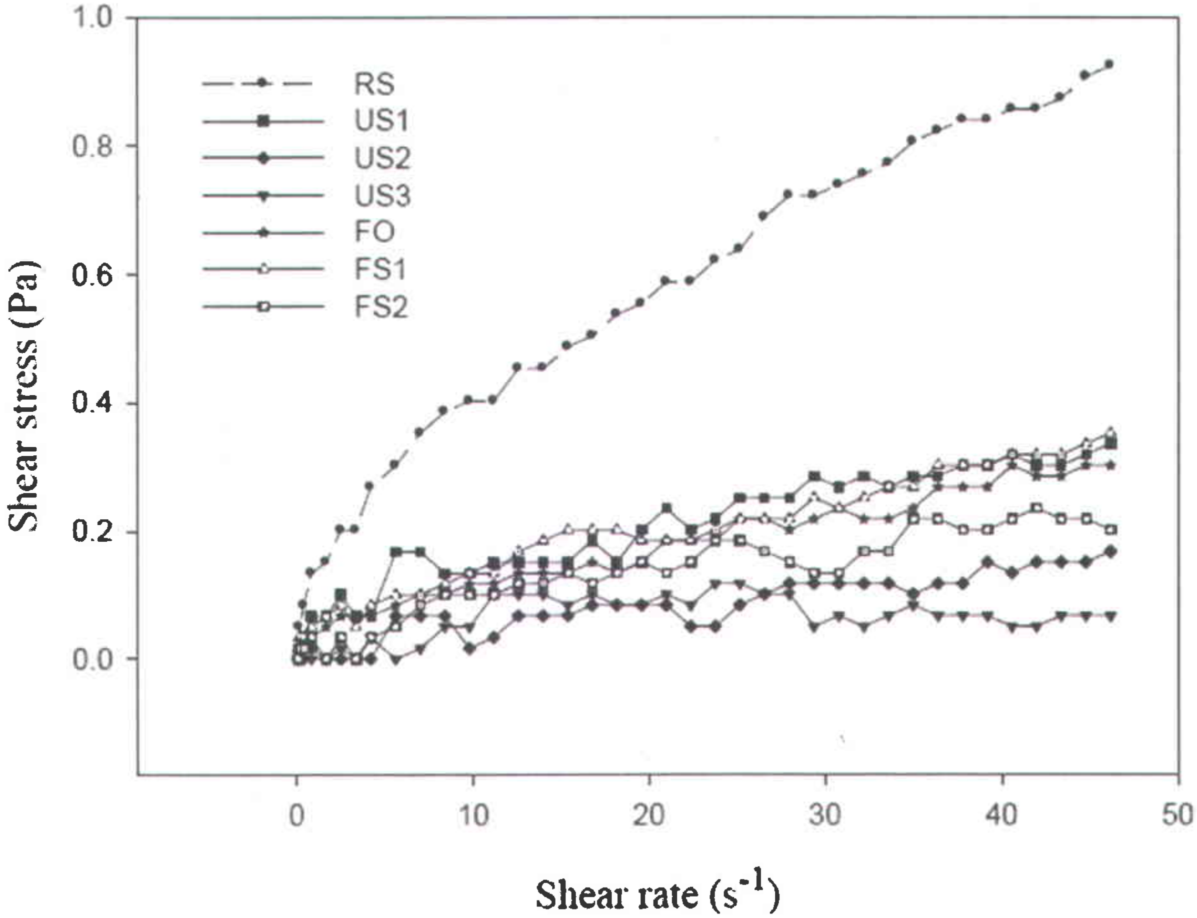

Figure 5. Shear stress vs shear rate profiles of raw (RS), ultrasonicated (US1, US2 and US3), Fenton's oxidized (FO) and ferro-sonicated (FS1 and FS2) sludge samples

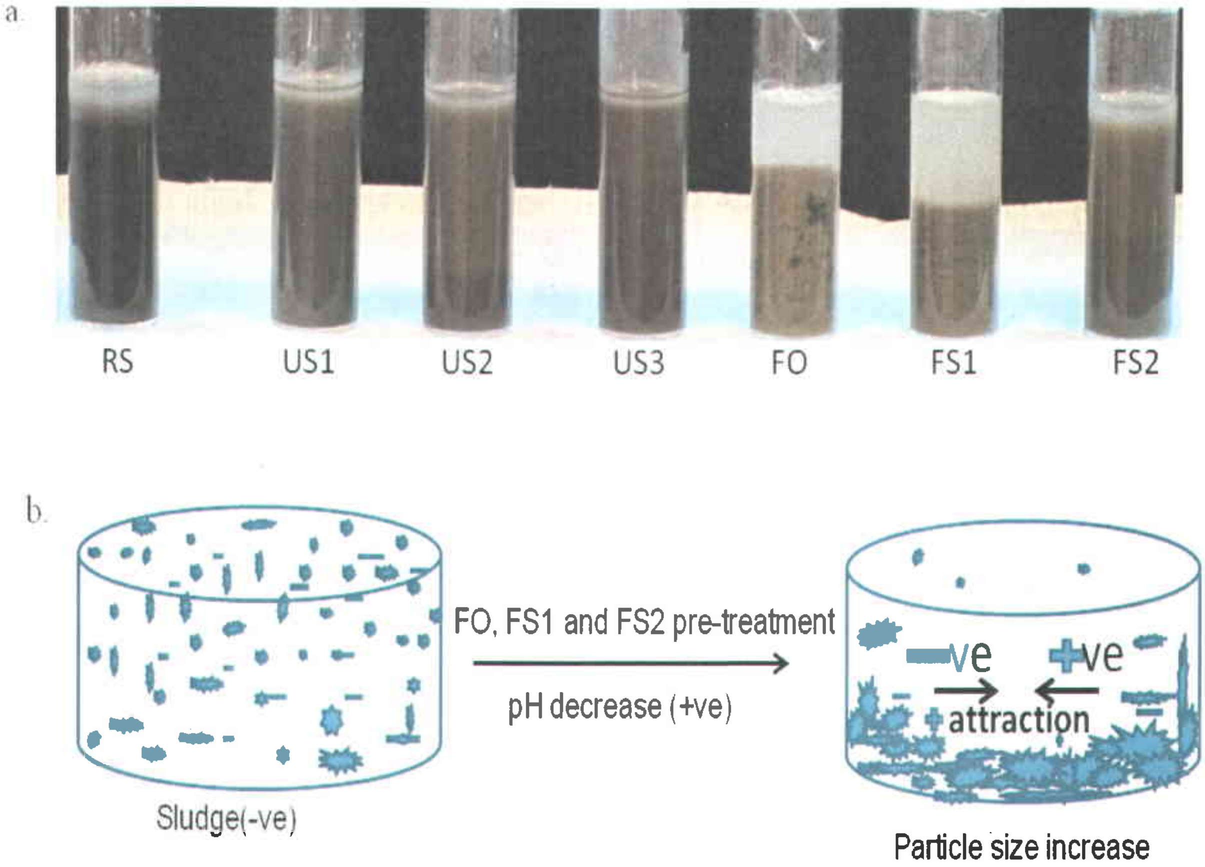

Figure 6. Change in particle size of raw sludge after different pre-treatment processes; a) change after 3 h of settlement, b) schematic presentation of increase in particle size in FO, FS1 and FS2 pre-treated sludge

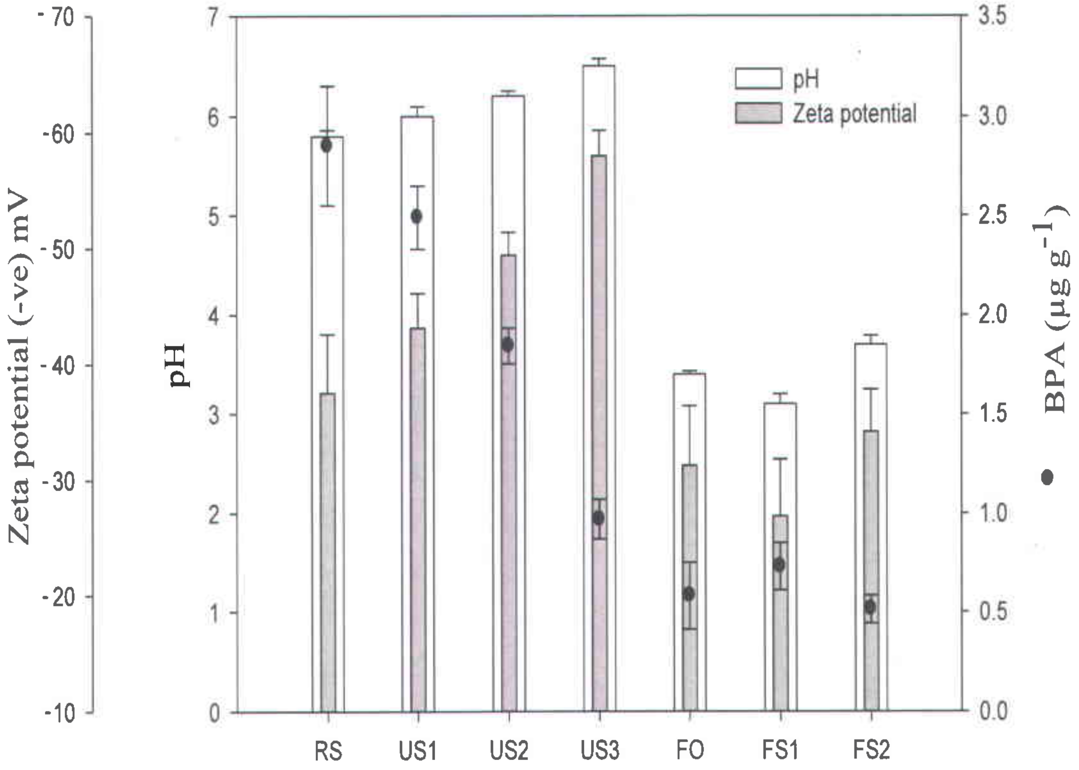

Figure 7. Correlation of zeta potential values of raw and pre-treated sludges at different pH with degradation of BPA.

(RS, Raw sludge; US1, Ultrasonicated sludge 1; US2, Ultrasonicated sludge 2; US3, Ultrasonicated sludge 3; FO, Fenton's oxidized sludge; FS1, Ferro-sonicated sludge 1 and FS2, Ferro-sonicated sludge 2)

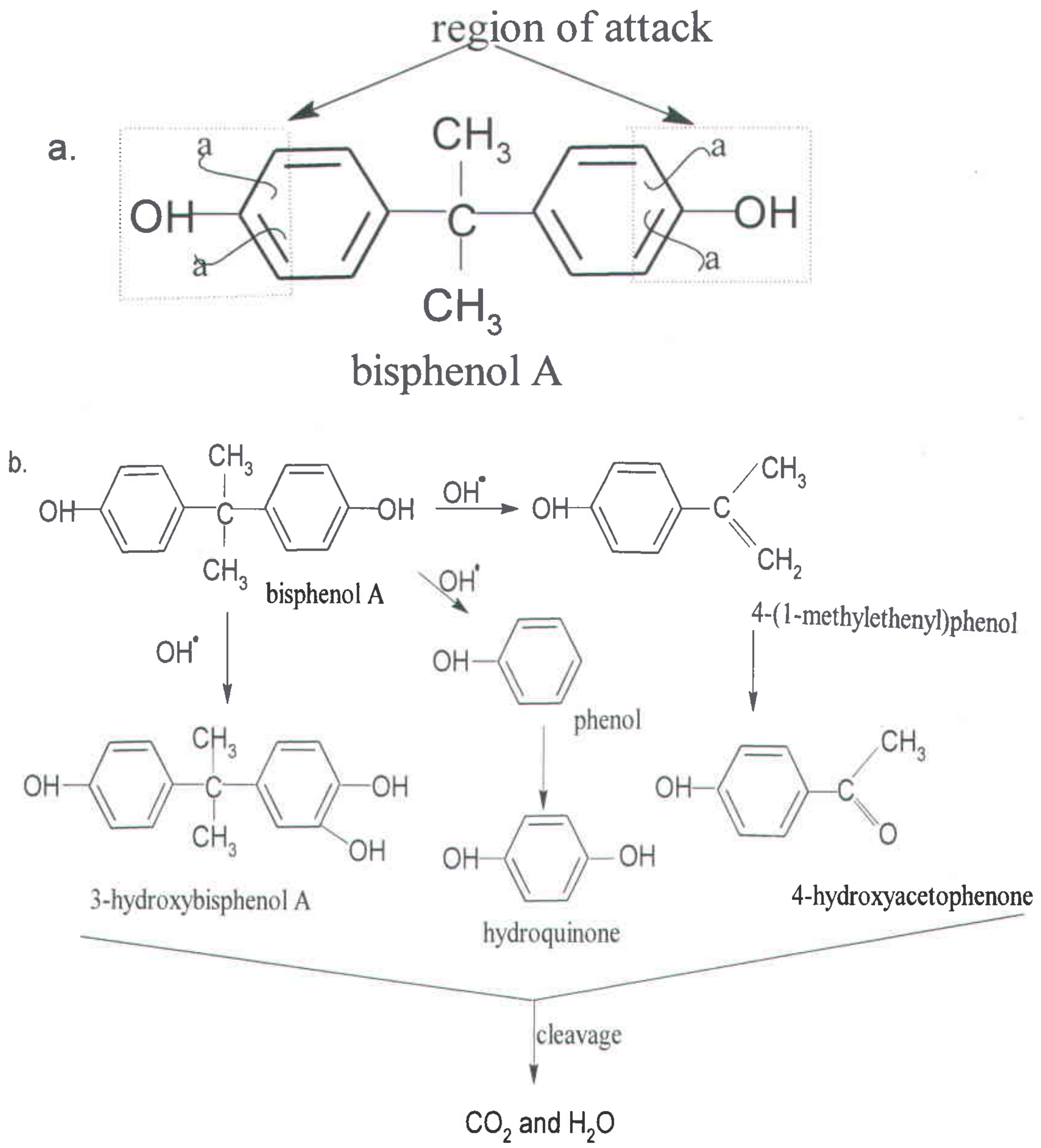

Figure 8. BPA degradation during ultrasonication, Fenton's oxidation and ferro sonication pre-treatment of WWS; a) proposed region of attack (a- the site of intraenol ring cleavage), b) proposed pathway of degradation based on M/Z ratios obtained during LC-MS/MS

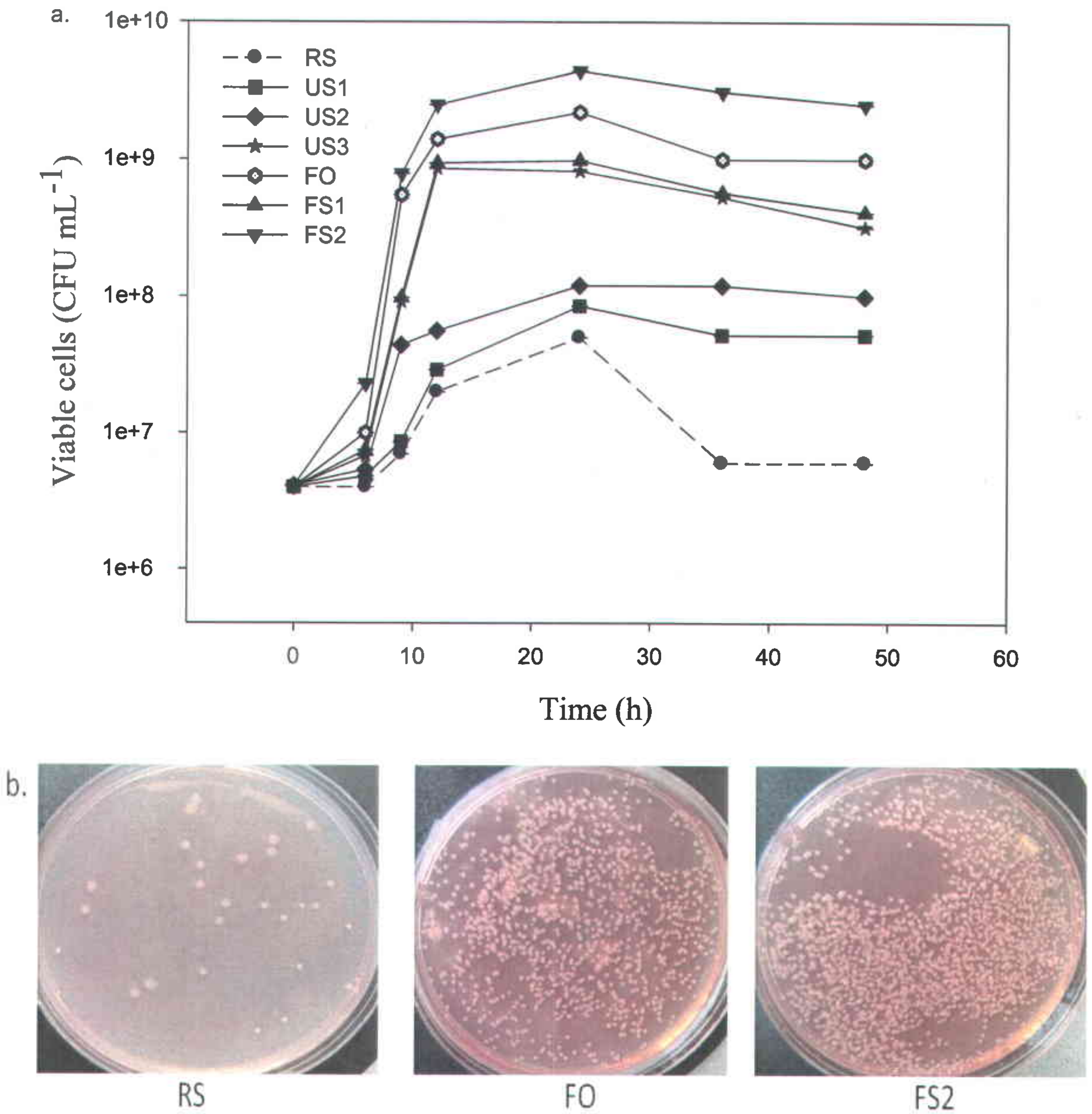

Figure 9. S. meliloti growth in raw and different pre-treated sludges; a) growth profile in raw and pre-treated sludges at different incubation times, b) growth in raw sludge, Fenton's oxidized sludge and ferro-sonicated sludge 2 after 24 h of incubation

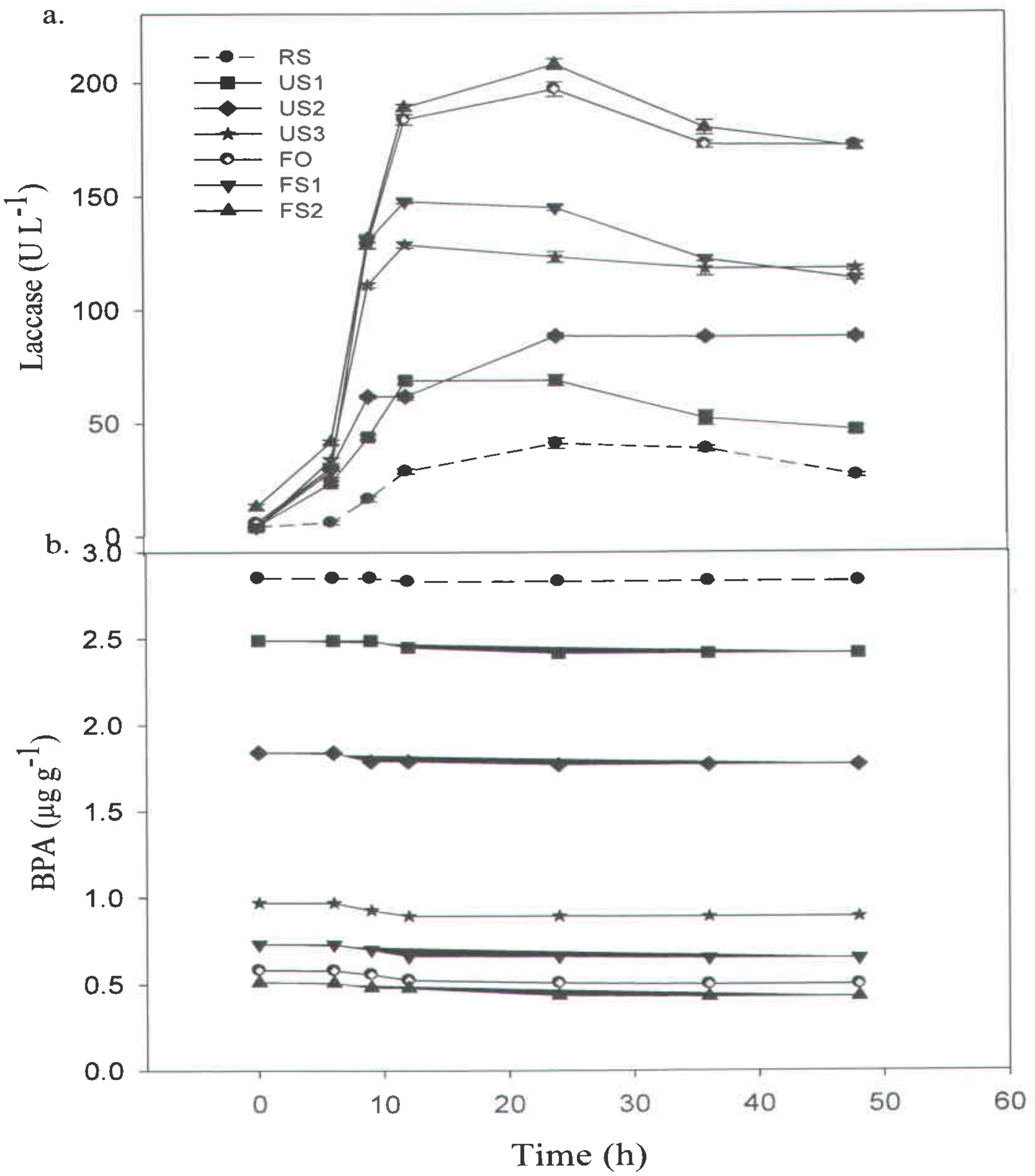

Figure 10. BPA degradation in raw and different pre-treated sludges by laccase activity produced by S. meliloti; (a) laccase activity, (b) BPA degradation

CHAPITRE V

ÉTUDES D'OPTIMISATION

PARTIE 1

PARAMETER OPTIMIZATION OF FERRO-SONICATION PRE-TREATMENT PROCESS FOR DEGRADATION OF BISPHENOL A AND BIODEGRADATION FROM WASTEWATER SLUDGE USING RESPONSE SURFACE MODEL

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RÉSUMÉ

Dans cette étude, l'application d'un modèle de surface de réponse pour prédire et optimiser la ferro-sonication comme un pré-traitement de dégradation du bisphénol A (BPA), un composé perturbateur endocrinien de boues d'épuration (WWS) a été étudiée. La ferro-sonication a été effectuée conformément à la methode de plan central composite (CCD) à quatre variables indépendantes, telles que les concentrations en matières solides des boues d'épuration, le pH, le temps de traitement aux ultrasons et la concentration de $FeSO_4$. L'effet de ferro-sonication a été évalué en termes des matières solides des boues (matières en suspension (SS) et de solides volatils (VS)) et de la matière organique (demande chimique en oxygène (COD) et de carbone organique soluble (SOC)) solubilisation et la dégradation simultanée BPA de WWS. Il a été observé que, parmi toutes les variables étudiées, le temps de traitement aux ultrasons avait plus touchées de façon importante l'efficacité du processus de ferro-sonication suivi par $FeSO_4$ et la concentration en solides. Grâce à ce processus d'optimisation, il a été constaté que la dégradation maximale BPA de 88% pourrait être obtenue avec 163 fois ultrasons/min, 2,71 mg / L $FeSO_4$, pH 2,81 à 22 g / L SS. En outre, l'effet de la ferro-sonication pré-traitement sur la biodégradation des WWS a également été étudié. Il a été observé que le temps ultrasonication à un effet significatif et la plus biodégradabilité (32,48%) a été observé à 180 ultrasons par min.

Mots-clés: Bisphénol A; La biodégradation; Ferro-sonication; La méthodologie de surface de réponse; Boues d'épuration

ABSTRACT

In this study, the application of response surface model in predicting and optimizing the ferro-sonication pre-treatment for degradation of bisphenol A (BPA), an endocrine disrupter compound from wastewater sludge (WWS) was investigated. The ferro-sonication pre-treatment process was carried out according to central composite design (CCD) with four independent variables such as wastewater sludge solids concentration, pH, ultrasonication time and $FeSO_4$ concentration. The effect of ferro-sonication pre-treatment was assessed in terms of increase in sludge solids (suspended solids (SS) and volatile solids (VS)) and organic matter (chemical oxygen demand (COD) and soluble organic carbon (SOC)) solubilization and simultaneous BPA degradation from WWS. It was observed that among all the variables studied, ultrasonication time had more significantly affected the efficiency of the ferro-sonication pre-treatment process followed by $FeSO_4$ and solids concentration. Through this optimization process, it was found that maximum BPA degradation of 88% could be obtained with 163 min ultrasonication time, 2.71 mg/L $FeSO_4$ concentration, pH 2.81 with 22 g/L SS. Further, the effect of ferro-sonication pre-treatment on biodegradation of WWS was also studied. It was observed that ultrasonication time had significant effect and the higher biodegradation (32.48%) was observed at 180 min ultrasonication time.

Keywords: Bisphenol A; Biodegradation; Ferro-sonication; Surface response methodology; Wastewater sludge

1. INTRODUCTION

Endocrine disrupting compounds (EDCs) have been found to cause adverse effects on the endocrine system of man and animals with particular environmental concern [1]. As better understanding of the biological effects of EDCs emerges, investigators have begun to study the potential effects of EDCs on human, which include malformations of newborns, undescended testicles, abnormal sperm, low sperm counts, abnormal thyroid function, breast cancer and testicular prostate cancer among others [2, 3]. Bisphenol A (BPA) has been identified as EDC by the U.S Environmental Protection Agency (EPA), World Wide Fund for Nature (WWF) and is declared as a social, environmental and global issue [4]. Recently, the report submitted by Canadian Health Measures Survey (CHMS) showed that Canadians aged 6 to 79 had a geometric mean concentration of urinary BPA of 1.16 µg/L. The report also showed that BPA was found in 91% of Canadians and higher concentrations were measured in teens aged 12 to 19 [5, 6].

BPA is a representative EDC due to its large consumption as a monomer for the production of polycarbonate and epoxy resins, unsaturated polyester-styrene resins and flame retardants. Due to the daily use of these products, higher concentration of BPA is observed in wastewater (WW) and finally in wastewater sludge (WWS) (0.004-1.36 mg kg^{-1}) [4]. Many treatments, such as dewatering, digestion, burning, landfilling and use in agriculture have been carried out for the disposal of excess sludge. However, high cost of these treatments and presence of varied organic matter including BPA in sludge, makes its final disposal a challenge. Therefore, interest for solutions allowing sludge volume and mass reduction and simultaneous degradation of organic compounds has a great demand [7]. The effective removal of BPA from WWS is usually limited to physico-chemical pre-treatments, such as photo-catalytic oxidation, Fenton's oxidation, ultrasonication, ferro-sonication and ozonation [8, 9, 10].

Ferro-sonication pretreatment is a combination of ultrasonication and addition of $FeSO_4.7H_2O$. Ultrasonic irradiation generates cavitation bubbles in medium, which repeats a cycle of formation, growth and collapse in accordance with ultrasonic waves. The induced cavitation that occurs during ultrasonication results in sudden and violent collapse of huge number of microbubbles, which generates powerful hydro-mechanical shear forces in the bulk liquid surrounding the bubbles [11, 12]. The high temperature produced during the bubble collapse (implosion) decomposes water (H_2O) into extremely reactive hydrogen atoms (H^+), and hydroxyl radicals ($OH^•$) and in the cooling phase, these radicals will recombine to form hydrogen peroxide and molecular hydrogen [11, 13]. Further, addition of $FeSO_4.7H_2O$ results in higher production of $OH^•$ radicals due to the reaction between hydrogen peroxide and Fe^{2+}.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^• + OH^- \qquad (1)$$

$OH^•$ radicals formed during ferro-sonication pre-treatment leads to oxidation and mineralization of organic compound present in WWS. However, no studies have been reported as yet on the degradation of BPA in WWS by ferro-sonication pre-treatment.

In fact, ferro-sonication would be a prospective method in augmenting sludge biodegradability for value-addition by solubilization of organic matter. To the best of our knowledge, none of the studies discussed the enhancement of biodegradability for sludge value-addition, such as production of biopesticides, bioplastics, enzymes, bioflocculants among others using ferro-sonication as pre-treatment.

Therefore, the objective of the present work is to study the optimization of ferro-sonication pre-treatment process to improve the solubilization, biodegradability and simultaneous degradation of BPA from WWS using response surface methodology.

2. MATERIALS AND METHODS

2.1. Chemicals

BPA (98% purity assay) was obtained from Sigma-Aldrich (Ontario, Canada). HPLC-grade methanol (MeOH), dichloromethane (DCM), acetone and chloroform, used for cleaning and extraction purposes, were purchased from Fisher Scientific (Ontario, Canada). Sep-Pak Plus C18 environmental cartridges used for solid phase extraction (SPE) clean-up was purchased from Waters (Milford, MA, USA). Acetic acid, hydrogen peroxide, sodium hydroxide and sulfuric acid were supplied by Fisher scientific (Ontario, Canada) and were of analytical grade. HPLC grade water was prepared in the laboratory using a Milli-Q/Milli-RO Millipore system (Milford, MA, USA).

2.2. Wastewater sludge

Secondary sludge used in the study was collected from Quebec Urban Community (CUQ) wastewater treatment plant (Beauport, Quebec city, Quebec, Canada). Samples were collected in pre-cleaned amber glass bottles with aluminum foil-lined caps and stored under dark conditions at 4±1 ^{0}C. The sludge suspended solids (SS) were concentrated from 1.5% (w/v) to higher SS concentrations by gravity settling and centrifugation of the settled sludge at 1600 × *g* for 3 min in a Sorvall RC 5C plus Macrocentrifuge (rotor SA-600). The supernatant was discarded in order to obtain 15, 20, 25, 30 and 35 g/L SS.

2.3. Ferro-sonication pre-treatment of wastewater sludge

Ferro-sonication pre-treatment was carried out by addition of $FeSO_4.7H_20$ solution in WWS followed by ultrasonic pre-treatment. Four hundred milliliters of WWS was acidified to pH ranging from 2 to 4 by using 10 N H_2SO_4 and placed in a 1 L flask. The ultrasonication was carried out using ultrasonic homogenizer Autotune 750W (Cole-Parmer Instruments, Vernon Hills, Illinois, US). The ultrasonication equipment was operated at a frequency of 20 kHz by using platinum probe with a tip diameter of 12 mm and ultrasonic intensity of 15 W/cm^2. The ultrasonic intensity was defined as the actual power dissipated by the equipment divided by the area of the transducer. The ultrasonic probe was dipped in such a way that it was immersed 2 cm into the sludge.

2.4. Analysis

2.4.1. General

Analysis of pH, total solids (TS), suspended solids (SS), volatile solids (VS), soluble chemical oxygen demand (SCOD), soluble organic carbon (SOC) was carried out as per the Standard Methods [14]. The SS, VS, SCOD and SOC solubility increment were calculated as follows:

$$S_{SS} = \frac{(SS_0 - SS_S)}{SS_0} \times 100\% \tag{2}$$

$$S_{VS} = \frac{(VS_0 - VS_S)}{VS_0} \times 100\% \tag{3}$$

$$SCOD_{increment} = \frac{(SCOD_S - SCOD_0)}{TCOD} \times 100\% \tag{4}$$

$$SOC_{increment} = \frac{(SOC_S - SOC_0)}{TOC} \times 100\% \tag{5}$$

Where, SS_0, VS_0, $SCOD_0$ and SOC_0 are referred to as the parameters of WWS before pre-treatment, and SS_S, VS_S, $SCOD_S$ and SOC_S are referred to as the parameters after pre-treatment.

2.4.2. BPA analysis

For quantification of BPA, the extraction was carried out by microwave assisted extraction method (MARS microwave extractor, CEM Corporation, North Carolina, USA). Sep-Pak Plus C18 environmental cartridges were pre-conditioned by passing 7 mL of methanol and 3 mL of HPLC water at a flow rate of 1 mL min^{-1} [10]. LC-MS/MS analyses were performed on a TSQ Quantum Access (Thermo Scientific, Mississauga, Ontario) with a Finnigan surveyor LC pump equipped with a 120-vial capacity sample management system. The analytes were separated on a 3 micrometer, 100 × 2.1 mm Hypersil Gold C18 reverse phase column (Thermo Scientific, Peterborough). The sample injection volume was set at 10 µL. A binary mobile phase gradient with water [A] and methanol [B] was used for analyte separation at a flow rate of 200 µL min^{-1}. The gradient was performed as isocratic 5% A and 95% B for 6 min. The identification of BPA was done in a full-scan mode by matching the retention time and mass spectrum with true standards. Quantitative LC-MS/MS analysis was carried out in negative ionization (NI) condition and in selective reactions monitoring (SRM) mode using BPA d_{16} as internal standard.

2.5. Response surface methodology

Application of response surface methodology for the optimization of ferro-sonication pre-treatment for degradation of BPA from WWS will help in overcoming the limitations of time consuming conventional optimization method of 'one factor-at-a-time' (at each step, a single factor is changed while other factors remain constant). Moreover, the statistical optimization method can evaluate the effective factors and help in building models to study interaction and select optimum conditions of variables for a desirable response. In the response surface method, the factors such as SS concentration (X_1), pH of sludge (X_2), $FeSO_4.7H_2O$ concentration (X_3) and ultrasonication time (X_4) were considered as independent variables, and SCOD, SOC increment, biodegradability and BPA degradation in pre-treated sludge as dependent variables. To begin with, the screening experiments were carried out to determine the direction of optimal domain of each process. Two-level fractional factorial design (FFD) was employed in the screening step. Once the provisional optimal values were determined, a central composite design (CCD) was used to find the optimal conditions of these four factors (X_1, X_2, X_3 and X_4). In this regard, a set of 31 experiments including, 7 center points (0, 0, 0) and 7 axial points (+2, -2) and 17 points corresponding to a matrix of 2^3 which incorporate 17 experiments (+1, 0, -1) including four variables, were carried out. The levels of each factor along with their codes and values of two experimental designs are listed in Table 1. A multiple regression analysis of the data was carried out by STATISTICA 7 of STATSOFT Inc. (Thulsa, U.S.) by surface response methodology. After running the CCD experiments, a second-order polynomial regression equation was fitted to the data (Eq. 6).

$$Y = \beta_0 + \sum_{i=1} \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum_{i=1} \sum_{j=i+1} \beta_{ij} X_i X_j \qquad (6)$$

where, Y is predicted response of the dependent variable, X_i and X_j are independent variables influencing the response of Y, β_0 is constant of the second order equation, β_i is linear regression coefficient of each independent variable, β_{ii} is quadratic regression coefficient of each independent variable, and β_{ij} is regression coefficient of interactions between two independent variables. The significance of the second-order model as shown in Eq. (6) was evaluated by analysis of variance (ANOVA). The insignificant coefficient was eliminated after the F (Fisher)-test and the final model was obtained. Combination of factors (such as X_iX_j) represents an interaction between the individual factors in the respective term. The various response surface graphs presented are function of level of factors and indicate the effect of variables individually and in combination and determine their optimum level for solid and organic matter solubilization with simultaneous biodegradation and degradation of BPA from WWS. The response surface graphs presented the second-order polynomial model which showed the predicted response of two factors at a time, holding the other two factors at fixed zero level and are in fact more helpful in interpreting the main effect and the interactions.

2.6. Biodegradability test

Examination of biodegradability was carried out by inoculating the pre-treated and raw sludges with microbial consortia of 2 % (v/v) of fresh activated wastewater sludge (1.5 ml) followed by incubation at 25 ± 1 ° C at 150 rpm on a rotary shaker for 20 days. Oxygen corresponded to the oxygen entrained from the atmosphere into the shaking flasks on a rotary shaker. The biodegradability was assessed by the decrease in TS consumed by the microorganisms after incubation (20 days) (Eq. (7)). At the end of incubation, the volume loss due to evaporation was readjusted to 75 ml with Milli-Q water.

$$\text{Biodegradability (\%)} = \left(1 - \frac{\text{TS concentration after biodegradation}}{\text{TS concentration before biodegradation}}\right) \times 100 \qquad (7)$$

WWS pre-treatments rupture suspended solids (microbial cells), liberate the nutrients, partially solubilize the suspended solids, increase the soluble chemical oxygen demand, decrease viscosity and improve the overall WWS biodegradability [15, 16]. Hence, in this research biodegradability was chosen as a parameter to assess the effectiveness of ferro-sonication pre-treatment process for value added products and simultaneous degradation of BPA.

3. RESULTS AND DISCUSSION

3.1. Screening experiments

The experimental results of screening experiments of ferro-sonication pre-treatment for studying the effect of solids concentration (X_1), pH (X_2), $FeSO_4.7H_2O$ concentration (X_3), and ultrasonication time (X_4) on biodegradation and degradation of BPA are presented in Table 2. From the data presented, the higher response of BPA degradation and biodegradability was observed in trial no. 24 at ultrasonication time of 180 min, $FeSO_4.7H_2O$ concentration of 2.43 mg/L at pH 3.0 and solids concentration 25 g/L. SS, VS and organic matter (SCOD and SOC) solubilization observed during trial no. 24 was 43.79%, 56.01%, 61.92% and 15.71%, respectively. BPA degradation and biodegradation observed during trial no. 24 was 82.99% and 32.48%, respectively. Higher degradation of BPA and increased biodegradation observed in trial no. 24 was due to higher SS, VS, SCOD and SOC solubilization. With the improvement of sludge solubilization during ferro-sonication pre-treatment process, organic matter will be transferred from solid to aqueous phase, which resulted in the increase in soluble protein and carbohydrate concentration in aqueous phase and decrease of SS, VS, SCOD and SOC in solid phase. Our previous study has demonstrated that increase in SS, VS, SCOD and SOC solubilization during hydrolysis and oxidative pre-treatment of WWS resulted in higher degradation of BPA [10].

The higher SS, VS, SCOD and SOC solubilization observed in trial no. 24 as compared to other trials was due to higher ultrasonication time leading to higher ultrasonication supplied energy. Higher energy input caused more acoustic cavitation in the medium, which then caused sludge floc disintegration and cell breakage leading to release of intracellular materials to aqueous phase [17, 18]. Hence, at higher specific energy, solubilization started with the hydrolysis of carbohydrate followed by aminoacids and proteins and finally, fats and lipids and the subsequent phase where most of the organic matter was solubilized [18]. The higher ultrasonication time during trial no. 24 also leads to higher temperature which induced transformation of solid-state bound organic compounds into a soluble state.

The other reason for higher degradation of BPA observed in trial no. 24 might be due to higher production of $OH^{\cdot}$ radicals with 180 min ultrasonication time. A comparative degradation of BPA

(80.04%) was also observed in trial no. 22 with ultrasonication time of 120 min. In both trial no. 22 and 24, the pH and solids concentration of sludge was same with 3.4 mg/L and 2.43 mg/L $FeSO_4.7H_2O$ concentration, respectively. The significant degradation of BPA in trial no. 22 with 120 min ultrasonication time compared to 180 min in trial no. 24 was due to the addition of higher concentration of $FeSO_4$, which enhanced the formation of $OH^•$ radicals in the solution (Equation 1) [19]. Taking into account the specific supplied energy (E), trial no. 22 was chosen as the best condition among the trials tested as it resulted in 39.62%, 52.03%, 56.01% and 14.78% SS solubilization, VS solubilization, SCOD and SOC increment, leading to 80.04% and 32.01% BPA degradation and biodegradation, respectively. Trial 22 was conducted at following conditions: 120 min ultrasonication time, 3.4 mg/L $FeSO_4.7H_2O$, pH 3 and 25 g/L of SS.

3.2. Optimization studies

3.2.1. Effect of variables on BPA degradation

Using the method of experimental factorial design and response surface analysis, the optimal conditions to obtain higher degradation of BPA during ferro-sonication pre-treatment of WWS was determined. The validity of the model was proved by fitting different values of the variables into the model equation and carrying out the experiment at these values of the variables. The data were fitted into a second-order polynomial function (Equation 6). The statistical significance of the second-order polynomial model was verified by ANOVA. The quality of the model fit was evaluated by the coefficient R^2 which represents the proportion of variation in the response data and it can be explained by the fitted model. High R^2 was considered as an evidence for the applicability of the model in the range of variables included. It should be noted that a R^2 value greater than 0.75 indicates the aptness of the model. The analysis indicated that the second-order polynomial model resulted in a determination coefficient R^2 higher than 0.75, which ensured a satisfactory adjustment of the quadratic model to the experimental data.

The evaluation of statistical significance of four factors, such as solid concentration (X_1), pH (X_2), $FeSO_4.7H_2O$ concentration (X_3) and ultrasonication time (X_4) and their interaction was based on probability (p) values. The regression model in terms of second-order polynomial model was fitted into the experimental data of BPA degradation. Analysis of variance (ANOVA) including the ratio of the level mean square (MS) and the residual MS followed a Fisher (F) distribution with degrees of freedom (df) are shown in Table 3. Except for linear effect of pH (X_2), all the main effects have MS higher than the residual MS which showed that most of the variation in the data of degradation of BPA is accounted by the separate effect of independent variables. The statistical significance of individual and interaction effects were also determined based on comparison of F-value (F) with critical F-value (Fc) at 95%, 99% and 99.9% confidence. Taking into account the effect of variables on BPA degradation from WWS, solids concentration (X_1) showed significant linear effect (F > Fc for probability (p) < 0.05) and highly significant quadratic effects (F > Fc for probability (p) < 0.01); pH (X_2) showed no linear effect (p > 0.05), but showed highly significant quadratic effect (F > Fc for p < 0.01); $FeSO_4.7H_2O$ concentration (X_3) has highly significant linear effect (F > Fc for p < 0.01) and significant quadratic effect (F > Fc for p < 0.05); and ultrasonication time (X_4) had very highly significant linear and quadratic effect (F > Fc for p < 0.001). In between combination of parameters, only $FeSO_4.7H_2O$ concentration and ultrasonication time (X_3X_4) showed highly significant effects (F > Fc for p < 0.01) on degradation of BPA from WWS.

The second-order polynomial regression Equation (Eq. (6)) fitted well into the experimental data to give the following models (Eqs. (9), (10) and (11)) including regression coefficients with coded terms of operational parameters for organic matter (COD and SOC) solubilization and BPA degradation. However, the regression coefficients which were found insignificant (p > 0.05) were excluded from the model.

$$Y_{SCOD} = -26.71 - 0.96\,X_1 - 0.06\,X_1^2 - 4.30\,X_2^2 + 0.8\,X_4 - 0.003\,X_4^2 \qquad (9)$$

$$Y_{SOC} = -6.30 - 0.99\,X_2^2 + 0.35\,X_4 - 0.009\,X_4^2 \qquad (10)$$

$$Y_{BPA} = -568.6 - 7.75\,X_1 - 0.17\,X_1^2 - 23.56\,X_2^2 - 78.37\,X_3 - 15.1\,X_3^2 + 3.3\,X_4 - 0.008X_4^2 - 0.01\,X_3X_4 \qquad (11)$$

Hence, among all the four variables, ultrasonication time had very high significant effect on degradation of BPA from WWS followed by $FeSO_4.7H_2O$ concentration, sludge solids concentration and pH. This could be explained by the fact that, higher ultrasonication time leads to: i) increase of temperature within the medium resulting in thermal hydrolysis; ii) higher solubilization of SS, VS and organic matters (SCOD and SOC); iii) higher formation of H_2O_2 due to bubble collapse and mixing effect resulting in higher production of $OH^{\bullet}$ in the medium. As the ultrasonication time increased, organic matter (SCOD and SOC) solubilization and the number of $OH^{\bullet}$ radicals in the medium increased leading to higher oxidation of BPA [20, 21, 22]. Similarly, Yan et al. [23] had observed that COD, organic matter and nitrogen solubilization increased with increasing ultrasonication time. The transformation of solid-state bound organic compounds into a soluble form could be induced continuously by the elevated bulk temperature during sonication. Higher ultrasonication time caused more acoustic cavitation in the medium, which then caused sludge floc disintegration and cell breakage leading to release of intracellular materials to aqueous phase. The cavitation threshold depends on the medium temperature, pressure, and ultrasonication time, among others [23].

Addition of $FeSO_4.7H_2O$ during ferro-sonication pre-treatment of WWS enhanced the formation of $OH^{\bullet}$ radicals in the solution. Hence, $FeSO_4.7H_2O$ concentration had significant effect on degradation of BPA due to enhancement of higher production of $OH^{\bullet}$ radicals during ultrasonication process. The hydroxyl radicals generated during ferro-sonication pre-treatment processes have an oxidizing potential of 2.8 V as compared to normal hydrogen electrode (NHE) and are capable of oxidizing a wide range of organic compounds including BPA in WW and WWS [24]. Ultrasonication is considered as a suitable method to improve the Fenton degradation of BPA [19, 25,26].

The response surface graph of SCOD and SOC increment affected by the ultrasonication time and $FeSO_4.7H_2O$ concentration at constant pH and solids concentration (Fig. 1a, b) showed that highest solubilization (61.92% and 15.71%, respectively) occurred at 180 min ultrasonication time and 2.43 mg/L $FeSO_4.7H_2O$. BPA degradation from WWS assessed as a function of different ferro-sonication pre-treatment operational parameters is presented in Fig. 2. The response surface graph of BPA degradation affected by the ultrasonication time and $FeSO_4.7H_2O$ concentration at constant pH and solids concentration (Fig. 2a) showed that highest degradation (82.99%) occurred at 180 min ultrasonication time and 2.43 mg/L $FeSO_4.7H_2O$. The results suggested that there was a correlation between organic matter solubilization and BPA degradation. Moreover, SCOD and SOC were mainly composed of soluble proteins and carbohydrates. Hence, at higher specific energy, solubilization started with the hydrolysis of carbohydrate followed by aminoacids and proteins and finally, fats and lipids and the subsequent phase where most of the organic matter was solubilized. Therefore, higher degradation of BPA from WWS during higher ultrasonication time and $FeSO_4.7H_2O$ concentration was due to higher production of $OH^{\bullet}$ radicals and solubilization of sludge organic matter (COD and SOC).

Other factors that showed significant effect on BPA degradation from WWS were solids concentration and quadratic effect of pH. Fig. 2b shows the response for the interactive factors, solids concentration (X_1) and ultrasonication time (X_4), when pH and $FeSO_4.7H_2O$ concentration were fixed at central point. Higher BPA degradation (80%) was obtained in the range of 20-25 g/L SS and 120-180 ultrasonication time. The same response could be predicted in the range of 120-180 min ultrasonication time and 2.5-3.5 pH at constant solids and $FeSO_4.7H_2O$ concentration (Fig. 2c). This can be explained by the fact that, due to high octanol–water partition coefficient (log K_{ow} = 2.3–3.82) and hydrophobic nature [27], BPA has the tendency to strongly associate with the solids. The initial BPA concentration was observed in wastewater sludge with five solids (X1) concentrations such as 15, 20, 25, 30 and 35 g/L. Trials were carried out with different parameters (X1, X2, X3 and X4) and the final BPA concentration was observed to determine the % BPA degradation. Higher concentration of BPA was observed with increasing solids concentration [10]. Increased ultrasonication time leads to increase in hydro-mechanical shear force within the medium resulting in dissociation of solids and organic matter so that the adsorbed BPA was released to the aqueous phase. As the size of the flocs decreased with increasing ultrasonication time, overall availability of sorbed BPA molecules to react with $OH^{\bullet}$ radicals increased resulting in higher degradation of BPA.

Fig. 2d shows the response for the interactive factors, $FeSO_4.7H_2O$ concentration (X_4) and pH (X_2), when ultrasonication time and solids concentration was fixed at central point. This suggested that, pH and $FeSO_4.7H_2O$ concentration lower than 2.2 and 1.6 mg/L respectively, produced less effect on degradation of BPA from WWS. Higher degradation of BPA (> 60%) was obtained at pH 2.6-3.8 and 2.0-3.2 mg/L $FeSO_4.7H_2O$ concentration. This can be explained by the fact that $FeSO_4.7H_2O$ concentration lower than 2 mg/L was not sufficient to release large amount of iron ions to react with H_2O_2 produced during ultrasonication and form reactive $OH^{\bullet}$ radicals that lead to BPA degradation. Ioan et al. [19] observed more than 80% of degradation of BPA at 2.5 mg/L $FeSO_4.7H_2O$ concentration and pH 4. However, when the pH increased to the range of 4.5 to 6.5 and $FeSO_4.7H_2O$ concentration decreased to 1.4 mg/L, BPA degradation decreased below 30%. Hence, a relative $FeSO_4.7H_2O$ concentration reduces the efficiency of the process by regulating the production of $OH^{\bullet}$ radicals (Eq. 1) that leads to BPA degradation.

Therefore, ferro-sonication pre-treatment process with chosen parameters can be considered an oxidation process that played a vital role in enhancement of solubilization of sludge solids and organic matter and simultaneous degradation of BPA from WWS. From the model based on response surface methodology, the optimal conditions of ferro-sonication pre-treatment of secondary sludge at 15 W/cm^2 ultrasonication intensity were identified as follows: 163 min ultrasonication time, 2.71 mg/L $FeSO_4.7H_2O$ concentration, pH 2.81 with 22 g/L SS, to obtain a maximum 88% of BPA degradation from WWS.

3.2.2. Effect of variables on biodegradation

WWS solids (SS and VS) and organic matter (COD and SOC) solubilization has been adopted as a measure of ferro-sonication pre-treatment efficiency to improve biodegradability. As shown in Table 3, solid concentration (X_1) and ultrasonic time (X_4) showed very highly significant linear effect ($p < 0.001$); $FeSO_4.7H_2O$ concentration (X_3) showed significant linear effect and other factors showed no effect on biodegradation. Combination of parameters of ferro-sonication pre-treatment process did not show any interaction effect on biodegradability. The regression coefficients were than fitted in Eq. (6) to give the following model (Eq. (12)).

$$Y_{\mathrm{Biodegradability}} = -1.32\,X_1 - 23.63\,X_3 - 0.18\,X_4 \qquad (12)$$

The above analysis of significance of the factor on response of biodegradability showed that ultrasonication time exhibited major effect on improving the biodegradability followed by solids concentration and $FeSO_4.7H_2O$ concentration. This can be explained by the fact that, increase in ultrasonication time leads to increase in power input resulting in higher disintegration of sludge solids and organic matter. The disintegration degree of sonicated sludge increased significantly with increasing specific energy [17, 28]. In fact, under powerful hydro-mechanical shear forces generated from sudden and violent collapse of microbubbles, extracellular polymeric substances (EPS) considered as matrix that is embedded in cell sludge was degraded [29]. Consequently, the organic matter contained in EPS and cells was solubilized, coming under attack of collapsing cavitation bubbles and increasing the tendency to augment biodegradability.

The response surface plots in Fig. 3 presented the predicted biodegradability by using the four combinations of ferro-sonication pre-treatment process. As shown in Fig. 3a, 30% of biodegradability was achieved with ultrasonic time more than 120 min and 50% can be achieved by increasing the ultrasonication time more than 200 min. While pH and $FeSO_4.7H_2O$ concentration were fixed at central point, highest biodegradability of 30% was obtained in the range of 20-25 g/L SS and 120-160 min ultrasonication time (Fig. 3b). The effective impact of the lower solids concentration on biodegradation of sludge was also in agreement with the results of Verma et al. [30] who reported the optimal SS concentration as 20 g/L for the biodegradability of alkaline thermal hydrolyzed sludge to produce *Trichoderma viride* based biopesticides. At higher solids concentration, the microorganisms could be inhibited by higher amount of substrate and limited mass and oxygen transfer (oxygen inhibition effect/substrate inhibition effect).

4. CONCLUSIONS

The present research work on ferro-sonication pre-treatment of secondary sludge for degradation of bisphenol A from wastewater sludge and enhancement of biodegradability led to following conclusions:

1. The second-order response surface model was adequate to predict the bisphenol A degradation and biodegradability enhancement from wastewater sludge within four independent variables namely, solids concentration, pH, $FeSO_4.7H_2O$ concentration and ultrasonication time.
2. All the four independent variables showed significant effect on degradation of bisphenol A from wastewater sludge. Ultrasonication time plays an important role for increase in sludge solids (SS and VS) and organic matter (COD and SOC) solubilization and $OH^{\bullet}$ radicals production in the medium for degradation of bisphenol A.
3. The optimal conditions developed by the response surface model in order to obtain maximum 88% of bisphenol A degradation from wastewater sludge by ferro-sonication pre-treatment were as follows: 163 min ultrasonication time, 2.71 mg/L $FeSO_4.7H_2O$ concentration, pH 2.81 at 22 g/L SS.
4. Ultrasonication time and solids concentration showed significant effect on biodegradability enhancement of wastewater sludge. Higher biodegradability (32.48%) was observed at 180 min and 25 g/L ultrasonication time and solid concentration, respectively.

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Table 1. Codes and values of experimental range of four variables for screening using response surface methodology

Variables	Symbol	Coded level				
		-2	-1	0	+1	+2
Suspended solid (g/L)	X_1	15	20	25	30	35
pH	X_2	2.0	2.5	3.0	3.5	4.0
$FeSO_4.7H_2O$ (mg/L)	X_3	1.46	1.95	2.43	2.92	3.41
Ultrasonication time (min)	X_4	60	90	120	150	180

Table 2. Results of experimental plan by central composite design for changes in analytical parameters and simultaneous change in BPA degradation and biodegradability

Trial	X_1	X_2	X_3	X_4	SSs (%)	VSs (%)	SCOD increment (%)	SOC increment (%)	BPA degradation (%)	Biodegradability (%)
1	20	2.5	1.95	90	14.37	26.89	32.56	8.0	24.59	8.01
2	20	2.5	1.95	150	33.10	51.10	54.11	14.19	71.36	14.93
3	20	2.5	2.92	90	14.89	26.89	32.01	8.4	33.4	11.18
4	20	2.5	2.92	150	33.81	51.19	53.91	14.86	78.21	22.05
5	20	3.5	1.95	90	14.05	22.17	28.75	7.51	22.99	6.49
6	20	3.5	1.95	150	32.11	49.87	44.16	11.99	67.55	13.56
7	20	3.5	2.92	90	19.07	22.19	28.11	7.5	26.38	6.67
8	20	3.5	2.92	150	33.52	46.73	44.85	11.27	71.93	13.0
9	30	2.5	1.95	90	16.13	25.51	26.75	8.10	18.77	7.98
10	30	2.5	1.95	150	35.19	38.13	52.14	13.71	60.49	19.56
11	30	2.5	2.92	90	16.18	25.97	27.21	8.17	21.56	6.19
12	30	2.5	2.92	150	35.19	38.64	56.95	13.99	68.73	24.10
13	30	3.5	1.95	90	13.97	18.55	23.71	9.10	18.73	8.32
14	30	3.5	1.95	150	33.52	45.92	45.76	14.73	54.47	13.56
15	30	3.5	2.92	90	14.15	23.33	24.11	9.11	32.61	6.77
16	30	3.5	2.92	150	32.19	47.59	56.93	14.70	61.11	24.45
17	15	3.0	2.43	120	34.72	49.63	51.11	17.11	69.73	19.76
18	35	3.0	2.43	120	28.11	41.05	43.01	13.56	53.16	14.18
19	25	2.0	2.43	120	27.34	52.71	46.92	15.78	57.9	17.01
20	25	4.0	2.43	120	28.75	48.11	51.03	13.11	53.11	18.96
21	25	3.0	1.46	120	36.19	49.71	43.78	14.49	49.72	13.3
22	25	3.0	3.4	120	39.62	52.03	56.01	14.78	80.04	31.01
23	25	3.0	2.43	60	9.19	17.11	20.18	8.23	14.93	6.11
24	25	3.0	2.43	180	43.79	56.01	61.92	15.71	82.99	32.48
25	25	3.0	2.43	120	34.26	46.8	48.11	13.99	71.58	14.53
26	25	3.0	2.43	120	34.10	46.39	48.0	13.99	71.03	14.22
27	25	3.0	2.43	120	34.26	46.8	48.92	12.51	69.73	14.69
28	25	3.0	2.43	120	34.26	46.77	48.57	13.47	71.68	14.01
29	25	3.0	2.43	120	34.29	46.58	48.19	12.14	71.05	14.22
30	25	3.0	2.43	120	34.00	46.07	48.77	12.83	71.0	14.37
31	25	3.0	2.43	120	34.33	46.62	48.52	13.27	71.6	14.09

Table 3. ANOVA for the regression model equation and coefficients

Model	BPA degradation					Biodegradability				
	SS	df	MS	F-test	p	SS	df	MS	F-test	p
X_1	360.66	1	360.663	5.7249	0.0293*	10.631	1	10.631	5.0285	0.0008*
X_1^2	554.79	1	554.787	8.8062	0.0090*	3.874	1	3.8740	0.1752	0.6810
X_2	39.81	1	39.813	0.6320	0.4382	12.472	1	12.472	0.5640	0.4635
X_2^2	991.93	1	991.932	15.7451	0.0011*	0.374	1	0.3737	0.0169	0.8981
X_3	545.09	1	545.091	8.6523	0.0095*	136.476	1	136.47	6.1721	0.0244*
X_3^2	362.40	1	362.401	5.7525	0.0290*	24.082	1	24.082	1.0891	0.3121
X_4	9240.34	1	9240.34	146.673	0.0000*	775.794	1	775.79	35.085	0.0000*
X_4^2	1619.64	1	1619.64	25.7089	0.0001*	1.300	1	1.2995	0.0587	0.8115
X_1X_2	16.16	1	16.160	0.2565	0.6194	8.585	1	8.5849	0.3882	0.5419
X_1X_3	4.14	1	4.143	0.0658	0.8008	0.333	1	0.3330	0.0150	0.9038
X_1X_4	50.98	1	50.980	0.8092	0.3816	28.143	1	28.143	1.2727	0.2758
X_2X_3	0.16	1	0.158	0.0025	0.9606	1.092	1	1.0918	0.0493	0.8269
X_2X_4	42.64	1	42.641	0.6768	0.4227	7.508	1	7.5076	0.3395	0.5682
X_3X_4	568.12	1	568.405	5.9640	0.0093*	29.988	1	29.987	1.3562	0.2612
Residual	1007.99	16	62.999	---	---	353.785	16	22.111	---	---

*significant values ($p < 0.05$); SS: Sum of Square; df: degrees of freedom; MS: Mean Square; p: probability

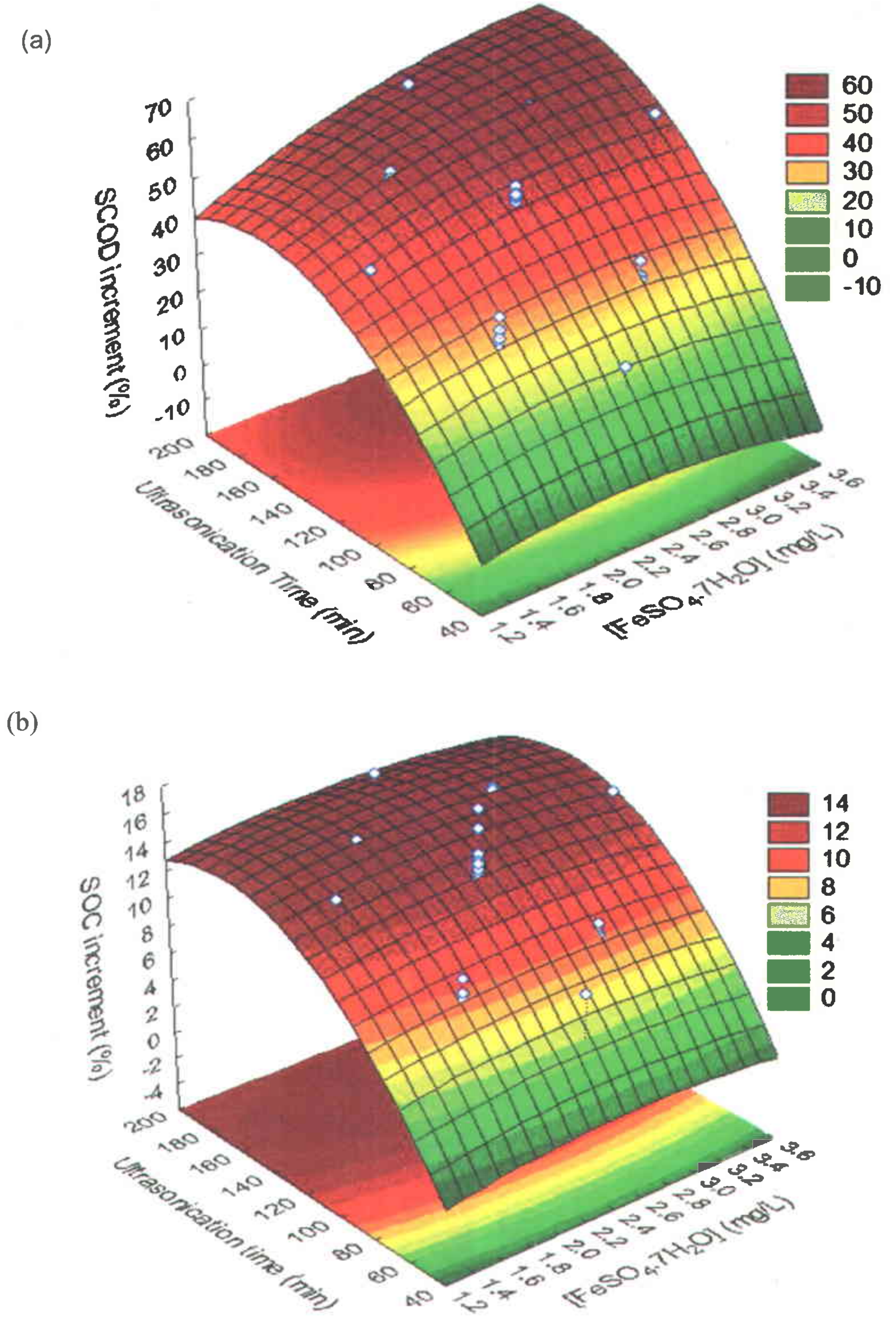


Figure 1. Response surface plot of organic matter solubilization as a function of ultrasonication time and ferrous sulphate concentration: a) COD solubilization; and b) SOC solubilization

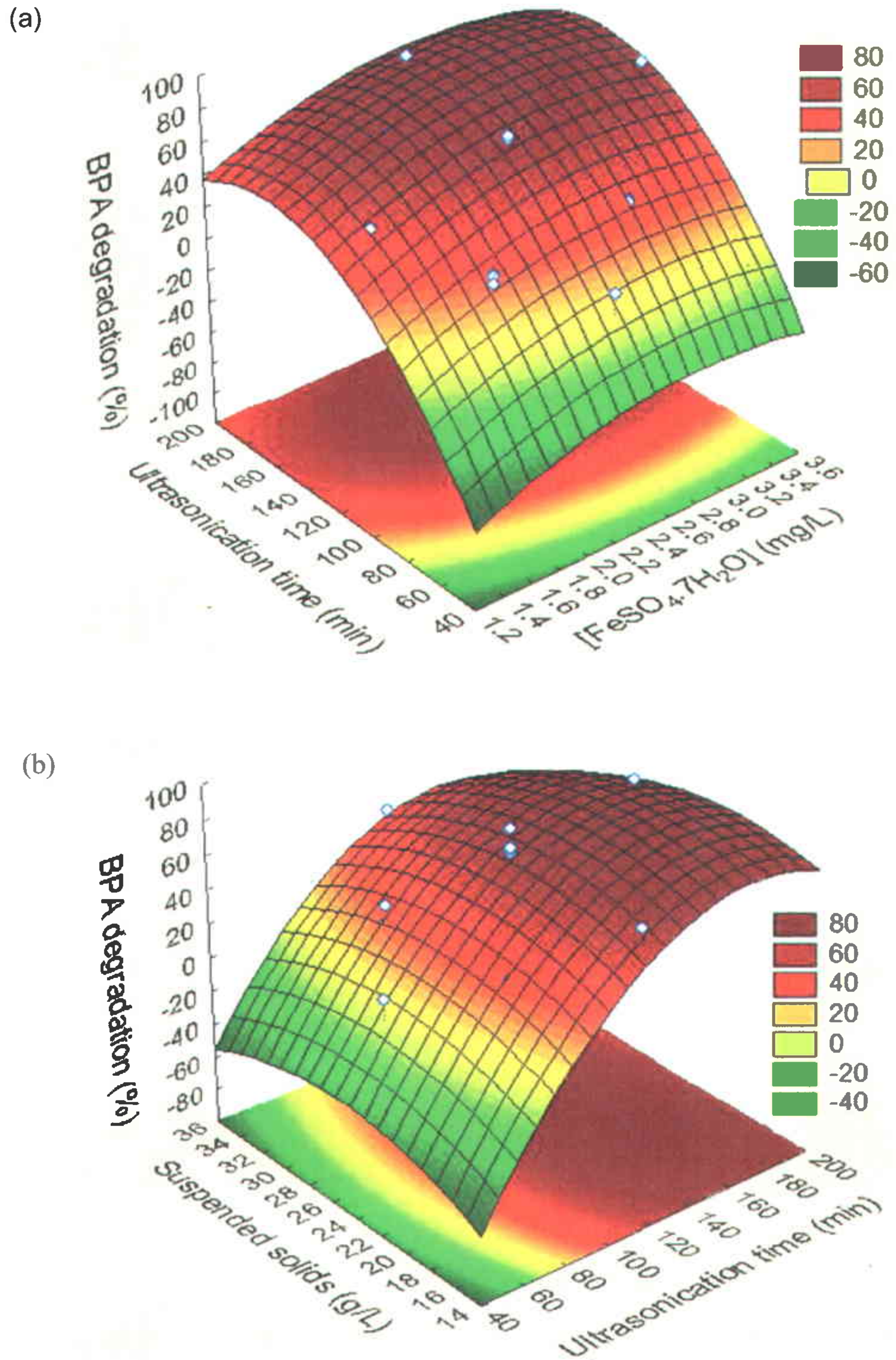
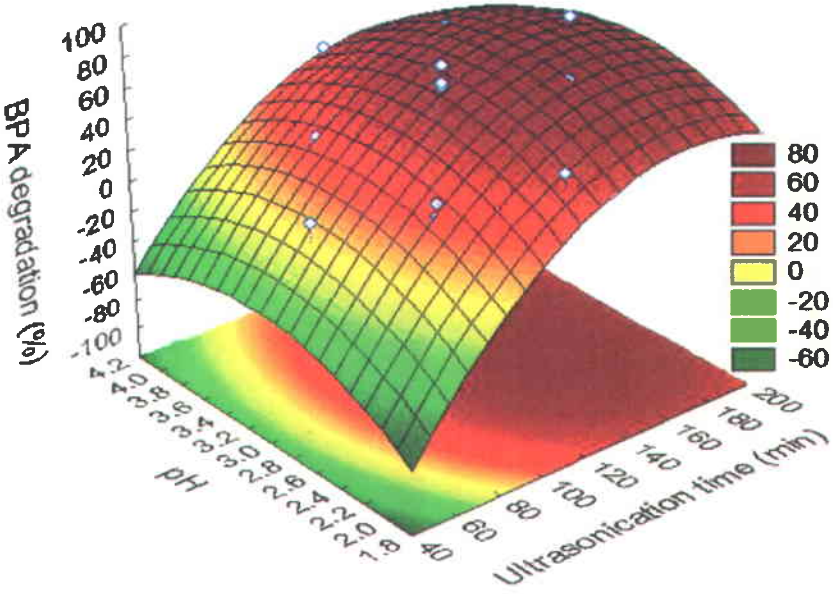


Figure 2. Response surface plots of BPA degradation as a function of: (a) ultrasonication time and FeSO4 concentration; (b) ultrasonication time and solids concentration

(c)



(d)

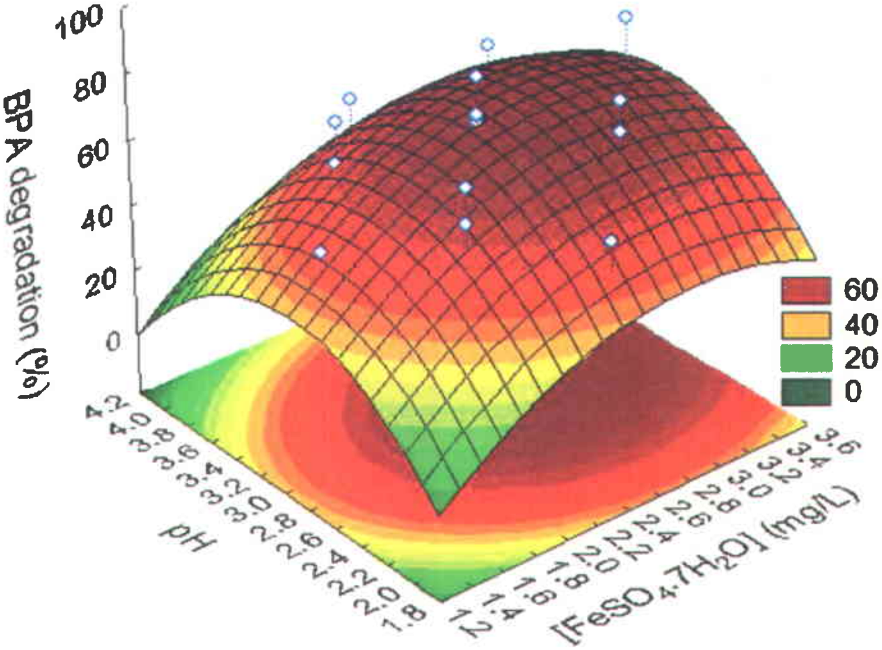


Figure 3. Response surface plots of BPA degradation as a function of: (c) ultrasonication time and pH (FeSO4 concentration and SS constant); (d) FeSO4 concentration and pH (ultrasonication time and SS constant)

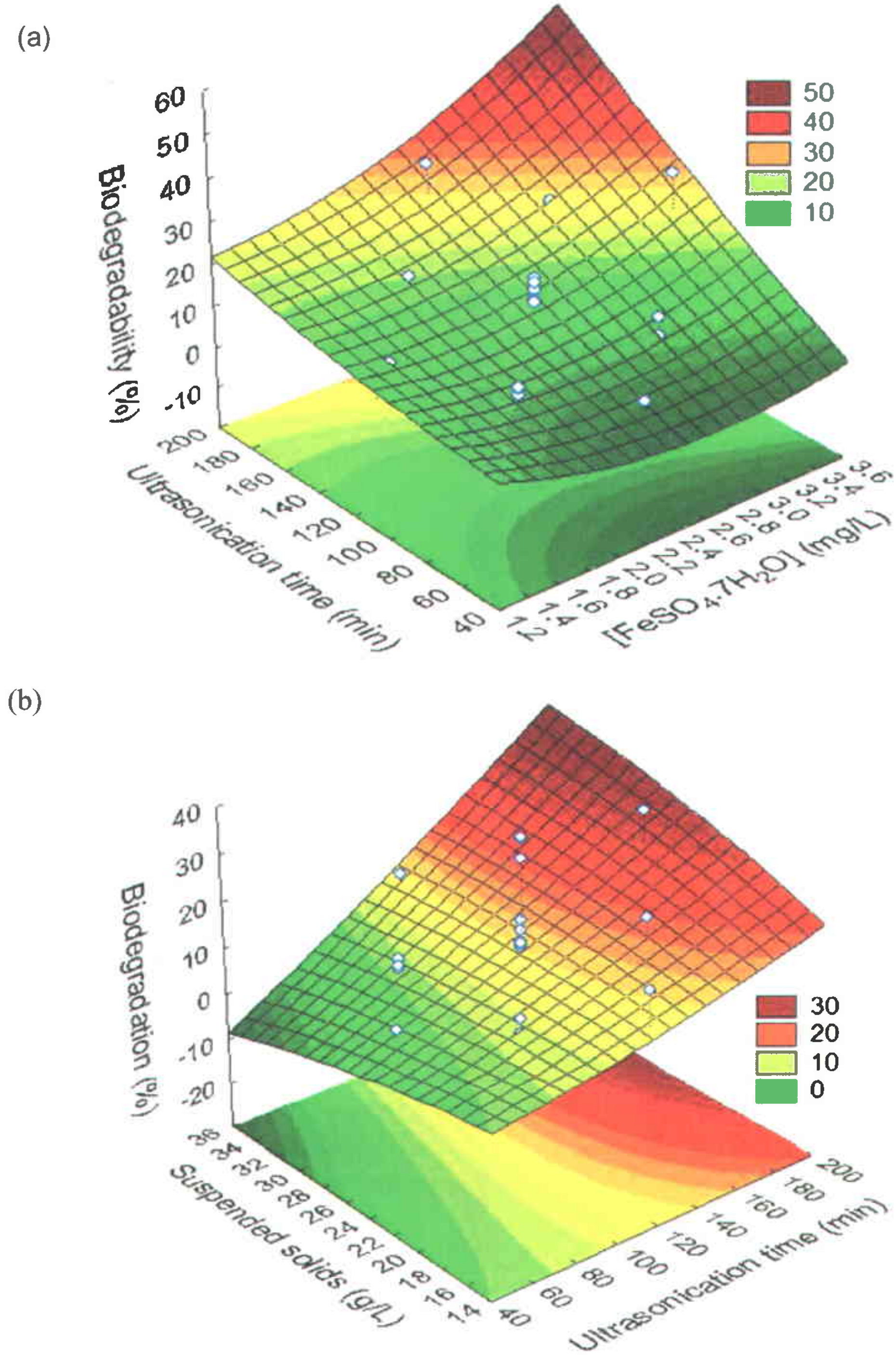


Figure 4. Response surface plots of biodegradability as a function of: (a) ultrasonication time and FeSO4 concentration (pH and SS constant); (b) ultrasonication time and solids concentration (pH and FeSO4 concentration constant)

PARTIE 2

PARTIAL OZONATION PRE-TREATMENT FOR SLUDGE SOLUBILIZATION AND SIMULTANEOUS DEGRADATION OF BISPHENOL A: QUANTIFICATION STUDIES

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RÉSUMÉ

L'ozonation a été étudiée comme un prétraitement pour l'amélioration de la solubilisation des solides de la boue, de la matière organique et la dégradation simultanée de bisphénol A (BPA), un composé perturbateur endocrinien de boues d'épuration (WWS). La méthode ultra-rapide utilisés pour l'analyse de BPA dans les WWS est basée sur la diode laser désorption thermique/ionisation chimique à pression atmosphérique (LDTD/APCI) couplée à la spectrométrie de masse en tandem (MS/MS). Les méthodes statistiques utilisées pour i'optimisation des tratrement comprend la surface methodologie de réponse avec plans factoriels fractionnaires (FFD) et plans composites centrés (CCD). L'ozonation a été réalisée avec quatre variables indépendantes, soit les concentrations des boues d'épuration en solides (15-35 g L^{-1}), le pH (5-7), la dose d'ozone (5-25 mg/g SS) et le temps d'ozonation (10-30 min). Il a été observé que, parmi toutes les variables étudiées, la dose d'ozone avait plus significative (la probabilité (p) <0,001) affecté l'efficacité de l'ozonation de pré-traitement par boues solides croissantes (matières en suspension (SS) et de solides volatils (VS)) solubilisation et la matière organique (soluble demande chimique en oxygène (SCOD) et du carbone organique (SOC)) et de la dégradation augmentation du BPA de WWS. Pendant le processus d'optimisation, il a été constaté qu'une dégradation de 100% du BPA pourrait être obtenue avec 24 g/L SS, 6,23 pH avec dose d'ozone de 26,14 mg/g SS pour 16,47 moment ozonation min. La plus forte dose d'ozone utilisée dans cette étude a été observée pour être rentable sur la base de solides et de solubilisation de la matière organique et de la dégradation du BPA.

Mots-clés: Bisphénol A; Ozonation; Solubilisation; La méthodologie de surface de réponse; Boues d'épuration

ABSTRACT

Ozonation pre-treatment was investigated for enhancement of sludge solids and organic matter solubilization and simultaneous degradation of bisphenol A (BPA), an endocrine disruptor compound from wastewater sludge (WWS). The ultrafast method used for analysis of BPA in WWS is based on the Laser Diode Thermal Desorption/Atmospheric Pressure Chemical Ionization (LDTD/APCI) coupled to tandem Mass Spectrometry (MS/MS). The statistical methods used for optimization studies comprised, response surface method with fractional factorial designs (FFD) and central composite designs (CCD). The ozonation pre-treatment process was carried out with four independent variables, such as wastewater sludge solids concentration (15-35 g/L), pH (5-7), ozone dose (5-25 mg/g SS) and ozonation time (10-30 min). It was observed that among all the variables studied, ozone dose had more significantly (probability (p) < 0.001) affected the efficiency of the ozonation pre-treatment by increasing sludge solids (suspended solids (SS) and volatile solids (VS)) solubilization and organic matter (soluble chemical oxygen demand (SCOD) and organic carbon (SOC)) increment and BPA degradation from WWS. During the optimization process, it was found that higher BPA degradation (100%) could be obtained with 24 g/L SS, 6.23 pH with ozone dose of 26.14 mg/g SS for 16.47 min ozonation time. The higher ozone dose used in this study was observed to be cost effective on the basis of solids and organic matter solubilization and degradation of BPA.

Keywords: Bisphenol A; Ozonation; Solubilization; Surface response methodology; Wastewater sludge

1. INTRODUCTION

Endocrine disrupting compounds (EDCs) have drawn much attention for their adverse health and ecological effects. An endocrine disrupter is defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development and/or behavior [1]. Bisphenol A (BPA) has been identified as EDC by the U.S Environmental Protection Agency (EPA), World Wide Fund for Nature (WWF) and is declared as a social, environmental and global issue [2]. The report submitted by Canadian Health Measures Survey (CHMS) showed that Canadians aged 6 to 79 had a geometric mean concentration of urinary BPA of 1.16 µg/L. The report also showed that BPA was found in 91% of Canadians and higher concentrations were measured in teens aged 12 to 19 [3].

BPA is a high-volume production chemical, with over 2 million metric tons global production in 2003. From 2003 to 2006, the global BPA consumption increased at an average annual rate of about 10% and the demand for BPA is expected to grow at annual rate of 7-8% during 2006-2011 [4]. BPA is used primarily in the production of epoxy resins and polycarbonate products. Polycarbonates have many applications in the manufacturing of different consumer products such as, thermal paper, paper coatings, different electrical and electronics parts, food and drink packaging, medical devices, among others [5]. Due to the daily use of these products, higher concentration of BPA is observed in wastewater (WW) and finally in wastewater sludge (WWS) [6].

Many treatments, such as digestion, dewatering, incineration, landfilling and use in agriculture have been carried out for the disposal of excess sludge. Meantime, disposal routes are subject to more legal and social constraints, such as land disposal is now restricted in some countries, incineration is quite expensive, and land application (agricultural use) is highly debated. Further, presence of various organic matter including BPA among others in sludge and high cost of these treatments makes the final disposal of sludge a challenge. Therefore, interest for solutions allowing sludge volume and mass reduction and simultaneous degradation of organic compounds has a great demand [7]. Mechanical pre-treatment was shown to be very effective in solubilizing microbial cells but turned out to be rather complicated and expensive [8]. Chemical and thermochemical pre-treatments have been shown to be efficient for enhancing sludge digestion but not efficient for degradation of organic compound including BPA [9, 10]. Oxidative treatments, such as ultrasonication, Fenton's oxidation, ferro-sonication and ozonation have been applied to disintegrate sludge cells and simultaneous degradation of organic compound including BPA [11, 12]. Among these processes, ozonation is of special interest because it has high oxidation potential, no oxidant residues remain and no increase in salt concentration occurs [13].

During ozonation, the degradation of the target compounds can be initiated by the direct reaction with hydroxyl radical ($^{\bullet}OH$) which is generated with chain reactions including initiations, propagation and termination steps [14].

$$O_3 + OH^- \rightarrow O_2^{\bullet -} + HO_2^{\bullet} \quad (1)$$

$$O_3 + O_2^{\bullet -} \rightarrow O_3^{\bullet -} + O_2 \quad (2)$$

$$HO_3 \rightarrow HO^{\bullet} + O_2 \quad (3)$$

$$O_3 + HO^{\bullet} \rightarrow HO_4^{\bullet} \quad (4)$$

$$HO_4^{\bullet} \rightarrow HO_2^{\bullet} + O_2 \quad (5)$$

WWS pre-treatment by ozonation causes the hydrolysis and partial oxidation of the organic matter. Therefore, partial ozonation is adopted in many treatment plants to avoid a complete oxidation. However, majority of research on ozonation for degradation of BPA has been reported in aqueous solutions. Further, little or no emphasis has been given to the effect of operational parameters, such as pH, solids concentration, ozonation time and ozone dose on the rate of degradation of BPA. However, process and operational parameters are critical variables in establishing the effectiveness and efficiency of any treatment technique.

Therefore, the objective of the present work is to study the optimization of ozonation pre-treatment process to improve the solubilization and simultaneous degradation of BPA from WWS using response surface methodology.

2. MATERIALS AND METHODS

2.1. Chemicals

BPA (98% purity assay) was obtained from Sigma-Aldrich (Ontario, Canada). HPLC-grade methanol (MeOH), dichloromethane (DCM), acetone and chloroform, used for cleaning and extraction purposes, were purchased from Fisher Scientific (Ontario, Canada). Sep-Pak Plus C18 environmental cartridges used for solid phase extraction (SPE) clean-up was purchased from Waters (Milford, MA, USA). Acetic acid, hydrogen peroxide, sodium hydroxide and sulfuric acid were supplied by Fisher scientific (Ontario, Canada) and were of analytical grade. HPLC grade water was prepared in the laboratory using a Milli-Q/Milli-RO Millipore system (Milford, MA, USA).

2.2. Wastewater sludge

Secondary sludge used in the study was collected from Quebec Urban Community (CUQ) wastewater treatment plant (Beauport, Quebec city, Quebec, Canada). Characteristics of secondary sludge used in the experiment is represented in Table 1. Samples were collected in pre-cleaned amber glass bottles with aluminum foil-lined caps and stored under dark conditions at 4±1 ^{0}C. The sludge suspended solids (SS) were concentrated from 1.5% (w/v) to higher SS concentrations by gravity settling and centrifugation of the settled sludge at 1600 × *g* for 3 min in a Sorvall RC 5C plus Macrocentrifuge (rotor SA-600). The supernatant was discarded in order to obtain 15, 20, 25, 30 and 35 g/L SS.

2.3. Ozonation pre-treatment of wastewater sludge

Ozonation pre-treatment was carried out by using an air tank, ozone gas generator (OZOMAX Ltd., Quebec, Canada), flow meter and a reaction vessel. Experimental set up for ozonation pre-treatment process is shown in Fig. 1. Four hundred milliliters of WWS was taken in the vessel and the pH was adjusted from 5 to 7 by addition of either 4N NaOH or 4N H_2SO_4. Production of ozone was controlled by adjusting the compressed air flow and by timer fitted with the ozonator. The air flow rate was adjustable by a valve. The experiments were carried out at different batch runs consisted of several ozone doses between 5 to 25 mg O_3 g^{-1} SS. The experiments were conducted at room temperature.

2.4. Analytical methods

Analysis of pH, total solids (TS), suspended solids (SS), volatile solids (VS), soluble chemical oxygen demand (SCOD), soluble organic carbon (SOC) was carried out as per the Standard Methods [15].

2.5. BPA analysis

2.5.1. Extraction and clean up

Sludge was frozen using liquid nitrogen prior to lyophilization by the freeze-dry system (Dura Freeze Dryer, Kinetics). About 0.5 grams of lyophilized and homogenized sample was transferred to a glass tube and 20 mL of dichloromethane-methanol (7:3, v/v) was added to the tube. The extraction was carried out by microwave assisted extraction method (MARS microwave extractor, CEM Corporation, North Carolina, USA). This system allowed simultaneous irradiation of 14 extraction vessels. Microwave power was 1200W (100%) and the extraction was performed in a temperature-controlled mode. The extraction temperature was 110±1 ^{0}C and programmed as follows: ramp to 110±1 ^{0}C for 10 min, holding at 110±1 ^{0}C for 10 min. The extract was separated by centrifugation at 7650 × g for 15 min and the procedure was repeated three times. The extracts were combined, concentrated to an approximate volume of 1 mL with gentle stream of nitrogen and redissolved in 100 mL of HPLC grade water.

Solid phase extraction (SPE) method was used for clean-up and pre-concentration of extract. Sep-Pak Plus C18 environmental cartridges were fitted into the vacuum manifold (Welch, USA) which was connected to a vacuum pump (Welch Rietschle Thomas, USA) to dispense samples through the cartridges. Cartridges were pre-conditioned by passing 7 mL of methanol and 3 mL of HPLC water at a flow rate of 1 mL min^{-1}. Sludge extracts (100 mL) were passed at a flow rate of 5 mL min^{-1}. After pre-concentration, the sorbents were dried by using a vacuum system set at (-15) psi.

The elution was performed by adding 2 x 4 mL of methanol/dichloromethane (3:1, v/v) mixture to the cartridge at a flow rate of 1mL min^{-1} and giving it a wait time of 10 min in order to give enough duration of contact between the solvent and the adsorbed compounds. The extracts were later evaporated to dryness with a gentle stream of nitrogen and reconstituted with methanol to a final volume of 200 µL prior to Laser Diode Thermal Desorption/ Atmospheric Pressure Chemical Ionization-Mass Spectrometry/Mass Spectrometry (LDTD/APCI-MS/MS) analysis. In order to confirm whether a higher matrix burden is affecting the extraction on SPE material due to competing matrix components, sludge spiked with BPA prior to SPE and LDTD/APCI-MS/MS analysis. The experiment was carried out in triplicates. Recovery observed in all the experiments varied in between 95 to 100%.

2.5.2.LDTD/APCI-MS/MS Analysis

Quantification of BPA in WWS was achieved with the LDTD/APCI ionization source (Phytronix Technologies, Quebec, Canada) mounted on a TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Quantification of BPA was performed by the internal standard method based on peak areas and relative retention time, using BPA d_{16} as internal standard. A five point standard addition calibration curve ranging from 0.01 µg L^{-1} 20 µg

L^{-1} was used. The area ratio (analyte:internal standard) was plotted against the analyte additions to obtain the calibration curve. Calibration curve was obtained using linear regression analysis and the established concentration range gave good correlation coefficients ($r^2 > 0.999$). Samples were first spotted (2 µL) into the LazWell 96-well polypropylene plate cavities containing inserts made of proprietary stainless steel alloy and left to dry at room temperature. The designed well shape allows the sample to concentrate in the heating zone while drying. The loaded plate is then transferred to an *X-Y* movable stage of the LDTD housing unit. An infrared (IR) laser diode (980 nm, 20 W, continuous) is then focalized to impact the back of the inserts, thermally desorbing the dried sample, which is vaporized into gas phase. The analysis was carried out in negative ionization mode with following conditions: laser pattern programming (2 s ramp from 0 to 45% (laser power), held for 2 s at 45% before shutting off); carrier gas flow rate (3 L/min); deposition volume (2 µL); sheath gas pressure (0 arbitrary units); auxiliary gas pressure (0 arbitrary units); skimmer offset (6 V); capillary temperature (270 °C); collision pressure (1.5 mTorr) and scan time (0.25 s).

2.5.3.Response surface methodology

The statistical optimization method can evaluate the effective factors and help in building models to study interaction and select optimum conditions of variables for a desirable response. Various stages of the optimization scheme are presented in Fig. 2. In the response surface method, the factors such as SS concentration (X_1), pH of sludge (X_2), ozone dose (X_3) and ozonation time (X_4) were considered as independent variables, and SCOD, SOC increment, and BPA degradation in pre-treated sludge as dependent variables. To begin with, the screening experiments were carried out to determine the direction of optimal domain of each process. Two-level fractional factorial design (FFD) was employed in the screening step. Once the provisional optimal values were determined, a central composite design (CCD) was used to find the optimal conditions of these four factors (X_1, X_2, X_3 and X_4). In this regard, a set of 31 experiments including, 7 center points (0, 0, 0) and 7 axial points (+2, -2) and 17 points corresponding to a matrix of 2^3 which incorporate 17 experiments (+1, 0, -1) including four variables, were carried out. The levels of each factor along with their codes and values of two experimental designs are listed in Table 2. A multiple regression analysis of the data was carried out by STATISTICA 7 of STATSOFT Inc. (Thulsa, U.S.) by surface response methodology. After running the CCD experiments, a second-order polynomial regression equation was fitted to the data (Eq. 6).

$$Y = \beta_0 + \sum_{i=1} \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum_{i=1} \sum_{j=i+1} \beta_{ij} X_i X_j \qquad (6)$$

where, Y is predicted response of the dependent variable, X_i and X_j are independent variables influencing the response of Y, β_0 is constant of the second order equation, β_i is linear regression coefficient of each independent variable, β_{ii} is quadratic regression coefficient of each independent variable, and β_{ij} is regression coefficient of interactions between two independent variables. The significance of the second-order model as shown in Eq. (7) was evaluated by analysis of variance (ANOVA). The insignificant coefficient was eliminated after the F (Fisher)-test and the final model was obtained. Combination of factors (such as X_iX_j) represents an interaction between the individual factors in the respective term. Various response surface graphs presented are a function of level of factors and indicate the effect of variables individually and in combination and determine their optimum level for solid and organic matter solubilization with simultaneous degradation of BPA from WWS. The response surface graphs presented the second-order polynomial model which showed the predicted response of two factors at a time, holding the other two factors at fixed zero level and are in fact more helpful in interpreting the main effect and the interactions.

3. RESULTS AND DISCUSSION

3.1. Screening experiments

The experimental design and results of screening experiments of ozonation pre-treatment with four variables, such as solids concentration (X_1), pH (X_2), ozone dose (X_3) and ozonation time (X_4) on degradation of BPA from WWS are presented in Table 3. Experiments also carried out in parallel by spiking a known concentration of BPA in secondary sludge. Ozonation pre-treatment experiments were carried out at pH ranging from 5 to 7 (acidic to neutral), as some studies showed that decomposition of aqueous ozone is known to occur very slowly at lower pH levels [16]. In the screening experiments, temporary optimal conditions of ozonation pre-treatment were determined at the experimental run which gave the highest solubilization of solids (SS and VS) and organic matter increment (SCOD and SOC) and simultaneous higher degradation of BPA from WWS. The higher response of BPA degradation (98.531%) was observed in trial no. 22 at ozone dose of 25 mg/g of SS, ozonation time of 20 min at pH 6 and solids concentration of 25 g/L. SS, VS solubilization and SCOD and SOC increment observed during trial no. 22 was 17.830%, 14.895%, 69.173% and 18.049%, respectively. Higher degradation of BPA observed in trial no. 22 was due to higher SS, VS, SCOD and SOC solubilization. Ozonation of sludge causes hydration of proteins, polysaccharides, lipids and other intracellular macromolecules secreted from disrupted sludge cells [17]. Muller [18] reported that the ozonation of sludge reached the highest degree of disintegration among several developing disintegration methods. With the improvement of sludge solubilization during ozonation pre-treatment process, organic matter will be transferred from solid to aqueous phase, which resulted in the increase in soluble protein and carbohydrate concentration in aqueous phase and decrease of SS, VS, COD and TOC in solid phase. Our previous study has demonstrated that increase in SS, VS solubilization and SCOD and SOC increment during hydrolysis and oxidative pre-treatment of WWS resulted in higher degradation of BPA [10, 11].

The higher SS, VS solubilization and SCOD and SOC increment observed in trial no. 24 as compared to other trials was due to higher ozone dose (25 mg/g SS). Higher ozone dose caused more disintegration of sludge cells in the medium, which then caused sludge floc disintegration and cell breakage leading to release of intracellular materials to aqueous phase. Scheminski et al. [19] reported that sludge particulates were transformed into soluble composition such as protein, lipid, and polysaccharide at an ozone dose of 0.05-0.5 gO_3/g DS and there is a phenomenon of mineralization for higher ozone dose [20]. When sludge is kept contact with ozone during ozonation pre-treatment, it reacts with solids in two different ways: direct and indirect reaction, both reactions occurring simultaneously [13]. Hence, at higher ozone dose, solubilization started with the hydrolysis of carbohydrate followed by aminoacids and proteins and finally, fats and lipids and the subsequent phase where most of the organic matter was solubilized.

The higher degradation of BPA observed in trial no. 22 may be also due to the higher production of $HO^{\bullet}$ radicals due to of higher ozone dose. A study by Lee et al. [21] showed that with increasing ozone dose, the degradation of BPA increases in aqueous medium. As BPA was found to be non-volatile in an air-sparging test, the decrease in BPA concentration in aqueous medium ascribed solely to the decomposition by molecular ozone [21]. A comparative degradation of BPA (94.17%) was also observed in trial no. 12 which was conducted at following conditions: ozone dose of 20 mg/g SS, 25 min ozonation time, pH 5.5 and 30 g/L of SS. The comparative degradation of BPA in trial no. 12 as compared to trial no.22 even at lower ozone dose was mainly attributed due to higher ozonation time. Increase in ozonation time increased the rate of production of hydroxyl radicals leading to higher degradation of BPA [16]. Taking into account the production cost of ozone (between 1.13 and 2.27 US $/kg of O_3 [13]), trial no. 12

was chosen as the best condition among the trials tested as it resulted in 22.89% SS solubilization, 19.17% VS solubilization, 65.3% SCOD increment and 17.3% SOC increment leading to 94.17% BPA degradation from WWS. However, trial no. 22 based on higher BPA degradation, was preferred to set the provisional optimum conditions among the trials tested for the optimization experiments.

3.2. Optimization studies for effect of variables on BPA degradation

Using the method of experimental factorial design and response surface analysis, the optimal conditions to obtain higher degradation of BPA during ozonation pre-treatment of WWS was determined. The validity of the model was proved by fitting different values of the variables into the model equation and carrying out the experiment at these values of the variables. The data were fitted into a second-order polynomial function (Equation 7). The statistical significance of the second-order polynomial model was verified by ANOVA. The quality of the model fit was evaluated by the coefficient R^2 which represents the proportion of variation in the response data and it can be explained by the fitted model. High R^2 was considered as an evidence for the applicability of the model in the range of variables included. It should be noted that a R^2 value greater than 0.75 indicates the aptness of the model. The analysis indicated that the second-order polynomial model resulted in a determination coefficient R^2 higher than 0.75, which ensured a satisfactory adjustment of the quadratic model to the experimental data.

The evaluation of statistical significance of four factors, such as solids concentration (X_1), pH (X_2), ozone dose (X_3) and ozonation time (X_4) and their interaction was based on probability (p) values. The regression model in terms of second-order polynomial model was fitted into the experimental data of BPA degradation. Analysis of variance (ANOVA) including the ratio of the level mean square (MS) and the residual MS followed a Fisher (F) distribution with degrees of freedom (df) are shown in Table 4. Except for linear effect of solids concentration (X_1), all the main effects have MS higher than the residual MS which showed that most of the variation in the data of degradation of BPA is accounted by the separate effect of independent variables. The statistical significance of individual and interaction effects were also determined based on comparison of F-value (F) with critical F-value (Fc) at 95%, 99% and 99.9% confidence. Taking into account the effect of variables on BPA degradation from WWS, solids concentration (X_1) showed no linear effect (probability (p) > 0.05) and significant quadratic effects (probability (p) < 0.05); pH (X_2) showed very highly significant linear effect ($p < 0.001$), but showed no quadratic effect ($p > 0.05$); ozone dose (X_3) had very highly significant linear as well as quadratic effect (p

< 0.001); and ozonation time (X_4) had no linear effect ($p > 0.05$) but significant quadratic effect ($p < 0.05$). In between combination of parameters, solids concentration and ozone dose (X_1X_3) and ozone dose and ozonation time (X_3X_4) showed very highly significant effects ($p < 0.001$) and pH and ozone dose (X_2X_3) *showed highly significant effect* ($p < 0.01$) on degradation of BPA from WWS.

The regression coefficients were then fitted in the Eq. (6) to give the following models (Eqs. (7)-(9)). The regression coefficients fitted into the Eqs. (7)-(9) are the ones that are significant based on the probability values ($p < 0.05$).

$$Y_{SCOD} = 53.99 + 13.45\,X_3 - 5.16\,X_3^2 + 2.9\,X_4 \qquad (7)$$

$$Y_{SOC} = 13.35 + 3.70\,X_3 - 1.28\,X_3^2 \qquad (8)$$

$$Y_{BPA} = 81.30 - 0.39\,X_1^2 - 4.37\,X_2 + 14.40\,X_3 - 3.36\,X_3^2 + 3.33\,X_4^2 - 0.81\,X_1X_3 + 2.84\,X_2X_3 - 1.35\,X_3X_4 \qquad (9)$$

Hence, among all the four variables, ozone dose (X_3) had very high significant effect on degradation of BPA from WWS followed by pH (X_2), ozonation time (X_4) and solids concentration (X_1). It was also observed that ozone dose had significant effect ($p < 0.05$) on solubilization of organic matter such as SCOD and SOC (Eq. (8) and (9)). The higher degradation of BPA with higher ozone dose could be explained by the facts that increase in ozone dose leads to: i) higher solubilization of SS, VS and organic matter (SCOD and SOC), ii) higher presence of molecular ozone and iii) higher production of hydroxyl radicals ($^{\bullet}$OH) in the medium. Ozone oxidizes target compounds by two different reaction pathways, direct reaction (molecular ozone) and radical reaction (OH radicals). At low pH, molecular ozone selectively attacks target compounds, whereas at high pH, ozone produces OH *radicals which is a strong and nonselective oxidizing* species [22]. Garoma and Matsumoto [14] observed that by keeping operational parameters such as initial BPA concentration, pH and bicarbonate ion concentration constant, the rate of BPA degradation linearly increased with ozone dose.

The response surface graph of SCOD and SOC increment affected by the ozone dose and ozonation time *at constant* pH *and solids concentration* are presented in Fig. 3a and b. The Fig. showed that highest SCOD and SOC solubilization (69.1% and 18.0%, respectively) occurred at ozone dose of 25 mg/g SS with ozonation time of 20 min. Fig. 4 presents the BPA degradation from WWS assessed as a function of different ozonation pre-treatment operational parameters.

The response surface graph of BPA degradation affected by the ozone dose and ozonation time at constant pH and solids concentration (Fig. 4a) showed that higher BPA degradation (98.53%) occurred at ozone dose of 25 mg/g SS with ozonation time of 20 min. The results suggested that there was a correlation between organic matter (SCOD and SOC) solubilization and BPA degradation. Moreover, SCOD and SOC are mainly composed of soluble proteins and carbohydrates. Hence, at higher ozone dose, solubilization started with the hydrolysis of carbohydrate followed by aminoacids and proteins and finally, fats and lipids and the subsequent phase where most of the organic matter was solubilized. Therefore, higher degradation of BPA from WWS during higher ozone dose was due to higher production of $OH^{\bullet}$ radicals and solubilization of sludge organic matter (COD and SOC). Martinez et al. [17] studied the effect of ozone pre-treatment on polycyclic aromatic hydrocarbons (PAH) removal from anaerobic digested sludge and observed that the removal of PAH increased with higher solubilization of organic matters.

Other factor that showed highly significant effect on BPA degradation from WWS during ozonation pre-treatment was pH (X_2). Fig. 4b shows the response for the interactive factors pH (X_2) and ozone dose (X_3) when ozonation time (X_4) and solids concentrations (X_1) were fixed at central points. Higher BPA degradation (> 80%) was obtained in the range of 5.5 to 6.5 pH and 15 to 25 mg/g SS ozone dose. At lower pH level the decomposition of molecular ozone in the medium is known to occur very slowly [16]. Thus, an increase in pH will result in an increase in hydroxyl radicals ($^{\bullet}OH$) in the medium leading to higher degradation of BPA. However, lower degradation of BPA at pH 7 could be attributed to an increase in the hydroxyl radical ($^{\bullet}OH$) scavenging by the carbonate ion which becomes a predominant species at higher pH levels [23].

The effect of ozonation time (X_4) and solids concentration (X_1) on BPA degradation from WWS was evaluated. Both the variables showed no linear effect on degradation of BPA from WWS. This can be explained by the fact that both ozonation time (X_4) and solids concentration (X_1) were effective when combined with ozone dose (X_3). From the different trials tested with this model it was observed that in some trials higher degradation of BPA was observed at lower ozonation time. This was due to the fact that at higher ozone dose even with lower ozonation time was capable of production of higher hydroxyl radicals in the medium leading to higher degradation of BPA.

Fig. 4c shows the response for the interactive factors ozone dose (X_3) and solids concentration (X_1), when ozonation time and pH were fixed at central point. Higher BPA degradation (> 80%) was obtained in the range of 20-30 g/L SS and 15-25 mg/g SS ozone dose. This can be explained by the fact that, due to high octanol–water partition coefficient (log K_{ow} = 2.3–3.82) and hydrophobic nature [24], BPA has the tendency to strongly associate with the solids. Increased ozone dose leads to increase in dissociation of solids and organic matter within the medium so that the adsorbed BPA was released to the aqueous phase. As the size of the flocs decreased with increasing ozone dose, overall availability of sorbed BPA molecules to react with hydroxyl radicals ($^{\bullet}$OH) increased resulting in higher degradation of BPA.

From the results obtained with different trials tested, ozonation pre-treatment process with chosen parameters can be considered as a partial oxidation process that played a vital role in enhancement of solubilization of sludge solids and organic matter with simultaneous degradation of BPA from WWS. From the model based on response surface methodology, the optimal conditions of ozonation pre-treatment of secondary sludge were identified as follows: 24 g/L SS, 6.23 pH with ozone dose of 26.14 mg/g SS for 16.47 min ozonation time in order to obtain 100% degradation of BPA from WWS. This is the optimum process condition for increasing solids and organic matter solubilization and simultaneous BPA degradation from WWS.

Considering the higher ozone dose used in this study (25 mg/g SS), the total expected cost for sludge ozonation (between 1.13 and 2.27 US $/kg of ozone dose [13]) was observed to be less (between 0.70 and 1.42 US $/kg of O_3) as compared to other studies. Many studies have been carried out with higher dose of ozone (>100 mg/ g SS or TS) [13, 20, 25] with an objective of increasing sludge solubilization, decreasing organic loading rate and ultimately increasing the biodegradability of sludge. However, in this study we observed considerable solubilization of solids and organic matter (17.8% SS, 14.9% VS and 69.1% SCOD) and simultaneous degradation of BPA (>98%) even at lower dose of ozone because of changing other operational parameters such as solids concentration, pH and ozonation time.

4. CONCLUSIONS

The present work on optimization of ozonation pre-treatment of secondary sludge for improvement of sludge solubilization and simultaneous degradation of bisphenol A from wastewater sludge led to following conclusions:

1. Central composite experimental design supplied enough information for optimization of bisphenol A degradation from wastewater sludge within four independent variables namely, solids concentration, pH, ozone dose and ozonation time.

2. The four independent variables showed effect on degradation of bisphenol A from wastewater sludge. Ozone dose had a very high significant effect on degradation of bisphenol A (probability (p) < 0.001) followed by pH, ozonation time and solids concentration of sludge, respectively.
3. Ozone dose played an important role in increase in sludge solids (SS and VS) and organic matter (SCOD and SOC) solubilization and hydroxyl radical ($^{\bullet}OH$) production in the medium for degradation of bisphenol A.
4. The optimal conditions developed by the response surface model in order to obtain 100% of bisphenol A degradation from wastewater sludge by ozonation pre-treatment were as follows: 24 g/L SS, 6.23 pH with ozone dose of 26.14 mg/g SS for 16.47 min ozonation time.

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Table 1. Characterstics of the secondary sludge used for experiment

Parameters	Secondary Sludge
pH	6.31±0.7
TS (g L^{-1})	13.46±2.3
SS (g L^{-1})	9.19±1.6
VS (g L^{-1})	7.81±3.2
VSS (g L^{-1})	5.73±1.4
TCOD (g L^{-1})	9.51±1.8
SCOD (g L^{-1})	0.78±0.09
Ammoniacal nitrogen (g L^{-1})	0.17±0.01
Phosphorus (g L^{-1})	0.311±0.07
TOC (g L^{-1})	282±10
SOC (g L^{-1})	0.21±0.08
Alkalinity (g L^{-1})	0.67±0.04

Table 2. Codes and values of experimental range of four variables for screening using response surface methodology

Variables	Symbol	Coded level				
		-2	-1	0	+1	+2
Suspended solids (g/L)	X_1	15	20	25	30	35
pH	X_2	5.0	5.5	6.0	6.5	7.0
Ozone dose (mg/g SS)	X_3	5.0	10.0	15.0	20.0	25.0
Ozonation time (min)	X_4	10	15	20	25	30

Table 3. Results of experimental plan by central composite design for changes in analytical parameters and simultaneous change in BPA degradation

Trial	X_1	X_2	X_3	X_4	SSs (%)	VSs (%)	SCOD increment (%)	SOC increment (%)	BPA degradation (%)
1	20	5.5	10.0	15	9.03	5.1	33	6.21	59.11
2	20	5.5	10.0	25	12.23	5.8	37.5	11	66.2
3	20	5.5	20.0	15	13.56	11.97	51.6	14.57	89.71
4	20	5.5	20.0	25	18.69	13.01	63	16	92.03
5	20	6.5	10.0	15	6.52	4.7	26.3	3.23	53.11
6	20	6.5	10.0	25	6	4.79	32.7	3.9	57.83
7	20	6.5	20.0	15	10.81	8.52	53.1	12.11	85.11
8	20	6.5	20.0	25	19.54	9.01	58.7	14	92.38
9	30	5.5	10.0	15	5.98	5.3	24.7	7.11	64.7
10	30	5.5	10.0	25	8.11	5.7	29.8	9.28	79.1
11	30	5.5	20.0	15	16.01	14.23	55.3	15.7	86.9
12	30	5.5	20.0	25	22.89	19.17	65.3	17.3	94.17
13	30	6.5	10.0	15	8.05	3.98	28.3	9.1	48.2
14	30	6.5	10.0	25	6.79	5.11	28.9	9.9	62.6
15	30	6.5	20.0	15	13.11	10.73	50.7	13.7	90.71
16	30	6.5	20.0	25	18.75	13.01	58.1	16	92.83
17	15	6.0	15.0	20	12.65	9.11	61.7	16.87	86.53
18	35	6.0	15.0	20	10.81	7.83	56.2	14.44	77.81
19	25	5.0	15.0	20	7.19	5.61	26.9	7.3	48.57
20	25	7.0	15.0	20	9.81	7.73	49.6	13.99	64.73
21	25	6.0	5.0	20	4.21	2.01	13.1	3.01	42.11
22	25	6.0	25.0	20	17.83	14.89	69.1	18	98.53
23	25	6.0	15.0	10	10.11	4.73	51.7	15.5	81.97
24	25	6.0	15.0	30	16.83	8.11	59	15.9	92.11
25	25	6.0	15.0	20	12.39	10.03	54.6	13.77	81.9
26	25	6.0	15.0	20	12.01	9.81	54.9	13.98	81.0
27	25	6.0	15.0	20	11.93	9.87	51.7	13.11	81.11
28	25	6.0	15.0	20	12.86	10	54	12.97	80.8
29	25	6.0	15.0	20	12.01	10	55.1	13	81.1
30	25	6.0	15.0	20	12.33	10.21	53.7	13.56	81.49
31	25	6.0	15.0	20	12.8	10.11	53.9	13	81.72

Table 4. ANOVA for the regression model equation and coefficients

Model	BPA degradation				
	SS	df	MS	F-test	p
X_1	1.649	1	1.649	0.0867	0.772256
X_1^2	4.298	1	4.298	0.2259	0.030976*
X_2	457.889	1	457.889	24.0698	0.000158*
X_2^2	45.955	1	45.955	2.4157	0.139680
X_3	4983.266	1	4983.266	261.9546	0.000000*
X_3^2	320.953	1	320.953	16.8715	0.000824*
X_4	265.801	1	265.801	13.9723	0.324111
X_4^2	17.690	1	17.690	1.0351	0.02793*
X_1X_2	8.866	1	8.866	0.4660	0.504583
X_1X_3	10.514	1	10.514	0.5527	0.000468*
X_1X_4	17.619	1	17.619	0.9262	0.350179
X_2X_3	129.903	1	129.903	6.8286	0.018830*
X_2X_4	0.413	1	0.413	0.0217	0.884728
X_3X_4	29.241	1	29.241	1.5371	0.000232*
Residual	304.374	16	19.023	--	--

*significant values ($p < 0.05$); SS: Sum of Square; df: degrees of freedom; MS: Mean Square; p: probability

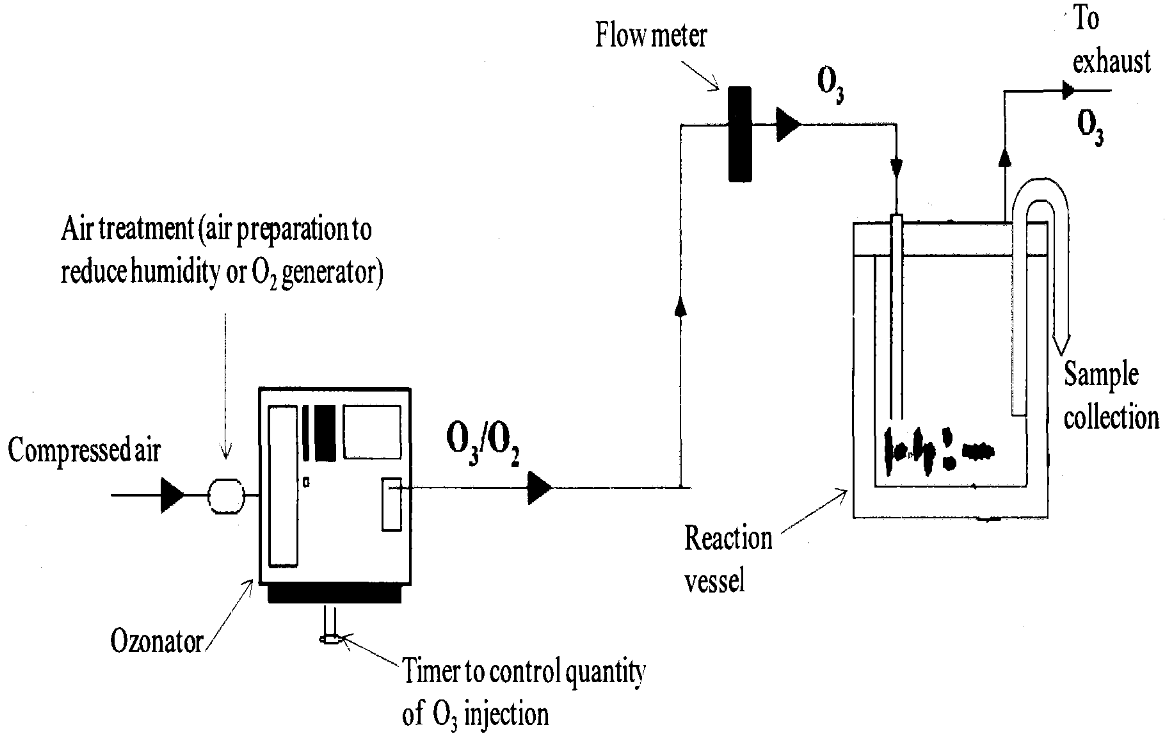

Figure 1. Experimental set up for ozonation pre-treatment

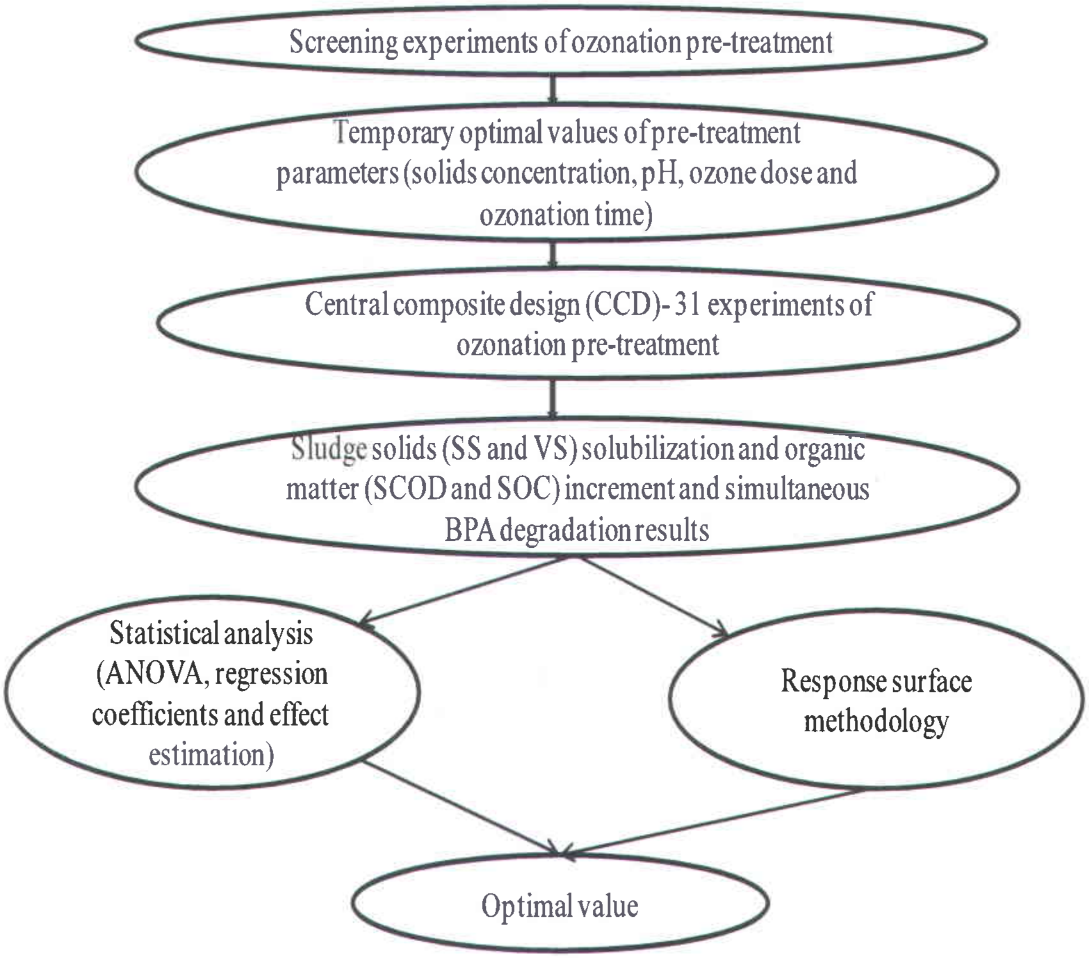

Figure 2. Schematic of optimization of ozonation pretreatment by response surface methodology

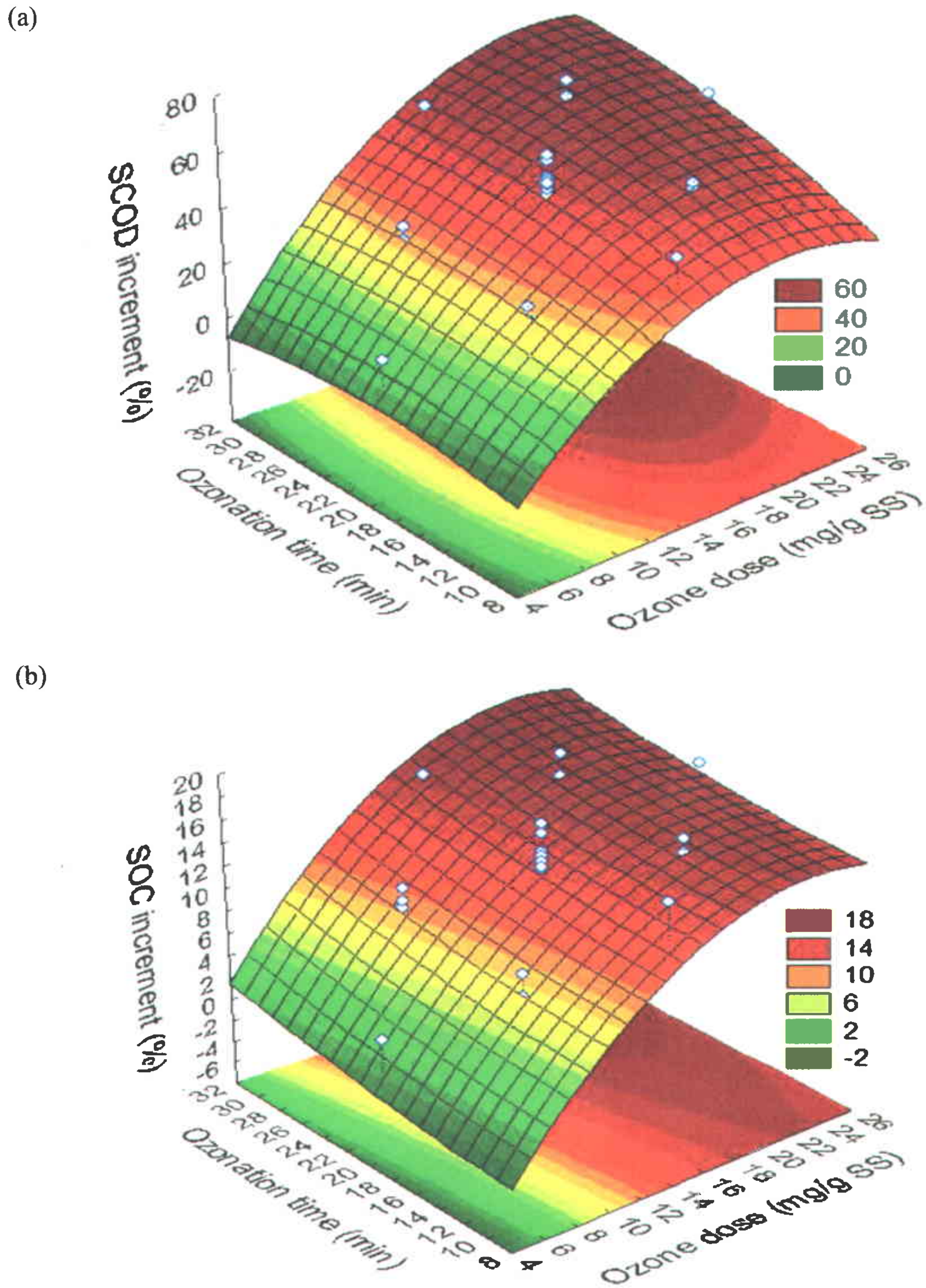

Figure 3. Response surface plot of organic matter solubilization as a function of ozone dose and ozonation time: a) COD solubilization; and b) SOC solubilization

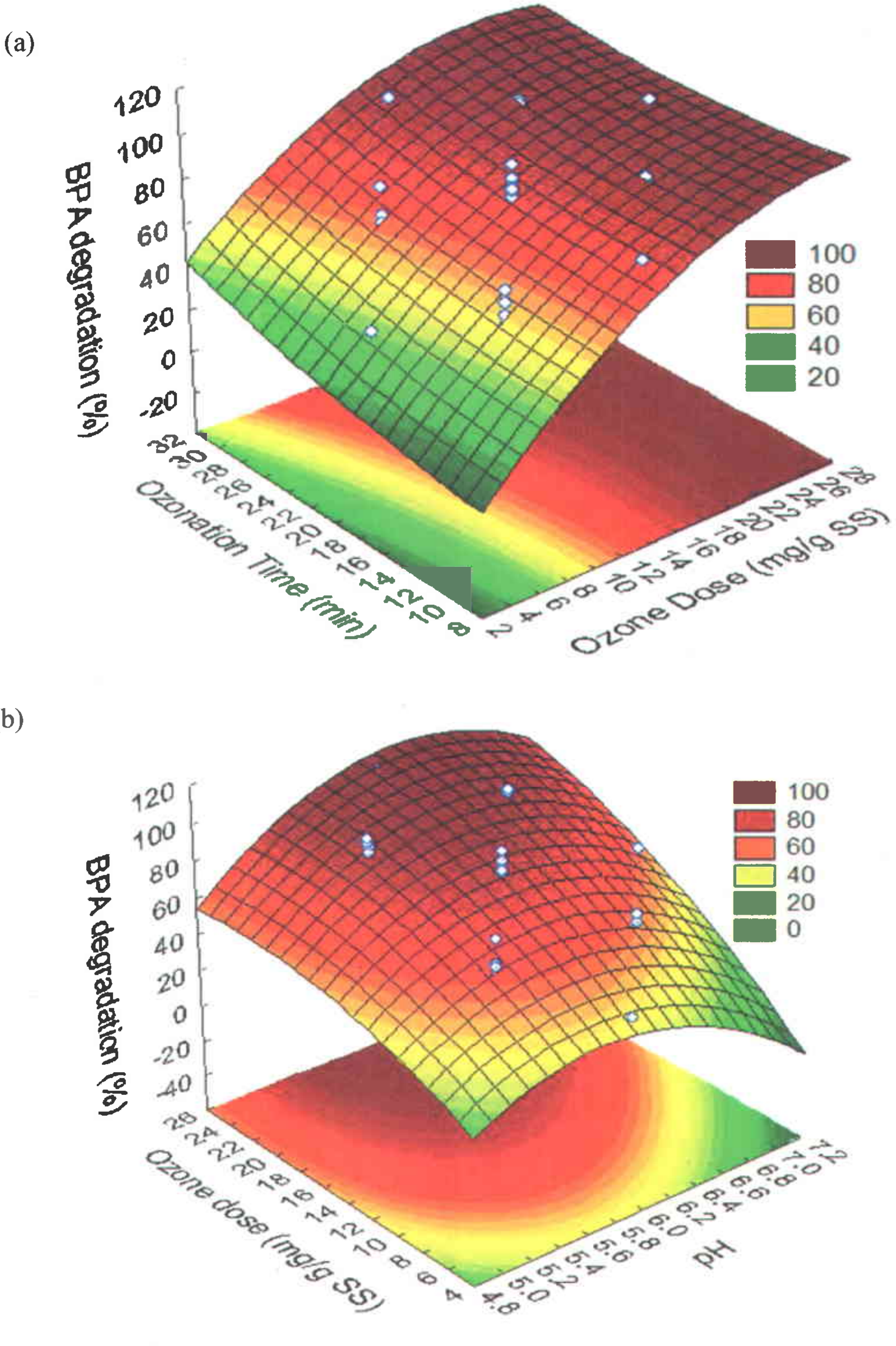

Figure 4. Response surface plots of BPA degradation as a function of: (a) ozone dose and ozonation time (pH and SS constant); (b) ozone dose and pH (SS and ozonation time constant); (c) ozone dose and solids concentration (pH and ozonation time constant)

(c)

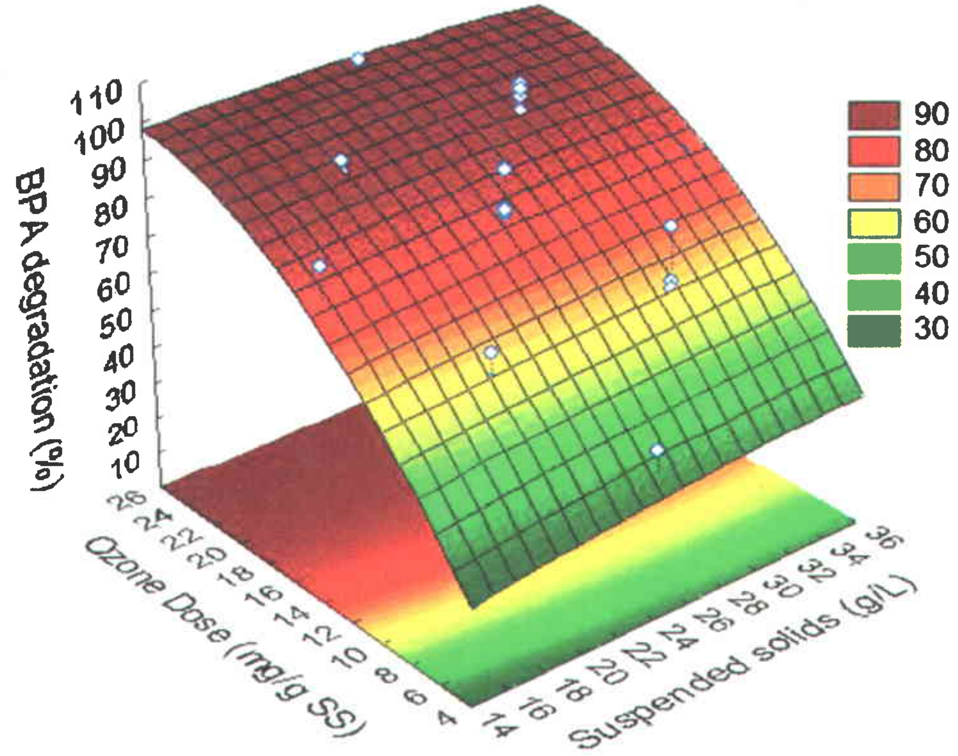

Figure 4. Response surface plots of BPA degradation as a function of: (a) ozone dose and ozonation time (pH and SS constant); (b) ozone dose and pH (SS and ozonation time constant); (c) ozone dose and solids concentration (pH and ozonation time constant)

PARTIE 3

FERRO-SONICATION AND PARTIAL OZONATION PRE-TREATMENT AND BIOTRANSFORMATION OF WASTEWATER SLUDGE FOR DEGRADATION OF BISPHENOL A: RHEOLOGY STUDIES

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RÉSUMÉ

L'effet des propriétés physico-chimiques de pré-traitement, y compris la ferro-sonication (FS) et l'ozonation partielle (OZ) sur la rhéologie des boues d'épuration (WWS) A été étudié. La ferro-sonication et l'ozonisation partielle ont été effectuées dans des conditions optimales: 163 d'ultrasons par minute, 2,71 mg L^{-1} $FeSO_4.7H_2O$ avec un pH=2,81 et 26,16 mg g^{-1} d'ozone SS, 16,47 min de durée d'ozonation et un pH de 6,23, respectivement. Les boues brutes et prétraitées décrit le comportement non-newtonien et pseudoplastique avec 85% à 97% de coefficient d'ajustement de Bingham, Casson et les modèles IPC pour la concentration de matières sèches varie de 15 g L^{-1} à 35 g L^{-1}. Parmi tous les modèles rhéologiques étudiées, la loi de puissance a été plus marquée (confiance de l'ajustement de 90% à 97%) pour décrire la rhéologie des boues brutes et prétraitées. Le pré-traitement des WWS a conduit à diminuer la pseudoplasticité en indice de consistance (K) et d'augmenter l'écoulement comportement de l'indice (n). En outre, les effets des FS et OZ sur le changement de taille des particules et sur la dégradation du bisphénol A (BPA), un perturbateur endocrinien, ont également été étudiés. Les résultats ont montré une diminution de la viscosité et de la taille des particules avec une plus grande dégradation du BPA à partir de WWS. La dégradation de BPA par les laccases produites par *Sinorhizobium meliloti* dans les boues brutes et pré-traitées a également été déterminée. Une plus grande activité de la laccase (366 U L^{-1}) a été observée dans les boues partiellement traitée à l'ozone, ce qui augmente l'élimination du BPA (0,16 mg g^{-1}) de WWS. Les résultats présentés dans ce manuscrit sont potentiellement intéressanes pour les chercheurs d'étudier le comportement des boues d'épuration en épuration des eaux usées et l'élimination des composés organiques en traces.

Mots-clés: Enzyme; Environnement; Fluides non-Newtoniens; Prétraitement; Rhéologie; Traitement des déchets

ABSTRACT

The effect of physico-chemical pre-treatment processes, including ferro-sonication (FS) and partial ozonation (OZ) on the rheology of wastewater sludge (WWS) was investigated. The ferro-sonication and partial ozonation pre-treatment was carried out at optimum conditions: 163 min ultrasonication time, 2.71 mg L^{-1} $FeSO_4.7H_2O$ with pH 2.81 and 26.16 mg g^{-1} SS ozone dose, 16.47 min ozonation time at pH 6.23, respectively. Raw and pre-treated sludge depicted non-Newtonian and pseudoplastic behavior with 85% to 97% confidence of fit into Bingham, Casson, Power and IPC paste models for solids concentration ranging from 15 g L^{-1} to 35 g L^{-1}. Among all the rheological models studied, the power law was more prominent (confidence of fit 90% to 97%) in describing the rheology of the raw and pre-treated sludge. Pre-treatment of WWS led to decrease in pseudoplasticity due to decrease in consistency index K and increase in flow behavior index n. Further, the effects of FS and OZ pre-treatment of WWS on particle size change and on the degradation of bisphenol A (BPA), an endocrine disruptor were also investigated. The results showed a decrease in viscosity and particle size together with a higher degradation of BPA from WWS. BPA degradation by laccases produced by *Sinorhizobium meliloti* in raw and pre-treated sludge was also determined. Higher activity of laccase (366 U L^{-1}) was observed in partially ozonated sludge, resulting in higher removal of BPA (0.16 µg g^{-1}) from WWS. The findings presented in this manuscript are potentially interesting to researcher studying the behavior of sewage sludge in wastewater treatment and removal of trace organic contaminants.

Keywords: Enzyme; Environment; Non-Newtonian fluids; Pre-treatment; Rheology; Waste treatment

1. INTRODUCTION

Pre-treatment processes, namely chemical treatment, mechanical treatment, oxidative treatments or combination of any two of these methods have been applied in various wastewater sludge (WWS) treatment processes, such as dewatering, digestion, and for degradation of different organic compounds including bisphenol A (BPA), an endocrine disruptor (Marttinen et al., 2003; Mohapatra et al., 2010a,b, 2011a). In order to degrade the refractory structure of WWS and increase its biodegradability, physico-chemical pre-treatment methods are commonly utilized to transform the particulate into soluble compounds (Lin et al., 1999). Out of the existing pre-treatment methods, ferro-sonication and partial ozonation pre-treatment are known for enhancing the mineralization of organic compounds and simultaneously increasing the biodegradability of sludge (Carballa et al., 2007). These pre-treatment processes break-up sludge flocs by destroying cell wall and membranes, resulting in release of intracellular organics in liquid phase and change in sludge composition. This enhances the overall solubilization and biodegradability of WWS for stabilization and reutilization processes (Tokumura et al., 2007).

The rheology describes the deformation of a flow under the influence of mechanical stress and is a useful tool for the characterization of WWS to control sludge treatment processes, such as dewatering, stabilization and advanced reuse processes, such as value addition (production of value-added products, such as biofertilizer, biopesticides, biocontrol agents and biopolymers among others) (Brar et al., 2007). WWS flows can be Newtonian or non-Newtonian, but all studies so far have demonstrated non-Newtonian regime (Bougrier et al., 2006; Brar et al., 2007). Rheological characterization of sludges represents one of the best examples of a fundamental property that has also been correlated to real time processes, such as sewage sludge treatment (disposal, dewatering, physico-chemical conditioning, anaerobic digestion, among others). Rheological parameters are very important in sludge management, not only as designing parameters in transporting, storing, landfill and spreading operations, but also as controlling ones in many treatments, such as stabilization and dewatering (Lotito et al., 1997). The hydrodynamic phenomenon in a typical unit operation can be easily modeled using apparent viscosity measurements as it influences the flow properties. Further, these measurements can also help in predicting the settling of flocs, oxygen transfer (for an aeration process) and pumping set installations etc. (Battistoni, 2000; Mikkelsen, 2001).

The rheological changes can also be due to change in surface chemistry of flocs or suspension forming WWS. The changes in surface chemistry can be brought out by pre-treatment. Ferro-sonication and partial ozonation pre-treatment resulting in the generation of hydroxyl radicals ($OH^{\bullet}$) in the medium which are very powerful and non-selective oxidizing agents (Staehelin and Hoigne, 1982). The hydroxyl radicals pass easily through the microbial cell wall in the sludge, leading to oxidative decomposition of cell membrane. Consequently, organic substances are discharged from the microbial cell into the liquid phase.

Thus, rheological changes of WWS during these pre-treatments will impact removal of BPA in several ways such as: i) decreased viscosity will increase oxygen transfer during microbial fermentation to produce value-added products and aid in biodegradation; ii) decreased particle size during pre-treatment will enhance the rate of reaction of BPA through formation of various active sites and thus degradation; and iii) decreased particle size will improve access by microbial enzymes during value-addition and thus biodegradation. However, no detailed studies have been carried out so far on the rheology change of WWS during ferro-sonication and partial ozonation as physico-chemical pre-treatment process considering the degradation of BPA and simultaneous enhancement of value-addition. Recently, WWS has been subjected to reuse for production of value-added products (VAPs) through the route of bioconversion. Bioconversion of WWS into VAPs (biopesticides or other bio-control agents, biofertilizer, microbial inoculants, industrial enzymes, bioplastics and other biopolymers) has been envisaged with successful and encouraging results (Verma et al., 2005; Brar et al., 2007).

Further, our previous studies were carried out on optimization conditions of ferro-sonication and partial ozonation pre-treatment process for the degradation of BPA from WWS. Hence, this becomes rationale for selection of the both type of pre-treatment. Further, BPA was selected as the model compound as it is an emerging contaminant and higher concentration was observed in WWS in most of the North American countries (Mohapatra et al., 2010a).

Hence, the present study will focus on the following objectives: (a) influence of ferro-sonication and partial ozonation pre-treatment on the rheological profile of wastewater sludge; (b) effect of viscosity and particle size on degradation of bisphenol A from wastewater sludge during pre-treatment and; (c) effect of laccase produced by *S. meliloti* on degradation of bisphenol A in oxidative pre-treated sludge.

2. MATERIALS AND METHODS

2.1. Chemicals

BPA (98% purity assay) was obtained from Sigma-Aldrich (Ontario, Canada). HPLC-grade methanol (MeOH), dichloromethane (DCM), acetone and chloroform, used for cleaning and extraction purposes, were purchased from Fisher Scientific (Ontario, Canada). Sep-Pak Plus C18 environmental cartridges used for solid phase extraction (SPE) clean-up were purchased from Waters (Milford, MA, USA). Acetic acid, hydrogen peroxide, sodium hydroxide and sulfuric acid were supplied by Fisher scientific (Ontario, Canada) and were of analytical grade. HPLC grade water was prepared in the laboratory using a Milli-Q/Milli-RO Millipore system (Milford, MA, USA).

2.2. Wastewater sludge

Physico-chemical treated sludge used in the study was collected from Quebec Urban Community (CUQ) wastewater treatment plant (Beauport, Québec city, Québec, Canada). CUQ treatment plant accomplishes primary and physical-chemical treatment of sewage before discharging the treated water into the Saint-Lawrence River. In phase one, primary and secondary sludge produced during primary and secondary settling of wastewater is forwarded to station East's four sludge thickeners. The proportion of solid matter in the sludge rises from 1% to 5%. In phase two, the sludge is transformed into dry matter at the treatment plant located at the CUQ incinerator, prior to burning with urban garbage.

Samples were collected in pre-cleaned amber glass bottles with aluminum foil-lined caps and stored under dark conditions at 4±1 °C. Characteristics of physico-chemical sludge used in the experiment are represented in Table 1 and the level of contaminants in the sludge used was discussed in Lee and Peart (2002). The sludge suspended solids (SS) were concentrated from 1.5% (w/v) to higher SS concentrations by gravity settling and centrifugation of the settled sludge at 1600 × *g for 3 min in a* Sorvall RC 5C plus Macrocentrifuge (rotor SA-600). The supernatant were discarded in order to obtain 15, 20, 25, 30 and 35 g L^{-1} SS. Analysis of pH, total solids (TS), suspended solids (SS), volatile solids (VS), soluble chemical oxygen demand (SCOD), soluble organic carbon (SOC) was carried out as per the Standard Methods (APHA, 2005).

2.3. Ferro-sonication pre-treatment of wastewater sludge

Ferro-sonication pre-treatment was carried out by addition of $FeSO_4.7H_2O$ solution in WWS followed by ultrasonic pre-treatment. The ferro-sonication pre-treatment was carried out at optimum conditions: 163 min ultrasonication time, 2.71 mg L^{-1} $FeSO_4.7H_2O$ with pH 2.81 (Mohapatra et al., 2011b). Four hundred milliliters of WWS was acidified to pH 2.81 by using 4 N H_2SO_4 and placed in a 1 L flask. The ultrasonication was carried out using ultrasonic homogenizer Autotune 750W (Cole-Parmer Instruments, Vernon Hills, Illinois, US). The ultrasonication equipment was operated at a frequency of 20 kHz by using platinum probe with a tip diameter of 12 mm and ultrasonic intensity of 15 W cm^{-2}. The ultrasonic probe was dipped in such a way that it was immersed 2 cm into the sludge. Further, ferro-sonication pre-treatment will be referred to FS.

2.4. Ozonation pre-treatment of wastewater sludge

Ozonation pre-treatment was carried out by using an air tank, ozone gas generator (OZOMAX Ltd., Quebec, Canada), flow meter and a reaction vessel. Ozonation pre-treatment was carried out at optimum conditions: 26.16 mg g^{-1} SS ozone dose, 16.47 min ozonation time at pH 6.23. Four hundred milliliters of WWS was taken in the vessel and the pH was adjusted by addition of 4N NaOH. Production of ozone was controlled by adjusting the compressed air flow and by timer fitted with the ozonator. The air flow rate was adjustable by a valve. The experiments were conducted at room temperature. Henceforth, ozonation pre-treatment will be referred as OZ.

2.5. BPA Analysis

2.5.1. Extraction and clean up

Sludge was frozen using liquid nitrogen prior to lyophilization by the freeze-dry system (Dura Freeze Dryer, Kinetics). About 0.5 grams of lyophilized and homogenized sample were transferred to a glass tube and 20 mL of dichloromethane-methanol (7:3, v/v) was added to the tube. The extraction was carried out by microwave assisted extraction method (MARS microwave extractor, CEM Corporation, North Carolina, USA). This system allowed simultaneous irradiation of 14 extraction vessels. Microwave power was 1200W (100%) and the extraction was performed in a temperature-controlled mode. The extraction temperature was 110±1 °C and programmed as follows: ramp to 110±1 °C for 10 min, holding at 110±1 °C for 10 min. The extract was separated by centrifugation at 7650 × g for 15 min and the procedure was repeated three times. The extracts were combined, concentrated to an approximate volume of 1 mL with gentle stream of nitrogen and redissolved in 100 mL of HPLC grade water.

Solid phase extraction (SPE) method was used for clean-up and pre-concentration of extract. Sep-Pak Plus C18 environmental cartridges were fitted into the vacuum manifold (Welch, USA) which was connected to a vacuum pump (Welch Rietschle Thomas, USA) to dispense samples through the cartridges. Cartridges were pre-conditioned by passing 7 mL of methanol and 3 mL of HPLC water at a flow rate of 1 mL min^{-1}. Sludge extracts (100 mL) were passed at a flow rate of 5 mL min^{-1}. After pre-concentration, the sorbents were dried by using a vacuum system set at (-15) psi.

The elution was performed by adding 2 x 4 mL of methanol/dichloromethane (3:1, v/v) mixture to the cartridge at a flow rate of 1mL min^{-1} and giving it a wait time of 10 min in order to give enough duration of contact between the solvent and the adsorbed compounds. The extracts were later evaporated to dryness with a gentle stream of nitrogen and reconstituted with methanol to a final volume of 200 µL prior to Laser Diode Thermal Desorption-Atmospheric Pressure Chemical Ionization-Mass Spectrometry/Mass Spectrometry (LDTD-APCI-MS/MS) analysis.

2.5.2. LDTD-APCI-MS/MS Analysis

Quantification of BPA in WWS was achieved with the LDTD-APCI ionization source (Phytronix Technologies, Quebec, Canada) mounted on a TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples were first spotted (2 µL) into the LazWell 96-well polypropylene plate cavities containing inserts made of proprietary stainless steel alloy and left to dry at room temperature. The designed well shape allows the sample to concentrate in the heating zone while drying. The loaded plate is then transferred to an *X-Y* movable stage of the LDTD housing unit. An infrared (IR) laser diode (980 nm, 20 W, continuous) is then focalized to impact the back of the inserts, thermally desorbing the dried sample, which is vaporized into gas phase. The analysis was carried out in negative ionization mode with following conditions: laser pattern programming (2 s ramp from 0 to 45% (laser power), held for 2 s at 45% before shutting off); carrier gas flow rate (3 L/min); deposition volume (2 µL); sheath gas pressure (15 arbitrary units); auxiliary gas pressure (5 arbitrary units); skimmer offset (6 V); capillary temperature (270 °C); collision pressure (1.5 mTorr) and scan time (0.25 s).

2.6. Rheology Study

Viscosity of raw and different pre-treated sludges was measured by using a rotational viscometer Brookefield DVII PRO+ (Brookfield Engineering Laboratories, Inc., Stoughton, MA, USA) equipped with Rheocalc32 software (for rheological models). Two different spindles, namely, SC-34 (small sample adaptor), and ultralow centipoise adapter were used with a sample cup volume of 18 mL/50 mL (spindle dependent). The gaps between spindle and respective sample chamber were 1.235 and 4.830 mm, respectively, for ultralow (viscosity range, 1.0×10^{-3} to 30×10^{-3} kg m^{-1} s^{-1}), and small sample (viscosity range $\geq 30 \times 10^{-3}$ kg m^{-1} s^{-1}) adapter spindle to accommodate sludge flocs. The calibration and viscosity testing procedure for each spindle was carried out as per instrument manual.

Time dependent profile was studied at lower shear rate (3.51 s^{-1}) and viscosity of sludge samples at different solids concentration was measured at 36.69 s^{-1}. The shear rate behavior was determined from 0.3 to 56 s^{-1}. All measurements were done at 25±1 °C, and viscosity was referred to as “apparent viscosity”.

Particle size analysis was carried out by using laser scattering particle size distribution analyzer (partica LA-950V2, HORIBA, CA, USA). The stirrer and recirculation pump speed were also kept moderate at 250 and 500 rpm, respectively to minimize the damage of sludge particles. For analysis, each sample was diluted approximately 400-fold in tap water and analyzed in triplicate. The results were then averaged to produce the particle size distribution, and the readings were recorded as particle volume percent in 51 discrete particle ranges between 0.1 to 1000 µm. Floc disruption at D_{43} (volume mean diameter) analogous to the particle size expressed as diameter 43% size distribution cutoff points was chosen as average particle size as it showed the volume mean diameter. A standard deviation of 6% and 8% was observed for viscosity and particle size measurements, respectively.

2.7. Rhizobial growth

The fast-growing *S. meliloti* strain A_2 (Agriculture and Agri-food Canada, Sainte-Foy, Quebec, Canada) was used throughout this study. Cultures were maintained at 4±1 °C on yeast mannitol agar (YMA) slants (Vincent, 1970). Cell production was carried out in different media: the standard medium yeast mannitol broth (YMB), raw sludge, ferro-sonicated and ozonated sludge and each sludge sample with spiked BPA. All sludge samples were sterilized at 121 ± 1 °C for

30 min prior to inoculation. The YMB medium contained the following constituents in grams per liter: K_2HPO_4, 0.5; $MgSO_4.7H_2O$, 0.2; NaCl, 0.1; yeast extract, 1; and mannitol, 10.

The strain A_2 was grown at 30 ± 1°C for 72 h on a rotary shaker at 200 rpm and the cell counts were determined on YMA plate supplemented with Congo red (0.25%) after a serial dilution on saline solution (NaCl, 0.85% (w/v)). The samples were plated in triplicates. For enumeration, 30–300 colonies were counted per plate. The results were expressed in colony forming units per mL (CFU/mL). Colonies were counted after incubation of plates for 0 h, 6 h, 9 h, 12 h, 24 h, 36 h and 48 h at 30 °C.

2.8. Laccase activity

Laccase activity was measured with 2,2-azino bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) in 0.1 M phosphate-citrate buffer (pH 4). Oxidation of ABTS was determined by the increase in A420 ($\varepsilon420 = 36 \text{ (mM cm)}^{-1}$) (Collins and Dobson, 1997). One unit of laccase activity was defined as the amount of enzyme required to oxidize 1 µmol of ABTS per min.

3. RESULTS AND DISCUSSION

3.1. Rheology of raw and pre-treated sludge

3.1.1. Shear thinning behavior

Fig. 1 illustrates rheogram and shear rate behavior of raw and pre-treated sludges at different solids concentrations (15-35 g L^{-1}). The rheograms of raw and pre-treated sludges showed non-linear relationship (strong non-Newtonian behavior) and viscosity vs. shear rate curves showed pseudoplastic (viscosity decreased with shear rate) behavior. The treated sludge curves (FS and OZ) were lower than the untreated one (RS), which showed that viscosity for a given shear rate decreased with pre-treatments. Pre-treatment processes, such as ferro-sonication and ozonation among others are known to bring about a change in the surface chemistry of WWS (proteins and polysaccharides) (Stasinakis, 2008). WWS pre-treatments rupture microbial cells, liberate the nutrients, partially solubilize the suspended solids, increase the soluble chemical oxygen demand, decrease viscosity and improve the overall WWS biodegradability (Barnabe, 2004).

Further, from Fig. 1, it was observed that the effect of pre-treatment on rheological behavior of sludge decreased with increasing solids concentration as the curves came closer to each other with increasing solids concentrations. The pre-treatment processes influence the rheological behavior by modifying overall sludge properties, including structure, strength and size of sludge flocs and composition (Bougrier et al., 2006). At high solids concentration, structural units of the suspension were typically larger in size and knitted close to each other. As particle concentration increases, a network of particles is formed due to increased rate of interactions leading to an increase in resistance to flow of sludge materials (Mikkelsen and Keiding, 2002). Therefore, the effect of pre-treatment on rheological behavior of sludge decreased as solids content increases, so does the viscosity at constant shear rate.

However, in between FS and OZ pre-treated sludge with all the solids concentrations (15-35 g L^{-1}), the lower viscosity and shear stress with respect to shear rate were observed in OZ pre-treated sludge, showing higher shear-thinning behavior of sludge. The effect of ozonation pre-treatment on rheological behavior was mainly attributed to the disintegration of the sludge aggregates and disruption of cells, leading to breaking of the microbial slurry where the particles constantly interact with each other that influence decrease in viscosity. Further, the higher production of hydroxyl radicals ($OH^{\bullet}$) during ozonation pre-treatment caused oxidative decomposition of the cell wall as it easily passes through the microbial cell membrane in the sludge (Bougrier et al., 2006). Consequently, organic substances are discharged from the microbial cell into the liquid phase leading to decrease in viscosity.

3.1.2. Rheological models

Table 2 demonstrates various rheological models with varying degree of confidence of fit for raw and pre-treated sludges at different solids concentrations. The purpose of studying different rheological models is to evaluate the existing models for characterizing the non-Newtonian properties of raw and pre-treated sludges with different solids concentration. The raw (RS) and pre-treated (FS and OZ) sludge strongly followed Bingham plastic model with high values of confidence of fit (81.2–94.7%) with the solids concentrations varying from 15 to 25 g L^{-1}, whereas a poor fit (51.2–72.1%) was observed with increased solids concentration to 30 and 35 g L^{-1}. Bingham model is a simple rheological model that relates shear stress and shear rate and quantifies yield stress and high-shear viscosity. The Bingham law is represented mathematically by Eq. (1):

$$\tau = \tau_0 + \eta\gamma \qquad (1)$$

Where, τ is shear stress, τ_0 is yield stress, η is viscosity and γ is shear rate. It was observed that the yield stress (stress needed to be applied to overcome the Van-der-Wall forces and induce the suspension to flow (Courraze and Grossiard, 1991)) for the model increased with increasing solids concentrations. Lower yield stress was observed in both FS and OZ pre-treated sludge as compared to raw sludge. However, in between FS and OZ pre-treated sludge, lower yield stress was observed in OZ pre-treated sludge. The results may be contributed by two factors, such as-higher disintegration of sludge cells during ozonation pre-treatment and hence less resistance (shear thinning); and the EPS formed by the natural sludge microorganisms were broken due to higher production of hydroxyl radicals ($OH^{\bullet}$) (Bougrier et al., 2006).

Further, Casson and NCA–CMA Casson (modified Casson) model was followed with good confidence of fits (78–96.1%) in both raw and pre-treated sludges. Casson model quantifies yield stress and high shear viscosity, a model which incorporates features of the power law and the Bingham plastic equations. The Casson and NCA–CMA Casson (modified Casson) model is mathematically represented by Eq. 2 and 3 respectively:

$$\sqrt{\tau} = \sqrt{\tau_0} + \sqrt{\eta\gamma} \qquad (2)$$

$$(1 + a_1)\sqrt{\tau} = 2\sqrt{\tau_0} + (1 + a_1)\sqrt{\eta\gamma} \qquad (3)$$

Where, τ is shear stress, τ_0 is yield stress, η is viscosity, γ is shear rate, and a_1 is ratio of spindle and inner cup radius. Considering Casson law model, plastic viscosity and yield stress increased with increasing solids concentration in both raw and pre-treated sludges. However, lower plastic viscosity and yield stress were observed in FS and OZ pre-treated sludge as compared to raw sludge.

Meanwhile, power and IPC paste (extension of power model) were followed with good confidence of fits (77.5–97.2%) for raw and pre-treated sludge. However, power law followed best fits (89.6–97.2%) for all types of sludge as compared to other models. Power law model is a useful rheological model that describes the relationship between viscosity or shear stress and shear rate over the range of shear rates where shear thinning occurs in a non-Newtonian fluid. It quantifies overall viscosity range and degree of deviation from Newtonian behavior of fluid. The power and IPC paste models are mathematically represented in Eq. 4 and 5 respectively:

$$\tau = K\gamma^n \qquad (4)$$

$$\eta = K\mathrm{R}^n \qquad (5)$$

Where, τ is shear stress, η is viscosity, γ is shear rate, K is consistency index, and R is rotational speed. The consistency index (K, a constant linked to structure) increased with increasing solids concentrations and lower value was observed in FS and OZ pre-treated sludge as compared to raw sludge. Lower K value was observed in OZ pre-treated sludge as compared to FS pre-treated sludge within the range of solids concentrations tested (15-35 g L^{-1}). Interestingly, for up to 20 g L^{-1}, the *K* value observed in OZ pretreated sludge was lower than 100, which showed higher tendency of sludge towards liquid. Meanwhile, flow behavior index (*n*) (lower than 1 indicating deviation from Newtonian behavior) of the sludges decreased with increasing sludge solids concentrations. Further, *n* values observed in OZ pre-treated sludge were higher as compared to raw and FS pre-treated sludge. The disintegration of sludge flocs, degradation of EPS and oxidation by hydroxyl radicals ($OH^{\bullet}$) during ozonation pre-treatment are the main reasons for the decrease in cohesiveness of sludge fluids (*K* values for OZ pre-treated sludge were lower than raw and FS pre-treated sludge) and increase in flowbility (*n* values for OZ pre-treated sludge were higher than raw and FS pre-treated sludge).

3.2. Analysis of viscosity and particle size changes and BPA degradation after pre-treatments

Fig. 2a and b present the effects of FS and OZ pre-treatment processes on viscosity and particle size on removal of BPA from raw sludge. The viscosity observed in raw sludge varied in between 52.7 × 10^{-3} kg m^{-1} s^{-1} to 156.3 × 10^{-3} kg m^{-1} s^{-1} for solids concentration of 15 to 35 g L^{-1}. The viscosity observed in FS and OZ pre-treated sludge was observed in between 44.75 × 10^{-3} kg m^{-1} s^{-1} to 120 × 10^{-3} kg m^{-1} s^{-1} and 23.1 × 10^{-3} kg m^{-1} s^{-1} to 48.7 × 10^{-3} kg m^{-1} s^{-1} respectively, for solids concentration ranging from 15 to 35 g L^{-1}. The lower viscosity observed during OZ pre-treated sludge was mainly due to higher solubilization of sludge solids (data not reported) and disintegration of flocs. As observed from the Fig. 2a, the viscosity of raw and pre-treated sludge increased with increasing the solids concentration. The higher viscosity observed in FS pre-treated sludge as compared to OZ pre-treated sludge was may be due to operating conditions, i.e. acidification (lower pH). The reaction conditions will lead to changes in inter-particle interactions between sludge flocs and their components. The decrease in pH will result in a decrease in electrostatic repulsions between flocs (which are mostly negatively charged) causing higher resistance to flow leading to higher viscosity (Mikkelsen, 2001).

The initial BPA concentration observed in raw sludge at five solids concentrations of 15, 20, 25, 30 and 35 g L^{-1} were 0.28 μg g^{-1}, 0.31 μg g^{-1}, 0.37 μg g^{-1}, 0.42 μg g^{-1} and 0.44 μg g^{-1}, respectively. The FS and OZ pre-treatment were carried out at optimum conditions and the final BPA concentration was observed to determine the percentage BPA degradation. Higher degradation (92.95–100%) of BPA was observed in OZ pre-treated sludge as compared to FS pre-treated sludge (79.26–94.1%). Higher degradation of BPA observed in OZ pre-treated sludge contributed towards change in sludge solids solubilization and particle size distribution. Further, the lower viscosity observed in OZ pre-treatment process also considered as an additional side effect for higher degradation of BPA from raw sludge. It is well known that sludge rheological parameters are strongly dependent on solids concentration and particle size (Mohapatra et al., 2010b). Lower viscosity increased mass transfer rate of BPA causing higher reaction rate (with $OH^{\bullet}$ formed during pre-treatment) and it's partitioning, leading to degradation within the medium. In order to verify this hypothesis, we used an Equation showing the relationship between liquid phase mass transfer coefficient (k_L) with viscosity (η) (Delaloye et al., 1991).

$$\frac{k_L a}{D^{0.5}} = 90L^{0.74}\eta^{-0.5} \qquad (6)$$

From the above Eq. it is clear that, k_L decreased with increase in viscosity as a result of the increasing resistance to mass transfer. The negative sign on the exponent of η indicated a decrease in k_L value with increasing liquid phase viscosity. The apparent viscosity value influences the global coefficient of mass transfer in an inverse relation (Schluter and Dekwer, 1992).

As shown in Fig. 2b, particle size varied in between 27.93 to 56.1 µm was observed in raw sludge. The particle size observed in the FS and OZ pre-treated sludge with different solids concentrations (15 to 35 g L^{-1}) was 24.07 to 50.31 µm and 5.93 to 14.7 µm, respectively. The increased particle size observed in FS pre-treated sludge was due to decrease in pH (pH = 2.81). Neyens et al. (2004) observed that particle size can be modified by the presence of acids, i.e. supracolloidal particles (1–100 µm range) decrease with acidic pH. The other reason for increase in particle size was due to formation of ferric hydroxide and ferric hydroxo complexes during reaction between ferrous ions with hydroxide ions. Ferric hydroxide and ferric hydroxo complexes are known to possess high capacity of coagulation and flocculation.

The decrease in particle size (formation of larger volume fine particles) during OZ pretreatment causes the overall availability of sorbed BPA to react with hydroxyl radicals ($OH^{\bullet}$) increased, resulting in higher degradation of BPA. The availability of sorbed BPA increased with decrease particle size because with a high octanol-water partition coefficient (log *Kow* = 3.82) and hydrophobic nature (Mohapatra et al., 2011a), BPA has the tendency to strongly associate with the solids.

Further, the two phenomena namely, decrease in viscosity and particle size occurred simultaneously within the media leading to increased reaction rate and partitioning of BPA thus causing its higher removal from raw sludge. The effects of volume fraction and maximum volume fraction on viscosity are described using the Krieger-Dougherty equation:

$$\frac{\eta}{\eta_{\mathrm{medium}}} = \left(1 - \frac{\varphi}{\varphi_{\mathrm{m}}}\right)^{-[\eta]\varphi_{\mathrm{m}}} \qquad (7)$$

where η is the viscosity of the suspension, η_{medium} is the viscosity of the base medium, φ is the volume fraction of solids in the suspension, φ_m is the maximum volume fraction of solids in the suspension and $[\eta]$ in the intrinsic viscosity of the medium. This correlation indicates an increase in viscosity with increasing volume fraction.

3.3. Biofertilizer production, simultaneous production of laccase and BPA degradation

The cell growth of *S. meliloti* in raw and different pre-treated sludges and each sludge with spiked BPA was observed at optimum solids concentration of 30 g L^{-1} (Barnabe, 2004). Spiked BPA sample was taken in order to compare the cell growth of *S. meliloti* with unspiked sample (data not reported). No difference was observed in cell growth of *S. meliloti* in spiked and unspiked samples. The viable cell count of *S. meliloti* growth in raw and different pre-treated sludges is presented in Fig. 3a. It was observed that the growth of *S. meliloti* in terms of viable cells enhanced in FS and OZ pre-treated sludge as compared to raw sludge. The increase in viable cell count in pre-treated sludges as compared to raw sludge may be contributed by two factors- (i) Higher solubilization of solids and organic matter during FS and OZ pre-treatment of raw sludge and; (ii) decrease in viscosity and particle size in pre-treated sludges. As *S. meliloti* is an aerobic bacteria, the increase in solubilization during different pre-treatment processes leads to higher oxygen diffusion and consequently increased cell count. The concentration of suspended solids in sludge affected the growth of *S. meliloti* in secondary sludge from municipal wastewater treatment plant (Quebec, Canada) (Ben Rebah et al., 2002). Further, decrease in viscosity and particle size also increased the oxygen transfer rate within the medium leading to higher cell multiplication.

Among FS and OZ pre-treatment, high viable cell count (9.5×10^{10} CFU mL^{-1}) was observed in OZ pre-treated sludge at 24h. The higher cell count of *S. meliloti* in OZ pre-treated sludge as compared to FS pre-treated sludge may be due to following reasons: (i) high disintegration of sludge cells; (ii) higher production of hydroxyl radicals ($OH^{\bullet}$); (iii) lower viscosity and particle size. During OZ pre-treatment, sludge aggregates were disintegrated, followed by disruption of sludge cells, thus inducing the transformation of solid-state bound organic compounds into a soluble form (Bougrier et al., 2006). This further resulted in augmentation of nutrient availability for subsequent cell production in sludge. Further, the higher production of hydroxyl radicals during OZ pre-treatment transformed organic recalcitrant compounds into easily biodegradable products (de Morais and Zamora, 2005). In particular, organic substances were partially oxidized by hydroxyl radicals making the sludge susceptible to utilization by *S. meliloti* microorganisms. In addition, lower viscosity and particle size observed in OZ pre-treated sludge consequently ameliorated oxygen and substrate mass transfer within the medium leading to higher cell count (Verma et al., 2005).

Fig. 3b presents the laccase activity profile in raw and pre-treated sludges. It was evident that *S. meliloti* was capable of producing laccase enzyme in raw and pre-treated sludges. An increased activity of laccase was observed in raw and pre-treated sludges with increasing viable cell count of *S. meliloti.* Rosconi et al. (2005) carried out a comparative study between *Rhizobium* sp. to identify the laccase strain and they observed higher identity (99%) was established with *S. meliloti.* They also observed the higher production of laccase with higher cell count of *S. meliloti.* Among FS and OZ pre-treated sludge, higher activity of laccase (366 U L^{-1}) was observed in OZ pre-treated sludge. This trend might be due to higher viable cell count of *S. meliloti* in OZ pre-treated sludge. As seen in Fig 3b, laccase activity started increasing from 0 h to 24 h of fermentation time in raw sludge and FS and OZ pre-treated sludge. The results were in agreement with the increase in cell count of *S. meliloti* upto 24 h in raw and pre-treated sludges.

The degradation of BPA in raw and pre-treated sludges due to laccase activity is presented in Fig. 3c. For the analysis of degradation of BPA by laccase activity in raw and pre-treated sludges, a known concentration of BPA (0.58 µg g^{-1}) was spiked in raw sludges and FS and OZ pre-treated sludges. Equivalent concentration was spiked in raw sludge, FS and OZ pre-treated sludge. However, due to the background presence of BPA in this type of sludge, the initial concentration was different. The average BPA concentration observed in WWS in Quebec City, Canada varied in between 0.04 to 1.78 µg g^{-1} (Mohapatra et al., 2010a). Hence, it becomes rationale to select the given range of spiking concentrations. Sample spiked with BPA was collected at 0, 6, 9, 12, 24, 36 and 48 h for analysis of *S. meliloti* growth, laccase activity and simultaneous BPA degradation. It was observed that laccase was capable of degrading BPA in raw and pre-treated sludges. Laccase is a blue oxidase capable of oxidizing phenols and aromatic amines by reducing molecular oxygen to water by a multicopper system. The catalytic center consists of three types of copper with different functions: type 1 (blue copper) catalyzes the electron transfer, type 2 activates molecular oxygen and type 3 a copper dimer, is responsible for oxygen uptake (Hublik and Schinner, 2000).

The concentration of BPA observed at 0 h of incubation in raw, FS and OZ pre-treated sludge were 0.85, 0.73 and 0.58 µg g^{-1}, respectively. The concentration of BPA observed after 48 h of laccase activity in raw, FS and OZ pre-treated sludge were 0.78, 0.6 and 0.42 µg g^{-1}, respectively. Significant concentration of BPA (0.16 µg g^{-1}) was degraded due to laccase activity in OZ pre-treated sludge. Among FS and OZ pre-treatment, higher degradation of BPA was observed in OZ pre-treated sludge due to relatively higher laccase activity induced by high viable cell count. Another reason might be lower particle size (larger fiormation of fine particles) observed in OZ pre-treated sludge resulting in release of sorbed BPA to medium increasing the access to laccase and finally favoring biodegradation.

4. CONCLUSIONS

The rheology study on ferro-sonication and partial ozonation pre-treatment and biotransformation of wastewater sludge for degradation of bisphenol A led to following conclusions:

1. Ferro-sonication and ozonation pre-treatment caused decrease in viscosity and shear-thinning behavior of sludge i.e. pre-treated sludge showed non-Newtonian pseudoplastic behavior.
2. The power law described flow characteristics of raw and pre-treated sludge in the whole range of solids concentration (15 to 35 g L^{-1}). The variation of power model constants, i.e. decrease in consistency index (K) and increase in flow behavior index (n) signified the effect of ferro-sonication and ozonation pre-treatment process on reduction in pseudoplasticity of sludge.
3. Among ferro-sonication and ozonation pre-treatment, decreased viscosity and particle size was observed in ozonated sludge with whole range of solids concentration (15 to 35 g L^{-1}) and also higher removal of bisphenol A (92.95–100%) from raw sludge.
4. The viable cell count of S. meliloti increased in ferro-sonicated and ozonated sludge and higher growth (9.5×10^{10} CFU mL^{-1}) was observed in ozonated sludge due to higher mass and oxygen transfer in the medium enhanced by lower viscosity and particle size.
5. Higher laccase activity (366 U L^{-1}) was observed in ozonated sludge leading to higher removal of bisphenol A (0.16 µg g^{-1}) from raw sludge as compared to ferro-sonicated sludge.

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NOMENCLATURE AND UNITS

η	Plastic viscosity and/or viscosity (10^{-3} kg m^{-1} s^{-1})
τ	Shear stress (10^{-1} kg m^{-1} s^{-2})
τ_0	Yield stress (10^{-1} kg m^{-1} s^{-2})
K	Consistency index (10^{-3} kg m^{-1} s^{-n})
n	Flow behavior index
n_S	Shear sensitivity factor
L	Liquid mass flux (kg m^{2} s^{-1})
φ_m	Maximum volume fraction of solids in the suspension
R	Rotational speed (rpm)
η_{10}	10 rpm viscosity (10^{-3}kg m^{-1} s^{-1})
γ	Shear rate (s^{-1})
a_1	Ratio of spindle and inner cup radius
k_L	Liquid phase mass transfer coefficient (m h^{-1})
D	Diffusion coefficient (m^{2} s^{-1})
φ	Volume fraction of solids in the suspension
a	Gas liquid interfacial area (m^{2} m^{-3})

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Table 1. Characterstics of the secondary sludge used for experiment

Parameters	Secondary Sludge
pH	6.29±0.7
TS (g L^{-1})	13.49±1.7
SS (g L^{-1})	9.35±2.3
VS (g L^{-1})	7.81±3.2
VSS (g L^{-1})	5.83±1.4
TCOD (g L^{-1})	9.01±2.3
SCOD (g L^{-1})	0.78±0.09
Ammoniacal nitrogen (g L^{-1})	0.17±0.01
Phosphorus (g L^{-1})	0.311±0.07
TOC (g L^{-1})	295±15
SOC (g L^{-1})	0.21±0.08
Alkalinity (g L^{-1})	0.67±0.14
Bisphenol A (BPA) concentration	0.26 µg g^{-1}

Table 2. Different rheological model fits of Raw sludge (RS), Ferro-sonication (FS) and Ozonation (OZ) pre-treated sludge

Solids concentrations (g L^{-1})	15			20			25			30			35		
	RS	FS	OZ	RS	FS	OZ	RS	FS	OZ	RS	FS	OZ	RS	FS	OZ
Bingham law															
Plastic viscosity (η, 10^{-3}kg m^{-1} s^{-1})	6.82	4.83	4.07	13.1	7.55	6.98	28.4	12.1	9.5	32.7	22.6	17.1	37.9	26.5	21.9
Yield stress (τ_0, 10^{-1}kg m^{-1} s^{-2})	0.85	0.15	0.17	1.83	1.71	0.98	5.2	3.71	2.59	8.33	4.41	3.01	8.96	6.1	3.7
Confidence of fit (%)	84	93	94.7	82	91.3	92.6	81.2	84	89.1	63	57.3	72.1	51.6	51.2	65.5
Casson law															
Plastic viscosity (η, 10^{-3}kg m^{-1} s^{-1})	1.57	1.03	0.74	5.47	3.99	2.01	14.9	2.71	1.9	22.7	14.1	10.8	31.7	28.1	23.7
Yield stress (τ_0, 10^{-1} kg m^{-1} s^{-2})	0.95	0.27	0.11	1.72	1.51	0.84	1.99	1.35	1.1	2.74	1.95	1.83	4.01	3.5	3.01
Confidence of fit (%)	88.3	94.1	95	91.4	90	95.3	86	88.3	90.5	81.3	83.7	87.4	80.1	79.3	80.9
NCA/CMA Casson															
Plastic viscosity (η, 10^{-3}kg m^{-1} s^{-1})	3.17	3.01	1.95	5.37	4.91	2.58	23.9	11.7	4.18	27.3	19.5	13.7	34.1	32.7	28.3
Yield stress (τ_0, 10^{-1}kg m^{-1} s^{-2})	1.16	0.83	0.29	1.72	1.57	0.96	2.05	2.91	1.7	2.99	1.59	0.41	2.89	2.93	2.05
Confidence of fit (%)	91.4	90.7	95.3	89.1	95	96.1	82.2	93.7	89	80.7	82	85.3	78	82.9	81.4
Power law															
Consistency index (*K*, 10^{-3} kg m^{-1} s^{-n})	63.1	57.8	29.3	184.7	163.1	47.3	410.	396.1	92.7	571.5	490.2	168.1	1073.9	893.1	371.5
Flow behavior index (*n*)	0.64	0.75	0.91	0.51	0.58	0.87	0.44	0.47	0.83	0.37	0.41	0.80	0.33	0.38	0.65
Confidence of fit (%)	92.9	95.3	97.2	90.3	92.7	95.8	91.9	93.1	94.9	89.9	91.9	94.2	89.6	90.1	91.3
IPC paste															
Shear sensitivity factor (n_s)	18.3	11.9	7.59	27.1	32.9	16.3	62.8	44.7	25.1	115.3	102.7	74.9	197.4	142.9	168.3
10 rpm viscosity (η_{10})	0.62	0.49	0.37	0.69	0.58	0.37	0.72	0.66	0.41	0.63	0.48	0.27	0.69	0.62	0.37
Confidence of fit (%)	79.1	85.3	94	82.5	91.7	89.3	76.7	83.5	80.2	77.5	93.5	96.2	81.5	80.1	85.7

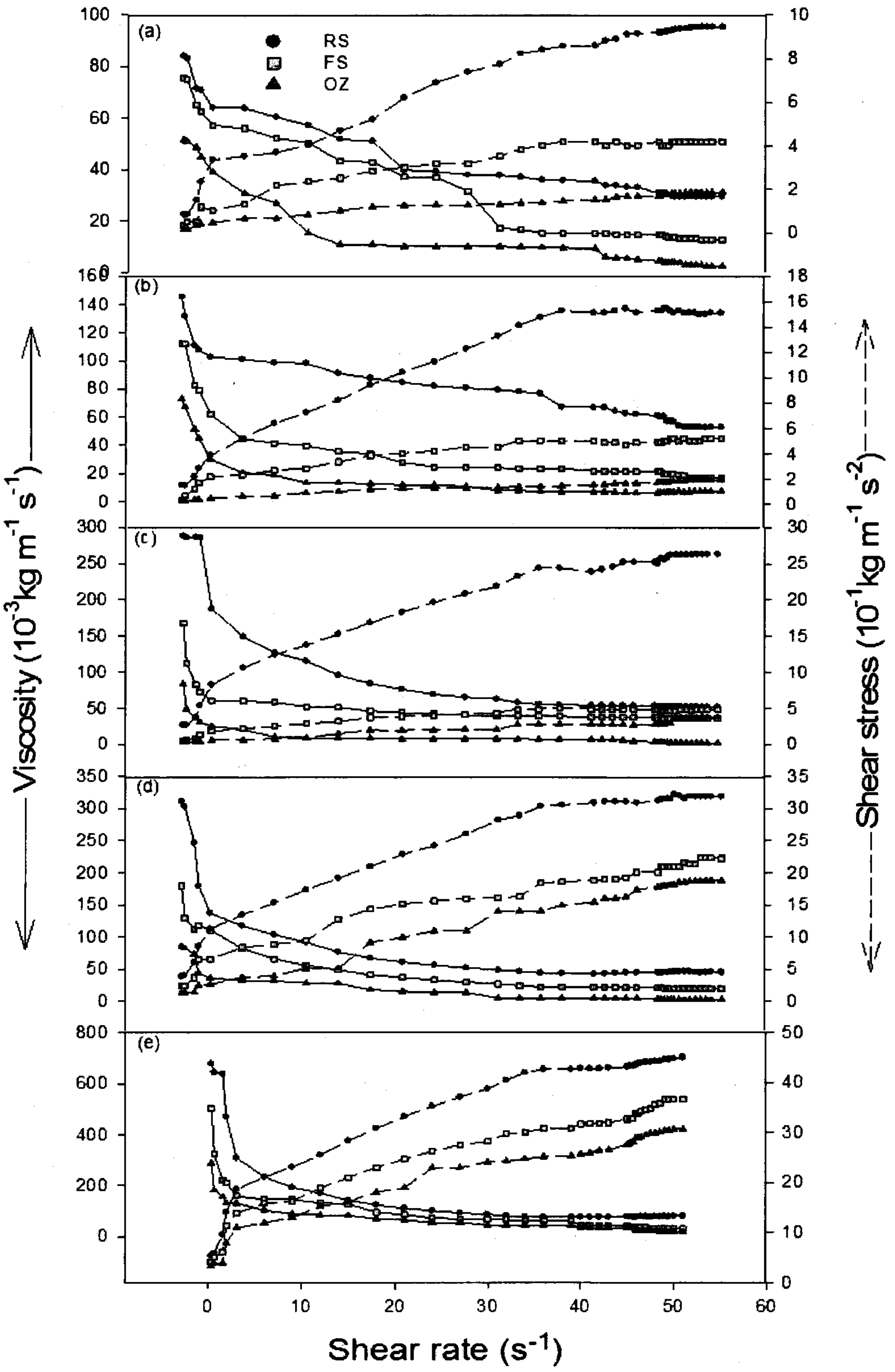

Figure 1. Rheograms of Raw sludge (RS), Ferro-sonication (FS) and Ozonation (OZ) pre-treated sludge at solids concentration of : (a) 15 g L-1; (b) 20 g L-1; (c) 25 g L-1; (d) 30 g L-1; (e) 35 g L-1

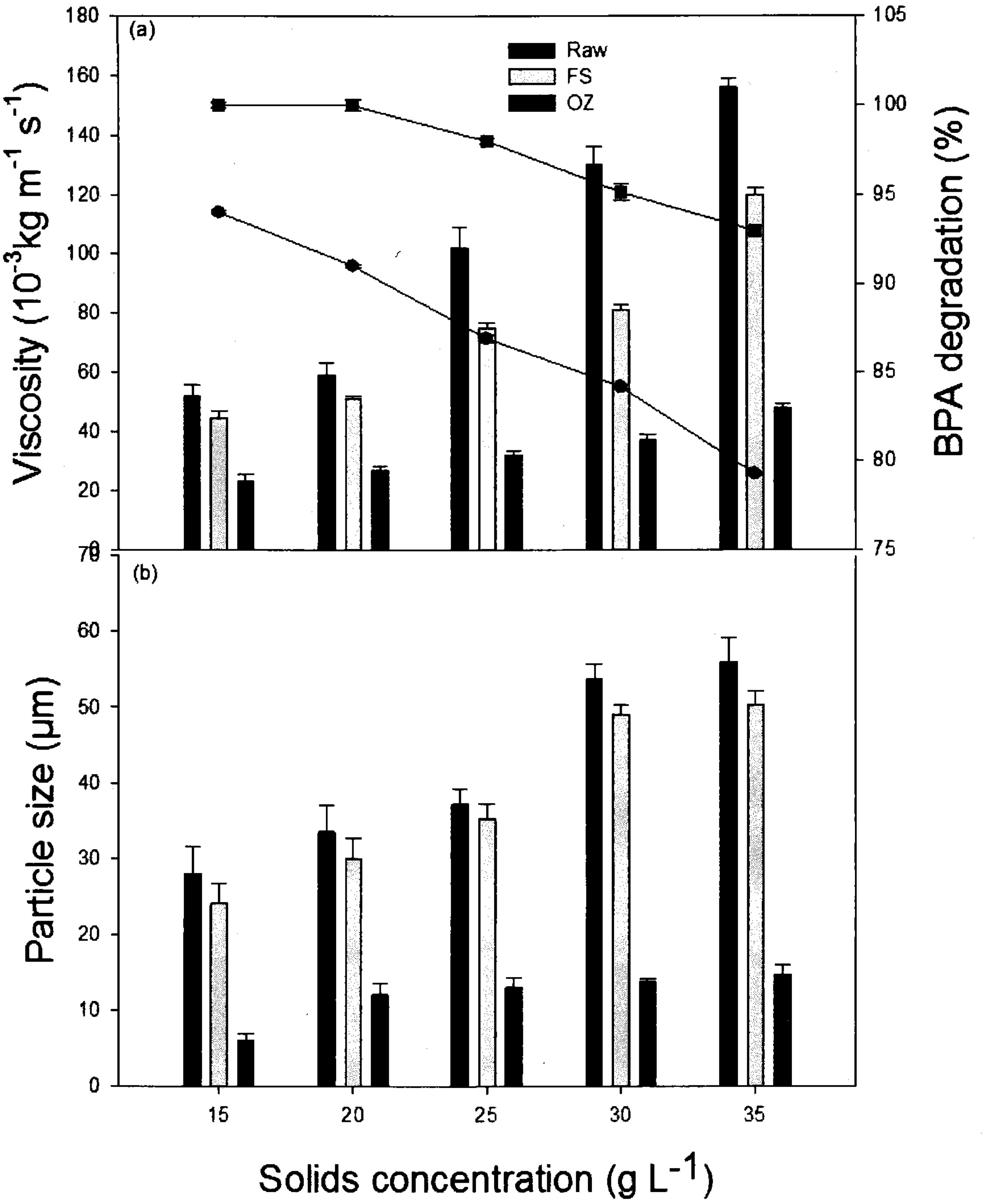

Figure 2. Effects of FS and OZ pre-treatment processes on viscosity and particle size on removal of BPA from raw sludge (■ozonation and •ferro-sonication pre-treatment): (a) viscosity; (b) particle size change

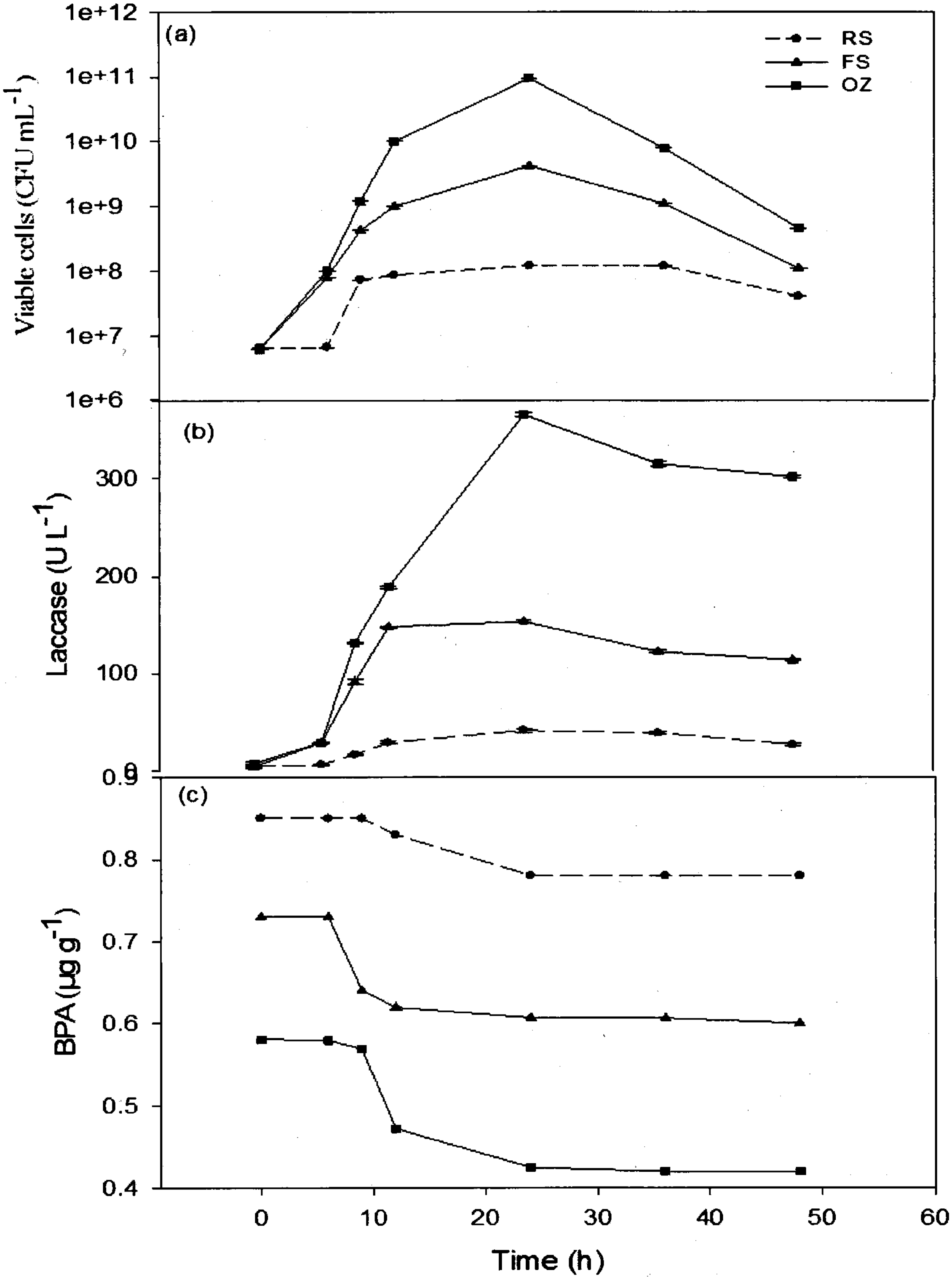

Figure 3. S. meliloti growth and production of laccase for degradation of BPA in raw sludge, ferro-sonication (FS) and ozonation (OZ) pre-treated sludge: (a) S. meliloti growth curve; (b) laccase activity; and (c) BPA degradation

CHAPITRE VI

PRÉTRAITEMENTS UTILISÉS POUR LA DÉGRADATION DE LA CBZ

A COMPARATIVE STUDY OF ULTRASONICATION, FENTON'S OXIDATION AND FERRO-SONICATION TREATMENT FOR DEGRADATION OF CARBAMAZEPINE FROM WASTEWATER AND TOXICITY TEST BY YEAST ESTROGEN SCREEN (YES) ASSAY

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RÉSUMÉ

Une étude comparative des procédés d'oxydation avancés (POAs) comme l'ultrasonication (US), l'oxydation de Fenton (OF) et ferro-sonication (FS) (la combinaison d'ultrasonication et d'oxydation de Fenton) pour la dégradation de carbamazepine (CBZ) des eaux usées (EU) est rapportée pour la première fois. Le CBZ est utilisé comme médicament antiépileptique dans le monde entier, trouvé comme un contaminant émergent persistant dans beaucoup de stations des d'épuration de traitement des eaux usées (STEU) et d'autres environnements aquatiques. Les traitements d'oxydation des EU ont causé une élimination efficace du médicament. Parmi les prétraitements d'US, de OF et de FS effectués, les taux les plus élevées en demande chimique en oxygène soluble (DCOs) et en carbone organique soluble (COs) (63 à 86 % et 21 à 34 %) ont été observés durant le prétraitement de OF, du à l'élimination la plus élevée de CBZ (84 à 100 %) des EU. En outre, l'analyse de sous-produits formés pendant les prétraitements d'US, de l'OF et du FS des EU a été effectuée en utilisant l'ionisation de produits chimiques par pression désorption-atmosphérique par laser à diode thermique (LDTD-APCI) couplé au tandem de la spectrométrie de masse (MS/MS). L'analyse au LDTD-APCI-MS/MS a indiqué la formation de deux sous-produits, comme epoxycarbamazepine et hydroxycarbamazepine du à la réaction des radicaux hydroxyles ($OH^{\bullet}$) avec CBZ pendant les trois type de procédés de prétraitement. De plus, l'activité oestrogénique des échantillons prétraités par l'US, l'OF et le FS avec le CBZ et ses sous-produits a été effectuée par la méthodologie des tests du criblage d'Œstrogène de levure (COL). Basé sur les résultats des tests de COL, aucun des échantillons prétraités n'a montré une activité oestrogénique.

Mot-clé : Procédés d'oxydation Avancés; sous-produits; Carbamazepine; Méthodologie des tests du criblage d'Œstrogène de levure; Eaux usées

ABSTRACT

A comparative study of ultrasonication (US), Fenton's oxidation (FO) and ferro-sonication (FS) (combination of ultrasonication and Fenton's oxidation) advanced oxidation processes (AOPs) for degradation of carbamazepine (CBZ) from wastewater (WW) is reported for the first time. CBZ is a worldwide used antiepileptic drug, found as a persistent emerging contaminant in many wastewater treatment plants (WWTPs) effluents and other aquatic environments. The oxidation treatments of WW caused an effective removal of the drug. Among the various US, FO and FS pre-treatments carried out, higher soluble chemical oxygen demand (SCOD) and soluble organic carbon (SOC) increment (63 to 86% and 21 to 34%, respectively) was observed during FO pre-treatment process, resulting in higher removal of CBZ (84 to 100%) from WW. Furthermore, analysis of by-products formed during US, FO and FS pre-treatment in WW was carried out by using laser diode thermal desorption-atmospheric pressure chemical ionization (LDTD-APCI) coupled to tandem mass spectrometry (MS/MS). LDTD-APCI-MS/MS analysis indicated formation of two by-products, such as epoxycarbamazepine and hydroxycarbamazepine due to the reaction of hydroxyl radicals ($OH^{\cdot}$) with CBZ during the three type of pre-treatment processes. In addition, the estrogenic activity of US, FO and FS pre-treated sample with CBZ and its by-products was carried out by Yeast Estrogen Screen (YES) assay method. Based upon the YES test results, none of the pre-treated samples showed estrogenic activity.

Keyword: Advanced oxidation processes; By-products; Carbamazepine; Yeast estrogenic screen assay; Wastewater

1. INTRODUCTION

Pharmaceutically active compounds (PhACs) are an emerging environmental issue due to their presence in the aquatic environment and potential for impacts on wildlife and humans (Kolpin et al., 2002). The presence of PhACs and their metabolites have been detected in wastewater (WW) because of their high production level and pharmacokinetical behavior during normal therapeutic use (e.g., half-life, metabolism and urinary and fecal excretion among others) and were pointed out as the major source of discharge of these compounds to the environment (Miao et al. 2005; Mohapatra et al. 2012). To date, most attention has been focused on identification, fate and distribution of PhACs in municipal wastewater treatment plants (WWTPs), which are commonly found at very low concentrations (ppb level or low) (Radjenovic et al., 2009).

Among PhACs, Carbamazepine (CBZ) was the most frequently detected pharmaceutical residue in water bodies (Zhang et al., 2008). CBZ is used alone or in combination with other medications to control certain types of seizures. It is also used to treat trigeminal neuralgia (a condition that causes facial nerve pain). CBZ is in a class of medications called anticonvulsants. CBZ is also sometimes used to treat mental illnesses, depression, post traumatic stress disorder, drug and alcohol withdrawal, restless leg syndrome, diabetes insipidus, certain pain syndromes, and a disease in children called chorea (Miao and Metcalfe, 2003).

Due to extensive use of CBZ in day to day life, the parent compound and some of its metabolites are subsequently released to wastewater treatment plants (WWTPs) and the conventional technology of treatment used in WWTPs appears insufficient to completely remove this compound (Clara et al., 2004; Mohapatra et al., 2012). Investigations found that CBZ is persistent and its removal efficiency by WWTPs was mostly below 10% (based on 19 published studies) (Zhang et al., 2008). Advanced treatment technologies are required to effectively eliminate organic pollutants including CBZ from WW (Zhang et al., 2008). Several authors have reported the successful application of ultrasonication and Fenton's oxidation pre-treatment process for degradation of CBZ in aqueous solution (Naddeo et al., 2009; Ghauch et al., 2011; Li et al., 2012). However, most of the studies are only fortification based rather than considering real time concentration of CBZ so that the study results are often overestimated.

Ultrasonic treatment is considered as an advanced oxidation processes (AOP) that generates $OH^{\cdot}$ radicals through the acoustic cavitation. Destruction of chemicals is usually achieved through a combination of pyrolytic reactions occurring inside or near the bubble and hydroxyl radical-mediated reactions occurring in the bulk liquid (Eq. 1-4).

$$H_2O \rightarrow H + HO^{\bullet} \qquad (1)$$

$$H + O_2 \rightarrow HO_2^{\bullet} \qquad (2)$$

$$O + H_2O \rightarrow 2HO^{\bullet} \qquad (3)$$

$$H + O_2 \rightarrow O + HO^{\bullet} \qquad (4)$$

Further, Fenton's oxidation has been exploited in the laboratory and WWTPs for stabilization of organic matter including removal of organic contaminants, such as PhACs (Zhang et al., 2008). Homogenous oxidation with the Fenton reagent occurs in the presence of ferrous or ferric ions with hydrogen peroxide via a free radical chain reaction which produces hydroxyl radicals. The organic substances are removed during two stages of oxidation and coagulation.

$$Fe^{3+} + e^{-} \rightarrow Fe^{2+} \qquad (5)$$

$$H_2O_2 + Fe^{2+} \rightarrow OH^{-} + HO^{\bullet} + Fe^{3+} \qquad (6)$$

However, majority of research on ultrasonication and Fenton's oxidation or combination of both (ferro-sonication) for degradation of CBZ so far has been carried out in water. Therefore, the present study was conducted in WW with real concentration of CBZ comprising following objectives: i) effect of ultrasonication, Fenton's oxidation and ferro-sonication pre-treatment process for the degradation of CBZ from WW; ii) determination of the reaction end-products as an important indicator of the treatment effectiveness; and iii) the estrogenic activity of treated WW using Yeast Estrogen Screen (YES) assay.

2. MATERIAL AND METHODS

2.1. Chemicals

Carbamazepine was obtained from Sigma-Aldrich (St Louis, MO, USA). The internal standard (IS), carbamazepine-d_{10} was purchased from C/D/N Isotopes Inc. (Montreal, Quebec, Canada). HPLC-grade methanol (MeOH), acetonitrile, ethyl acetate, and acetone, were purchased from Fisher Scientific (Ontario, Canada). Sep-Pak Plus C18 environmental cartridges used for solid phase extraction (SPE) clean-up was purchased from Waters (Milford, MA, USA). HPLC grade water was prepared in the laboratory using a Milli-Q/Milli-RO Millipore system (Milford, MA, USA).

2.2. Wastewater sample

Samples were collected from Quebec Urban Community (CUQ) wastewater treatment plant (Beauport, Quebec city, Quebec, Canada) which receives wastewater originating from domestic zones, industries, commercial enterprises and institutions present in the city. The eastern station of WWTP serves a population of 528,016 (2006 estimate) and has a treatment capacity of 400,000 m^3 day^{-1}. The CUQ treatment plant accomplishes primary and physical-chemical treatment of sewage before discharging the treated water into the Saint-Lawrence River.

Samples of WW (post-secondary treatment effluent before UV treatment) were collected in pre-cleaned glass amber bottles with aluminum foil-lined caps. Furthermore, the WW samples were immediately stored at 4±1 ^0C until treatment. The characteristics of the WW used for the experiment is presented in Table 1. The efficiency of WWTP to remove CBZ from WW was previously determined as 38% (Mohapatra et al., 2012).

2.3. Pre-treatment of wastewater

Different pre-treatments, such as ultrasonication, Fenton's oxidation and ferro- sonication were conducted at room temperature. The concentration of CBZ was measured in WW before and after each pre-treatment. In each case, a spiked CBZ sample (100 μg L^{-1}) was treated to compare the results with the unspiked sample (real WWTP samples). All the pre-treatments for degradation of CBZ from WW were carried out for 180 min as a steady state was observed in each case after this reaction time under different conditions used in this experiment.

2.3.1. Ultrasonic pre-treatment

The ultrasonication was carried out using ultrasonic homogenizer Autotune 750W (Cole-Parmer Instruments, Vernon Hills, Illinois, US). The ultrasonication equipment was operated at a frequency of 20 kHz by using titanium probe with a tip diameter of 12 mm. The ultrasonication was carried out with ultrasonic intensity of 5.8 W cm^{-2}, 12.4 W cm^{-2} and 16 W cm^{-2} for 180 min. The ultrasonic intensity was defined as the actual power dissipated by the apparatus divided by the area of the transducer. For each sonication experiment, 400 mL of WW was filled in a 1 L beaker and the ultrasonic probe was dipped 2 cm into the solution. Batch experiments were carried out without temperature regulation (no cooling). Henceforth, ultrasonication carried out with ultrasonic intensity of 5.8 W cm^{-2}, 12.4 W cm^{-2} and 16 W cm^{-2} was referred to as US1, US2 and US3, respectively.

2.3.2. Fenton's oxidation pre-treatment

WW sample of 400 mL was acidified to pH 3 by using 10 N H_2SO_4 and placed in a 1 L flask. Fenton's oxidation process was conducted at pH 3 to avoid iron hydroxide precipitation. To initiate Fenton's oxidation reaction, H_2O_2 and $FeSO_4$ solution were added to the WW samples with three different conditions: 0.2 mM, 0.4 mM, and 0.8 mM H_2O_2 and $[H_2O_2]_0/[Fe^{2+}]_0 = 50$. Fenton's oxidation was carried out at ambient temperature for 180 min using a shaker at 150 rpm. Henceforth, Fenton's oxidation with 0.2 mM, 0.4 mM, and 0.8 mM H_2O_2 concentration will be referred as FO1, FO2 and FO3, respectively.

2.3.3. Ferro-sonication pre-treatment

Four hundred milliliters of WW was acidified to pH 3 by using 10 N H_2SO_4 and placed in a 1 L flask. Ferro-sonication pre-treatment was carried out by addition of 0.2 mM of $FeSO_4$ solution in WW followed by ultrasonic pre-treatment under three conditions of ultrasonic intensity of 5.8 W cm^{-2}, 12.4 W cm^{-2} and 16 W cm^{-2} for 180 min. Ferro-sonication pre-treatment carried out with 5.8 W cm^{-2}, 12.4 W cm^{-2} and 16 W cm^{-2} ultrasonic intensity will be henceforth referred to FS1, FS2 and FS3, respectively.

2.4. Analysis

2.4.1. General

Analyses of total and soluble chemical oxygen demand (TCOD and SCOD), total and soluble organic carbon (TOC and SOC) were carried out as per the Standard Methods (APHA, 2005). The SCOD and SOC solubility increment were calculated as follows:

$$\mathrm{SCOD_{increment}} = \frac{(\mathrm{SCOD_S} - \mathrm{SCOD_0})}{\mathrm{TCOD}} \times 100 \qquad (7)$$

$$\mathrm{SOC_{increment}} = \frac{(\mathrm{SOC_S} - \mathrm{SOC_0})}{\mathrm{TOC}} \times 100 \qquad (8)$$

2.4.2. Carbamazepine analysis

The WW samples were filtered through a 0.45 µm glass-fiber (Fisherbrand G6 filter circles, Fischer Scientific, Ontario, Canada) and stored immediately at 4±1 °C, until analysis. Solid phase extraction (SPE) method was used for clean-up. Sep-Pak Plus C18 environmental cartridges were fitted into the vacuum manifold (Welch, USA) which was connected to a vacuum pump (Welch Rietschle Thomas, USA) to dispense samples through the cartridges. Cartridges were pre-conditioned by passing 7 mL of methanol and 3 mL of HPLC water at a flow rate of 1 mL min^{-1}. Filtered samples (200 mL) were passed at a flow rate of 5 mL min^{-1}. After pre-concentration, the sorbents were dried by using a vacuum system set at (-15) psi.

The elution was performed by adding 2 x 4 mL of methanol to the cartridge at a flow rate of 1 mL min^{-1} and giving it a wait time of 10 min in order to give enough duration of contact between the solvent and the adsorbed compounds. The extracts were later evaporated to dryness with a gentle stream of nitrogen and reconstituted with methanol to a final volume of 200 µL prior to Laser Diode Thermal Desorption-Atmospheric Pressure Chemical Ionization-Mass Spectrometry/Mass Spectrometry (LDTD-APCI-MS/MS) analysis.

2.4.3. LDTD-APCI-MS/MS Analysis

Quantification of CBZ was achieved with the LDTD-APCI ionization source (Phytronix Technologies, Quebec, Canada) mounted on a TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples were first spotted (2 µL) into the LazWell 96-well polypropylene plate cavities containing inserts made of proprietary stainless steel alloy and left to dry at room temperature. The designed well shape allows the sample to concentrate in the heating zone while drying. The loaded plate is then transferred to an *X-Y* movable stage of the LDTD housing unit. An infrared (IR) laser diode (980 nm, 20 W, continuous) is then focalized to impact the back of the inserts, thermally desorbing the dried sample, which is vaporized into gas phase. Quantification of CBZ was performed by the internal standard method based on peak areas and relative retention time, using carbamazepine d_{10} as internal standard. A five point standard addition calibration curve ranging from 0.01 µg L^{-1} to 20 µg L^{-1} was used. The area ratio (analyte:internal standard) was plotted against the analyte additions to obtain the calibration curve. Calibration curve was obtained using linear regression analysis and the established concentration range gave good correlation coefficients ($r^2 > 0.999$).

The analysis was carried out in negative ionization mode with following conditions: laser pattern programming of 1 s ramp from 0% to 25%, held for 3 s in 25% before shutting off the laser.; carrier gas flow rate (3 L/min); deposition volume (2 µL); sheath gas pressure (0 arbitrary units); auxiliary gas pressure (0 arbitrary units); skimmer offset (6 V); capillary temperature (270 °C); collision pressure (1.5 mTorr) and scan time (0.25 s).

2.5. Yeast Estrogen Screen (YES) assay

The YES assay described by Routledge and Sumpter (1996) was used to measure estrogenic activity of pre-treated (ultrasonication, Fenton's oxidation and ferro-sonication) wastewater samples. All samples were sterilized at 121 ± 1 °C for 30 min prior to YES assay. Standard stock solutions of CBZ were made in ethanol and all serial dilutions were carried out in ethanol. The procedure for measurement of the total estrogenic activity was carried out by serial dilution of each sample concentrate (100 µL) in ethanol across 12 wells in a 96-well plate (Costar Brand, NY, USA). Each dilution series was initiated by placing 200 µL of the sample concentrate in the first well of the single row. The volume of 100 µL was transferred to the second column and mixed with 100 µL of ethanol. The process was repeated across the row. Aliquots of 5 µL of each concentration were transferred to a 96-well plate. Further, aliquots (195 µL) of the seeded assay medium containing the yeast (hER-transfected recombinant yeast) and chlorophenol red-β-d-galactopyranoside (CPRG) were then dispensed into each sample well. Each plate contained at least one row of blanks (assay medium and solvent ethanol) and a standard curve for 17β-estradiol. The plates were sealed with parafilm and shaken at 150 rpm (to promote suspension of the yeast cells and constant mixing with the assay medium) for a 3-d incubation period at 32 ± 1 °C. The color development of the medium was checked periodically at an absorbance of 540 nm using a multireader microplate spectrophotometer (Epoch, BioTek, USA).

3. RESULTS AND DISCUSSION

3.1. Carbamazepine removal in wastewater

The effectiveness of ultrasonication (US), Fenton's oxidation (FO) and ferro-sonication (FS) processes was analyzed in terms of removal of CBZ from WW. Fig. 1 presents the SCOD and SOC increment and removal of CBZ from WW during different pre-treatment methods. Higher removal of CBZ was observed in Fenton's oxidation (84 to 100%) as compared to ultrasonic (22 to 51%) and ferro-sonication (62 to 93%) processes. The order of efficiency of pre-treatment methods for CBZ removal from WW was: FO3 > FO2 > FO1 >FS3 >FS2 >FS1 >US3 >US2 >US1, respectively. The reason for higher removal of CBZ during Fenton and ferro-sonication pre-treatment processes as compared to ultrasonic pre-treatment process, was due to the addition of $FeSO_4$, which enhanced the formation of hydroxyl ($OH^{\cdot}$) radicals in the solution (Equation 6). The hydroxyl radicals generated during Fenton and ferro-sonication pre-treatment processes have an oxidizing potential of 2.8 V as compared to normal hydrogen electrode

(NHE) and are capable of oxidizing a wide range of organic compounds including CBZ in WW (Kavitha and Palanivelu, 2004). Further, higher removal of CBZ in Fenton's oxidation processes as compared to ferro-sonication is believed to be due to the higher efficiency of production of $OH^{\bullet}$ radicals.

The initial TCOD, SCOD, TOC and SOC value observed in WW were 0.56 g L^{-1}, 0.13 g L^{-1}, 0.27 g L^{-1} and 0.12 g L^{-1}, respectively. Higher SCOD and SOC increment (Eq. 7 and 8) was observed during Fenton's oxidation (63 to 86% and 21 to 34%, respectively) pre-treatment processes as compared to ultrasonic (12 to 28% and 7 to 15%, respectively) and ferro-sonication (34 to 58% and 18 to 29%, respectively) pre-treatment processes (Fig. 1). The increased SCOD and SOC increment during Fenton and ferro-sonication pre-treatment processes as compared to ultrasonic pre-treatment processes was due to acid hydrolysis and partial oxidation (hydroxyl radicals) of organic matter. During these pre-treatment processes, organic substances were transferred from solid to the aqueous phase, which resulted in the increase of soluble protein and soluble carbohydrate concentration in the aqueous phase and decrease of COD and SOC in the solid phase.

3.2. Analysis of by-products formed during ultrasonication, Fenton's oxidation and ferro-sonication pre-treatment of wastewater

To identify different by-products formed during CBZ degradation by ultrasonication, Fenton's oxidation and ferro-sonication treatment of WW, the experiments were carried out in samples with; i) CBZ spiked in milli-Q water, ii) CBZ spiked in WW, and iii) real WW sample (without spiking). A comparison was done in each case to identify the intermediates. The study of CBZ oxidation by-products was carried out by using LDTD- -MS/MS experiments that were run in both positive and negative electrospray modes.

LDTD-MS/MS chromatography of CBZ oxidation by-products formed during ultrasonication, Fenton's oxidation and ferro-sonication pre-treatment of WW is presented in Fig. 2. Similar by-products were observed in all the three advanced oxidation processes (AOPs), epoxycarbamazepine (retention time of 2.9 min) and hydroxycarbamazepine (retention time of 5.1 min) due to the reaction of $OH^{\bullet}$ radicals with CBZ. Both by-pafter with 180 min of reaction time. In order to conform that these by-products were formed during oxidative treatments, a comparison was made between LDTD-MS/MS chromatography with and without treatment of WW.

A proposed possible pathway of degradation of CBZ during ultrasonication, Fenton's oxidation and ferro-sonication pre-treatment processes is presented in Fig. 3. However, a study by Ghauch et al. (2011) showed both, epoxycarbamazepine and hydroxycarbamazepine as by-products which disappeared at the end of reaction during degradation of CBZ by ultrasonic/Fe^0/H_2O_2 systems.

3.3. Estrogenic activity of ultrasonic, Fenton's oxidation and ferro-sonication pre-treated wastewater

Estrogenic activity was carried out in spiked synthetic wastewater. Before starting YES assay, each pre-treated (US3, FO3 and FS3) WW samples (spiked and unspiked) were filtered through a 0.45 µm glass-fiber (Fisherbrand G6 filter circles, Fischer Scientific, Ontario, Canada) and autoclaved at 121 ±1 °C for 30 min to inactivate bacterial growth. The YES test is an assay system in which the human estrogen receptor (hER) is expressed in yeast in a form capable of activating transcription of a promoter carrying estrogen-responsive sequences (ERE), in an estrogen-dependent manner (Routledge et al., 1998). The sensitivity and reproducibility of the estrogen screen were assessed by measuring the response of the yeast to solution with 17β-estradiol and different pre-treated WW spiked with CBZ. The change in color due to addition of 17β-estradiol and different pre-treated WW samples is presented in Fig. 4. The estrogenicity appeared with 17β-estradiol, as the wells are red in color after 3 d incubation. However, none of the pre-treated samples spiked and unspiked with CBZ showed any change in color.

Fig. 5 shows the response of the yeast to ultrasonication, Fenton's oxidation and ferro-sonication pre-treated WW spiked and unspiked with CBZ. The blank remained within the absorbance range of 0.8 to 0.9 (yellow to light orange) for a 3 d incubation. The results showed that CBZ has no estrogenic activity. None of the pre-treated WW samples tested were estrogenic (absorbance range 0.8 to 1.2). The presented results proved that ultrasonication, Fenton's oxidation and ferro-sonication pre-treated WW samples with CBZ and its different by-products (epoxycarbamazepine and hydroxycarbamazepine) are not estrogenic. Hence, these pre-treatments may be important and essential for safe disposal of wastewater, however more studies recommended on toxicity test (battery of assays) to conform results obtained from testing procedures. Furthermore, complementary experiments should be performed to obtain other data from these compounds, especially their toxicity. If it appears that these by-products are more toxic than their precursor (which is often observed with the primary oxidation by-products), it would indicate that longer treatment duration is necessary.

3.4. Cost-efficiency of pre-treatment methods

The economic study included chemical and energy consumption. The electric cost was estimated to be US \$ 0.09 kW h^{-1}. The total cost was evaluated in terms of U.S. dollars spent per cubic meter of the treated solution (US\$ m^{-3}). A comparison of three pre-treatment methods on the basis of cost analysis showed that the expensive setup and hardware costs, operational skills as well as higher treatment time are limiting factors for the applicability of an ultrasonicator. Therefore, Fenton method may be consider as an effective for treatment of wastewater due to shorter treatment time and reduced operational cost for real wastewater treatment plant.

4. CONCLUSIONS

The study on removal of CBZ in WW by ultrasonication, Feton's oxidation and ferro-sonication pre-treatment processes and toxicity test led to following conclusions:

(1) Among ultrasonication, Fenton's oxidation and ferro-sonication pre-treatment, higher SCOD and SOC increment (63 to 86% and 21 to 34%, respectively) was observed during Fenton's oxidation of wastewater resulting in higher removal (84 to 100%) of carbamazepine.

(2) Similar oxidative by-products of carbamazepine degradation, epoxycarbamazepine and hydroxycarbamazepine were observed during ultrasonication, Fenton's oxidation and ferro-sonication pre-treatment of wastewater.

(3) The Yeast Estrogen Screen (YES) assay results showed that ultrasonication, Fenton's oxidation and ferro-sonication pre-treated wastewater with carbamazepine were not toxic to the yeast (not estrogenic).

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Table 1. Characteristics of the wastewater used for experiment

Parameters	**Value**
pH	7.27±0.4
TS (g L^{-1})	1 ± 0.77
SS (g L^{-1})	0.3 ± 0.05
VS (g L^{-1})	0.07 ± 0.03
VSS (g L^{-1})	0.01 ± 0.002
TCOD (g L^{-1})	0.56 ± 0.02
SCOD (g L^{-1})	0.13 ± 0.05
Ammoniacal nitrogen (g L^{-1})	0.003 ± 0.0
Phosphorus (g L^{-1})	0.001 ± 0.0
TOC (g L^{-1})	0.27 ± 0.05
SOC (g L^{-1})	0.12 ± 0.06
Alkalinity (g L^{-1})	0.16 ± 0.01
Carbamazepine (CBZ) (ng L^{-1})	281 ± 7

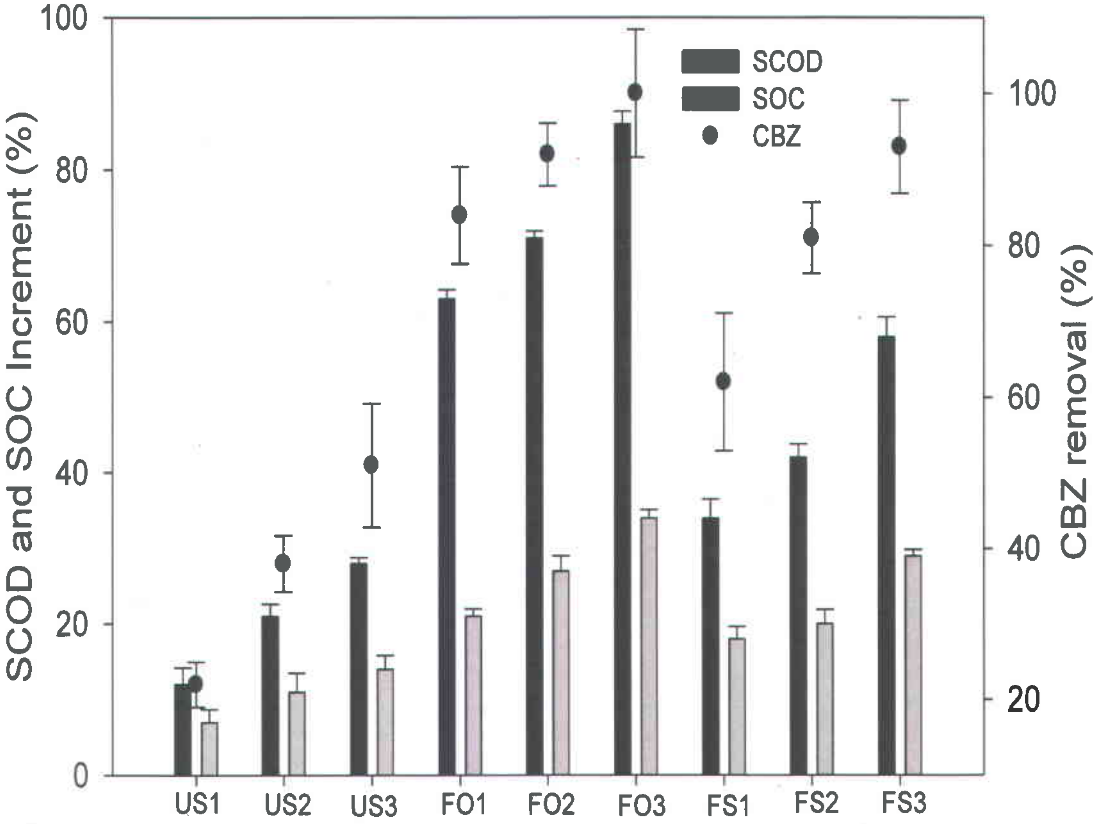

Figure 1. **CBZ removal, SCOD and SOC increment during the different conditions of ultrasonication, Fenton's oxidation and ferro-sonication (combination of ultrasonic and Fenton's oxidation) treatment of wastewater**

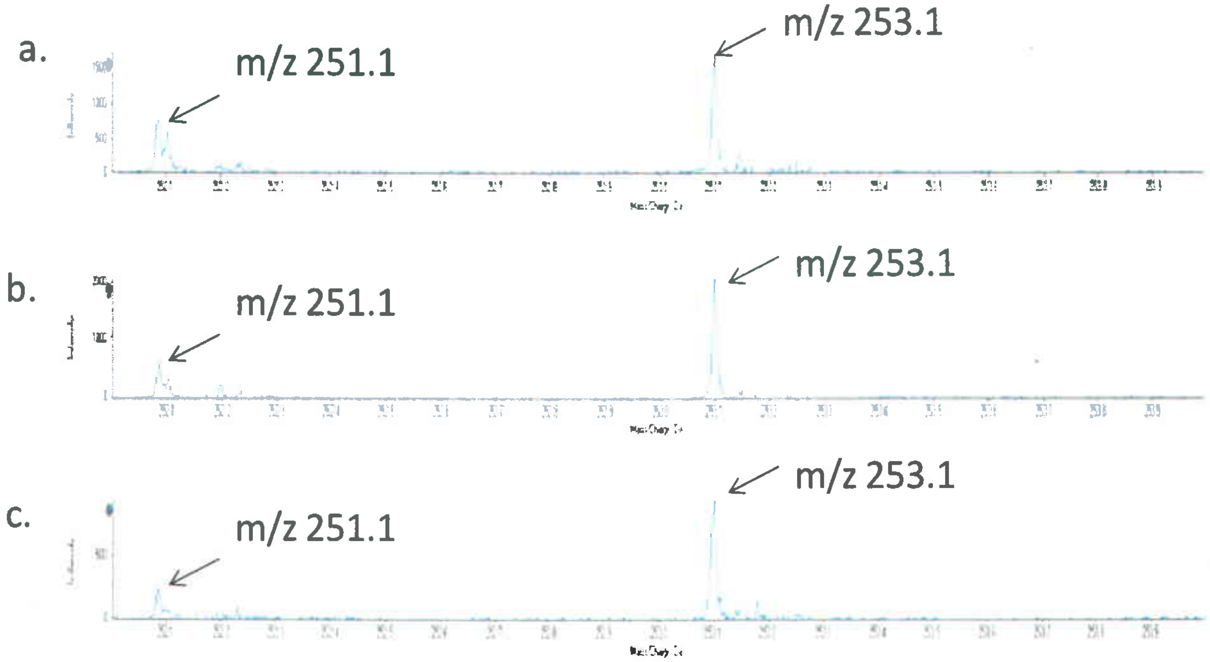

Figure 2 . Mass spectra of CBZ oxidation products formed with 180 min reaction time (epoxycarbamazepine (m/z 251.1) and hydroxycarbamazepine (m/z 253.)) in WW during different pre-treatments: (a) ultrasonication; (b) Fenton's oxidation, and (c) ferro-sonication

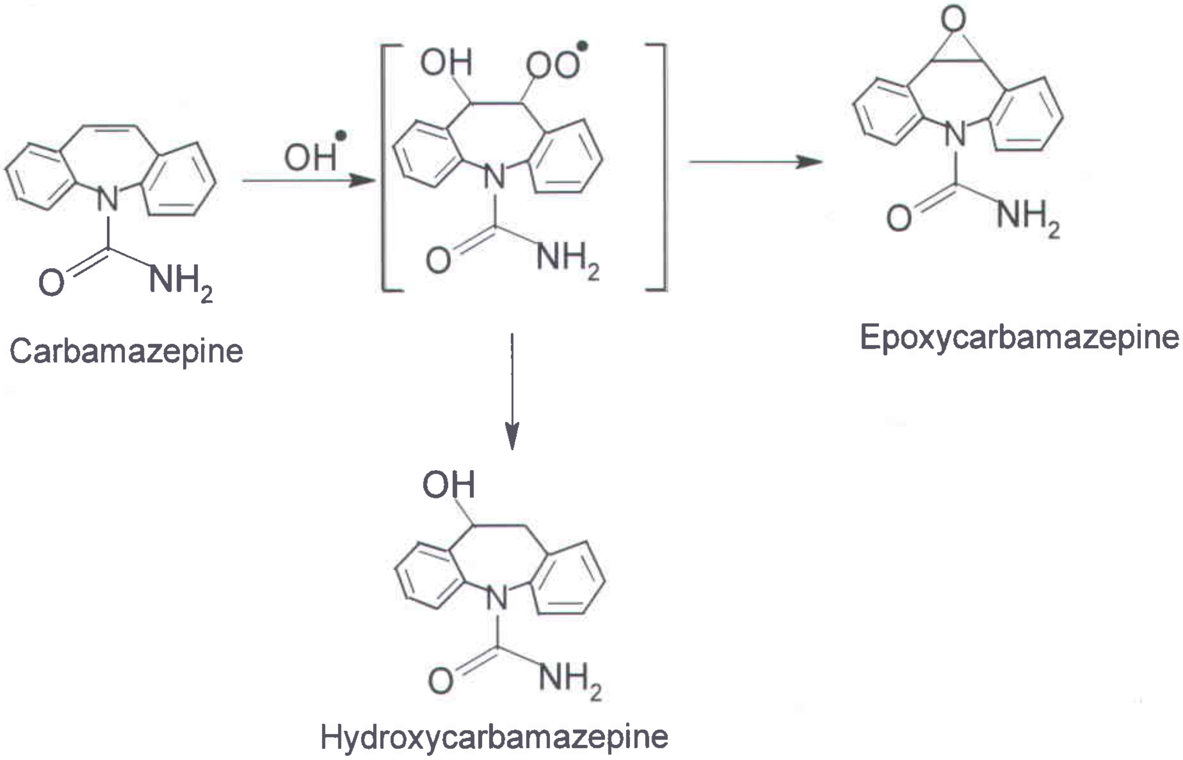

Figure 3. Carbamazepine degradation pathways induced by reaction with hydroxyl radical produced during ultrasonication, Fenton's oxidation and ferro-sonication treatment

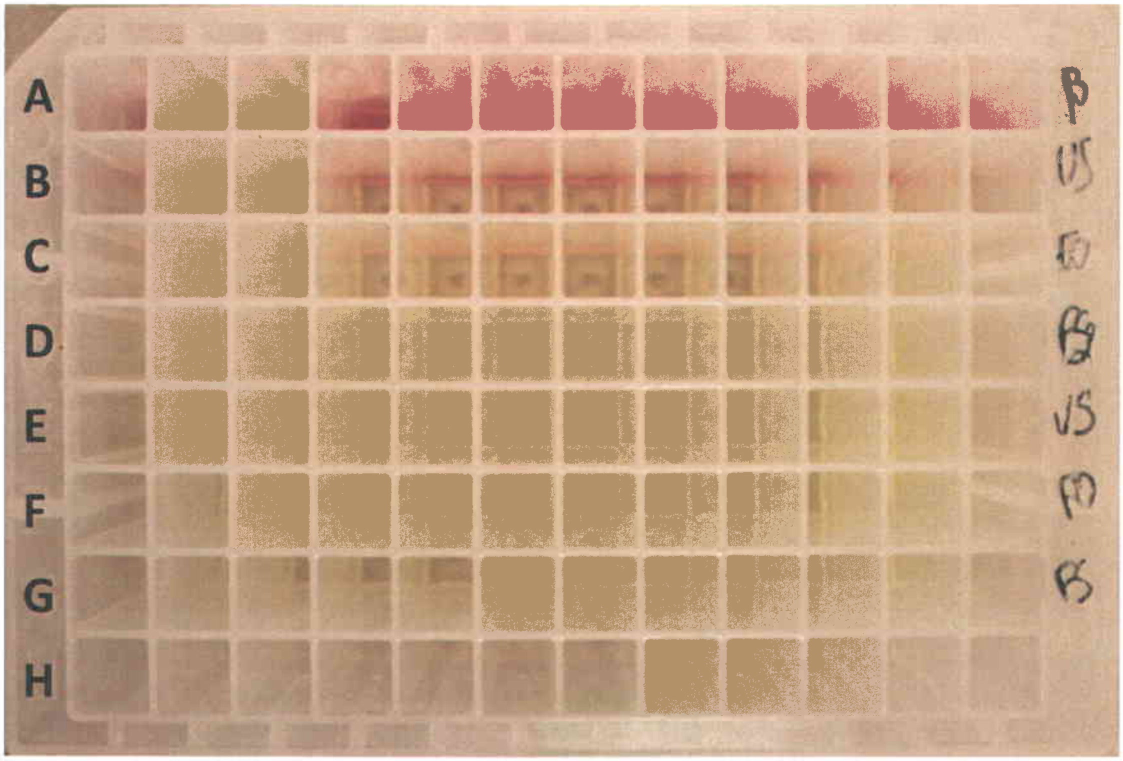

Figure 4. YES assay of E2 (17-β estradiol) (row A) and US, FO and FS pre-treated wastewater spiked (row B-D) and unspiked (row E-G) with carbamazepine and blank (row H)

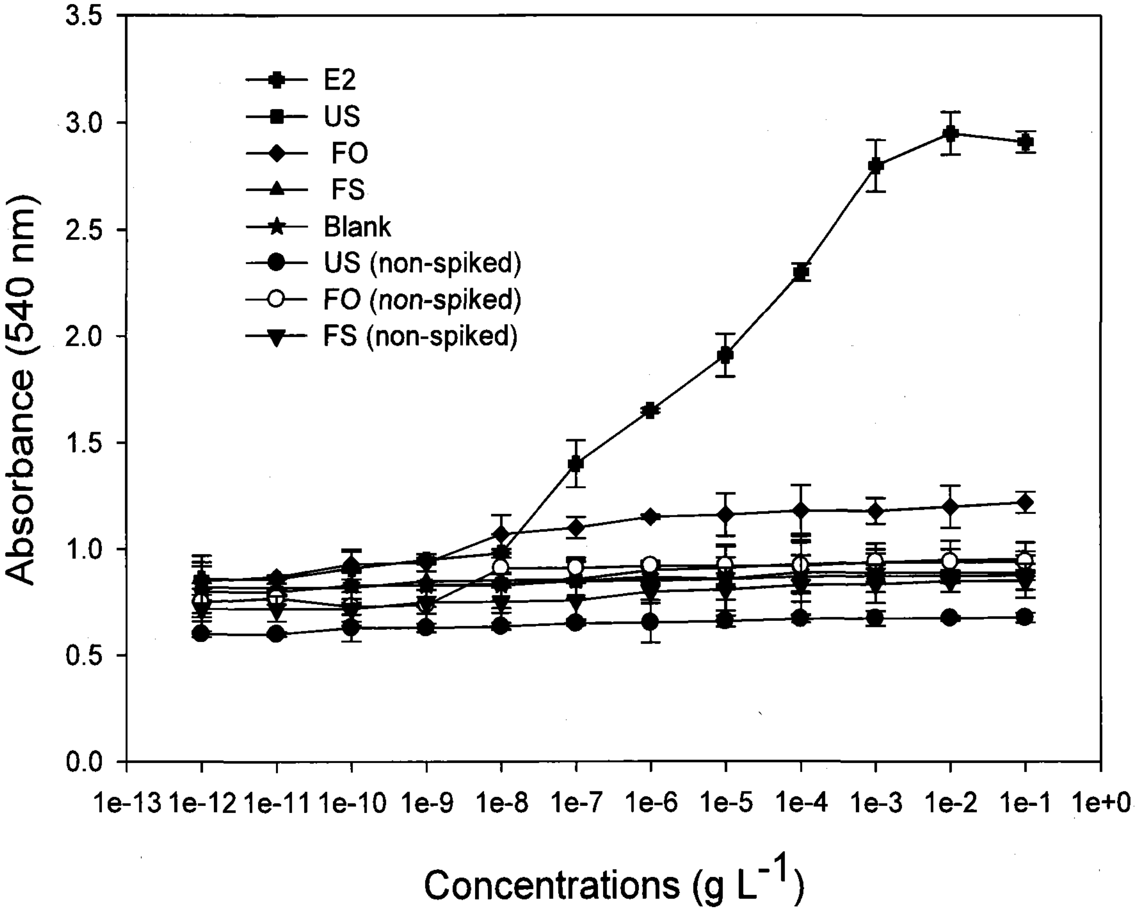

Figure 5. YES assay curves for E2 and US, FO and FS pre-treated wastewater spiked and non-spiked with carbamazepine

CHAPITRE VII

DÉGRADATION DU BPA ET DE LA CBZ PAR LES NANOPARTICULES

PARTIE 1

PREPARATION OF A NEW CLASS OF WHEY-STABILIZED ZnO NANOPARTICLES FOR DEGRADATION OF BISPHENOL A IN WATER

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PARTIE 2

PHOTOCATALYTIC DEGRADATION OF CARBAMAZEPINE IN WASTEWATER BY USING A NEW CLASS OF WHEY-STABILIZED NANOCRYSTALLINE TiO_2 AND ZnO

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APPLIED CATALYSIS B: ENVIRONMENTAL (UNDER REVIEW)

RÉSUMÉ

Les photocatalyseurs nanométriques ont attiré beaucoup d'attention en raison de leur grand ratio surface/volume. Toutefois, en raison de leur réactivité très élevée, les nanoparticules de TiO_2 et ZnO préparées selon différents procédés ont tendance soit à réagir avec les *milieux* environnant ou avec les agglomérats, ce qui entraîne la formation de flocs beaucoup plus grands et la perte significative de réactivité. Ce travail étudie la dégradation photocatalytique de la carbamazépine (CBZ), un composé pharmaceutique persistant dans les eaux usées (WW) à l'aide de nanoparticules de TiO_2 et de ZnO préparées en présence d'une poudre de lactosérum soluble dans l'eau, utilisée comme agent stabilisant. Les nanoparticules de TiO_2 et de ZnO préparées en présence d'un agent stabilisant (lactosérum) ont fait preuve d'une agglomération beaucoup moins et une capacité de dégradation beaucoup plus élevée que celle des mêmes nanoparticules préparées sans stabilisant. La dégradation photocatalytique (temps d'irradiation = 55 min) de la carbamazépine à l'aide de nanoparticules de TiO_2 stabilisées par la poudre de lactosérum (100%) était plus élevée que celle à l'aide des nanoparticules de ZnO (92%). La dégradation de la CBZ dans les eaux usées à l'aide de nanoparticules de TiO_2 était plus élevée que celle à l'aide de nanoparticules de ZnO. Cela était dû à la formation d'un nombre plus grand de trous photo-générés, vu que le pouvoir oxydant de TiO_2 est plus élevé. La capacité photocatalytique du ZnO était prévue égale à celle de TiO_2 comme, ils ont le même intervalle de bande d'énergie (3,2 eV). Toutefois, dans le cas de ZnO, une photocorrosion se produit fréquemment avec l'illumination de la lumière UV, et ce phénomène est considéré comme l'une des raisons principales de la baisse de l'activité photocatalytique de ZnO dans des solutions aqueuses. En outre, l'activité oestrogénique des échantillons prétraités contenant la CBZ et ses sous-produits a été analysée en utilisant le test d'œstrogénicité sur les levures (YES). En se basant sur les résultats de ce test, tous les échantillons prétraités n'ont pas montré une activité oestrogénique.

Mots-clés: Carbamazépine; Photocatalyse; test d'œstrogénicité sur levures; agent stabilisant (lactosérum) lactosérum; eaux usées

ABSTRACT

Nanoscale photocatalysts have attracted much attention due to their high surface area to volume ratios. However, due to extremely high reactivity, TiO_2 and ZnO nanoparticles prepared using different methods tend to either react with surrounding media or agglomerate, resulting in the formation of much larger flocs and significant loss in reactivity. This work investigates the photocatalytic degradation of carbamazepine (CBZ), a persistent pharmaceutical compound from wastewater (WW) using TiO_2 and ZnO nanoparticles prepared in the presence of a water-soluble whey powder as stabilizer. The TiO_2 and ZnO nanoparticles prepared in the presence of whey stabilizer displayed much less agglomeration and higher degradation power than those prepared without a stabilizer. Higher photocatalytic degradation of carbamazepine was observed (100%) by using whey stabilized TiO_2 nanoparticles with 55 min irradiation time as compared to ZnO nanoparticles (92%). The higher degradation of CBZ in wastewater by using TiO_2 nanoparticles as compared to ZnO nanoparticles was due to the formation of higher photo-generated holes with high oxidizing power of TiO_2. The photocatalytic capacity of ZnO anticipated as similar to that of TiO_2 as it has the same band gap energy (3.2 eV) as TiO_2. However, in the case of ZnO, photocorrosion frequently occurs with the illumination of UV light and this phenomenon is considered as one of the main reasons for the decrease of ZnO photocatalytic activity in aqueous solutions. Further, the estrogenic activity of photocatalyzed WW sample with CBZ and its by-products was carried out by Yeast Estrogen Screen (YES) assay method. Based upon the YES test results, none of the samples showed estrogenic activity.

Keywords: Carbamazepine; Photocatalysis; Yeast Estrogen screen assay; Whey stabilizer; Wastewater

1. INTRODUCTION

Pharmaceutically active compounds (PhACs) have become a subject of great interest to environmental researchers worldwide (Hao et al., 2007). The worldwide average per capita consumption of pharmaceuticals per year is estimated to be about 15 g and in industrialized countries, the value is expected to be in between 50 to 150 g (Alder et al., 2006). Due to their extensive use, presence in the aquatic environment and their potential for impacts on wildlife and humans, it is becoming an important environmental issue (Kolpin et al., 2002). To date, most attention has been focused on identification, fate and distribution of PhACs in municipal wastewater treatment plants (WWTPs), which are commonly found at very low concentrations (ppb level or low) (Radjenovic et al., 2009).

Carbamazepine (CBZ) is an established drug for the control of grand mal and psychomotor epilepsy and it is also effective in the treatment of trigeminal neuralgia. CBZ is also sometimes used to treat mental illnesses, depression, posttraumatic stress disorder, drug and alcohol withdrawal, restless leg syndrome, diabetes insipidus, certain pain syndromes, and a disease in children called chorea (Miao and Metcalfe, 2003). Once administered, the absorption of CBZ in the body is slow and unpredictable with approximately 2-3% of the applied dose discharged via urine (Elmquist et al. 1991). Approximately 72% of orally administered CBZ is absorbed, while 28% is unchanged and subsequently discharged through the faeces (RxList, 2006).

Due to extensive use of CBZ in day to day life, the parent compound and some of its metabolites are subsequently released to wastewater treatment plants (WWTPs). Due to the high production level and pharmacokinetic behavior of CBZ during normal therapeutic use (e.g., half-life, urinary and fecal excretion, and metabolism), the presence of CBZ and its metabolites have been detected in WWTPs and they were considered to be major source of discharge of CBZ to the environment (Daughton and Ternes, 1999). PhACs including CBZ are often released directly into the environment after use owing to a low removal rate in WWTPs, affecting the aquatic ecosystem (Andreozzi et al., 2002). Furthermore, instead of being discharged, effluents are being reused increasingly in many parts of the world, especially in arid and semi-arid countries, for irrigation and/or for aquifer replenishment in coastal regions where aquifers are subjected to overuse. Spreading wastewater (WW) on soil during reuse may result in the transfer of some PhACs including CBZ and their metabolites. Recent studies have revealed that CBZ was present in wastewater-irrigated soil with concentrations ranging from 0.02 to 15 ng g^{-1} dry matter (Kinney et al., 2006). Further, investigations found that CBZ is persistent and its removal efficiency by the WWTPs is mostly below 10% (based on 19 published studies) (Zhang et al., 2008). Therefore, it is necessary to treat the effluents containing PhACs including CBZ adequately before discharging or treating the water for drinking purposes by the water treatment plants.

Advanced oxidation processes (AOPs) have recently emerged as an important class of technologies for the oxidation and destruction of a wide range of organic pollutants including CBZ from WW (Chong and Jin, 2012). Different AOPs methods include heterogeneous and homogenous photocatalysis based on near ultraviolet (UV) or solar visible irradiation, electrolysis, ozonation, ultrasonication (US), Fenton's reagent, and wet air oxidation (WAO), while less conventional but evolving processes include ionizing radiation, microwaves, pulsed plasma and the ferrate reagent. In recent years, semiconductor photocatalysis has become more and more attractive and important since it has a great potential to contribute to such environmental problems. One of the most important aspects of environmental photocatalysis is in the selection of semiconductor materials, such as ZnO and TiO_2, which are close to being two of the ideal photocatalysts in several respects. For example, they are relatively inexpensive, and they provide photo-generated holes with high oxidizing power due to their wide band gap energy. As a well-known photocatalyst, TiO_2 and ZnO have received much attention in the degradation and complete mineralization of environmental pollutants including CBZ (Avisar et al., 2012). However, literature on the photocatalytic degradation of CBZ in WW is scarce as most of the studies were carried out in water. Furthermore, to the best of our knowledge there are no studies reported on estrogenic activity of WW after photocatalytic treatment for degradation of CBZ.

Due to the extremely high reactivity, TiO_2 and ZnO nanoparticles prepared by using different methods tend to either react with surrounding media or agglomerate, resulting in the formation of much larger flocs than desired and significantly loss in reactivity. To overcome these drawbacks, we developed a simple and green approach for synthesizing TiO_2 and ZnO nanopaerticles. We modified the conventional harsher chemical methods by applying a water soluble whey powder as a stabilizer.

The present study was conducted in WW with following objectives: i) preparation and characterization of whey-stabilized TiO_2 and ZnO nanoparticles; ii) suitability of whey-stabilized TiO_2 and ZnO nanoparticles for photocatalytic degradation of carbamazepine from wastewater; iii) determination of the reaction end-products as an important indicator of the treatment effectiveness; and iv) the estrogenic activity of treated wastewater using Yeast Estrogen Screen (YES) assay.

2. MATERIALS AND METHODS

2.1. Chemicals

Carbamazepine was obtained from Sigma-Aldrich (St Louis, MO, USA). The internal standard (IS), carbamazepine-d_{10} was purchased from C/D/N Isotopes Inc. (Montreal, Quebec, Canada). HPLC-grade methanol (MeOH), acetonitrile, ethyl acetate, and acetone, were purchased from Fisher Scientific (Ontario, Canada). Sep-Pak Plus C18 environmental cartridges used for solid phase extraction (SPE) clean-up was purchased from Waters (Milford, MA, USA). HPLC grade water was prepared in the laboratory using a Milli-Q/Milli-RO Millipore system (Milford, MA, USA).

2.2. Wastewater samples

Samples were collected from Quebec Urban Community (CUQ) wastewater treatment plant (Beauport, Quebec city, Quebec, Canada) which receives wastewater originating from domestic zones, industries, commercial enterprises and institutions present in the city. The eastern station of WWTP serves a population of 528,016 (2006 estimate) and has a treatment capacity of 400,000 m^3 day^{-1}. The CUQ treatment plant accomplishes primary and physical-chemical treatment of sewage before discharging the treated water into the Saint-Lawrence River.

Samples of WW were collected in pre-cleaned glass amber bottles with aluminum foil-lined caps. Furthermore, the WW samples immediately stored at 4±1 ^{0}C until analysis. The experiments were carried out in both real and synthetic WW. The concentration of CBZ observed in real WW (effluent before UV treatment) was 295 ng L^{-1}. So same amount of CBZ (295 ng L^{-1}) was spiked in the synthetic WW.

2.3. Preparation, characterization and measurements of TiO_2 and ZnO nanoparticles

2.3.1. Preparation of whey stabilized ZnO nanoparticles

The solution was prepared by dissolving 0.6 g of whey in 200 mL of milli Q water. Then, one gram of zinc nitrate ($Zn(NO_3)_2.4H_2O$) was dissolved in the solution and the pH of the solution was brought to 7.5 (Baruwati et al., 2006) by drop wise addition of 2 M NaOH aqueous solution. The reaction mixture was then stirred for 1 h at room temperature and transferred for heating by using a Multiwave-microwave sample preparation system (Paar Physica, Perkin Elmer) with infrared temperature control and hydraulic pressure control with feedback function capable of reducing microwave power near the limit of operating pressure. The microwave was operated at microwave power of 1000 W, operating pressure of 3000 kPa and temperature 160 ± 1 °C for 30 min. The hydrothermal reaction is represented in terms of two reaction steps: zinc hydroxide formation (Eq. 1) and dehydration of zinc hydroxide (Eq. 2)

$$\mathrm{Zn(NO_3)_2} + \mathrm{2NaOH} \rightarrow \mathrm{Zn(OH)_2} + \mathrm{2NaNO_3} \qquad (1)$$

$$\mathrm{Zn(OH)_2} \rightarrow \mathrm{ZnO} + \mathrm{H_2O} \qquad (2)$$

Further, after the hydrothermal process, the resultant white solid products were centrifugalized and washed several times with mili-Q water and finally dried at 80 °C for overnight. For comparison, ZnO nanoparticles in the absence of whey were prepared following similar procedure. Henceforth, the ZnO nanoparticles prepared in the presence and absence of whey will be referred as Whey-ZnO nano and ZnO nano, respectively.

2.3.2. Preparation of whey stabilized TiO_2 nanoparticles

Analytical grade titanium tetrachloride was adopted as the source material and sodium hydroxide as mineralizer. An aqueous solution of titanium was obtained by mixing one molar stoichiometric ratio of titanium isopropoxide (TTIP) in 200 mL of mili Q water mixed with 0.6 g of whey. The pH of the solution was brought to 7.5 (Vijayalakshmi et al., 2012) by drop wise addition of 2 M NaOH aqueous solution. The reaction mixture was then stirred for 1 h at room temperature and transferred for heating by using a Multiwave-microwave sample preparation system (Paar Physica, Perkin Elmer). The microwave was operated at microwave power of 1000 W, operating pressure of 3000 kPa and temperature 160 ± 1 °C for 30 min. Further, the resultant precipitate was centrifugalized and washed several times with mili-Q water and finally dried at 80

°C for overnight. For comparison, TiO_2 nanoparticles in the absence of whey were prepared following similar procedure. Henceforth, the TiO_2 nanoparticles prepared in the presence and absence of whey will be referred as Whey-TiO_2 nano and TiO_2 nano, respectively.

Further, for the comparision purpose, the TiO_2 and ZnO nanoparticles were prepared from commercial TiO_2 and ZnO (particle size in mm) (Fisher Scientific, Canada) by using Nano Spray Dryer (Buchi, Germany). Henceforth, TiO_2 and ZnO nanoparticles prepared by this method will be labeled as commercial TiO_2-nano and commercial ZnO-nano.

2.3.3. Characterization and measurement of ZnO and TiO_2 nanoparticles

Scanning electron micrographs were obtained using a Zeiss EM10 scanning electron microscope (SEM) (Zeiss, Thornwood, NJ) operated at 25 and 40 kV. The following general procedures were followed for the SEM measurements. A small amount of sample containing the nanoparticles was placed on a Formvar-carbon coated copper grid. The nanoparticles were then allowed for full contact/spreading on the grid. The bulk droplet was then wicked away with filter paper to remove the excess volume of the sample, and the grid was allowed to air-dry for 5 min. The residual nanoparticles attached to the grid were then imaged and photographed using the SEM.

The mean size and size distribution of nanoparticles were determined by using Zetasizer Nano ZS (Malvern Instruments Ltd., UK) (Mohapatra et al. 2013).

2.4. Photocatalytic degradation experiments

2.4.1. Casting of TiO_2 and ZnO films

One milligram of nanoparticles (Whey-TiO_2 nano; TiO_2 nano; commercial TiO_2-nano; Whey-ZnO nano; ZnO nano; and commercial ZnO-nano) was suspended in 1 mL of mili-Q water and sonicated for 15 min. The nanoparticle suspension was used for casting films on plain glass or quartz plates. A small aliquot (150 µL) was spread over an area of 1 cm^2 and air-dried. The semiconductor coated glass plates were then annealed in the oven for 1 h. The immobilized semiconductor films were used for photodegradation studies.

2.4.2. Photolysis experiments

All the photocatalysis experiments were carried out in a reactor made up of acrylic material with a dimension of 12.7 cm (width), 16.51 cm (length), and 11.43 cm (depth). A quartz window

(8.89 cm × 8.89 cm) was disposed on one face of the reactor for the photocatalysis experiments. The experimental set-up is presented in Fig. 1. All the assays were conducted in batch mode. A Heraeus TQ 150 medium-pressure Hg-vapor lamp (light intensity = 6.9 mW cm^{-2}; λ_{max} = 254 nm) was focused through the quartz window. The lamp was installed vertically outside or inside the reactor. The photocatalytic reactor was placed in a dark chamber in order to avoid interferences from the daylight. Mixing in the cell was achieved by a Teflon-covered stirring bar installed between the perforated plate and the bottom of the cell. All experiments were carried out at room temperature (25±1 °C).

2.5. Carbamazepine analysis

2.5.1. Preparation of samples

The WW samples were filtered through a 0.45 μm glass-fiber (Fisherbrand G6 filter circles, Fischer Scientific, Ontario, Canada) and immediately stored at 4±1 ^{0}C until analysis. Solid phase extraction (SPE) method was used for clean-up. Sep-Pak Plus C18 environmental cartridges were fitted into the vacuum manifold (Welch, USA) which was connected to a vacuum pump (Welch Rietschle Thomas, USA) to dispense samples through the cartridges. Cartridges were pre-conditioned by passing 7 mL of methanol and 3 mL of HPLC water at a flow rate of 1 mL min^{-1}. Filtered samples (200 mL) were passed at a flow rate of 5 mL min^{-1}. After pre-concentration, the sorbents were dried by using a vacuum system set at (-15) psi.

The elution was performed by adding 2 x 4 mL of methanol to the cartridge at a flow rate of 1 mL min^{-1} and giving it a wait time of 10 min in order to give enough duration of contact between the solvent and the adsorbed compounds. The extracts were later evaporated to dryness with a gentle stream of nitrogen and reconstituted with methanol to a final volume of 200 μL prior to Laser Diode Thermal Desorption-Atmospheric Pressure Chemical Ionization-Mass Spectrometry/Mass Spectrometry (LDTD-APCI-MS/MS) analysis.

2.5.2. LDTD-APCI-MS/MS Analysis

Quantification of CBZ was achieved with the LDTD-APCI ionization source (Phytronix Technologies, Quebec, Canada) mounted on a TSQ Quantum Ultra AM Mass Spectrometer (Thermo Fisher Scientific, Waltham, MA, USA). Samples were first spotted (2 μL) into the LazWell 96-well polypropylene plate cavities containing inserts made of proprietary stainless steel alloy and left to dry at room temperature. The designed well shape allows the sample to concentrate in the heating zone while drying. The loaded plate is then transferred to an *X-Y* movable stage of the LDTD housing unit. An infrared (IR) laser diode (980 nm, 20 W, continuous) is then focalized to impact the back of the inserts, thermally desorbing the dried sample, which is vaporized into gas phase. Quantification of CBZ was performed by the internal standard method based on peak areas and relative retention time, using carbamazepine d_{10} as internal standard. A five point standard addition calibration curve ranging from 0.01 µg L^{-1} to 20 µg L^{-1} was used. The area ratio (analyte:internal standard) was plotted against the analyte additions to obtain the calibration curve. Calibration curve was obtained using linear regression analysis and the established concentration range gave good correlation coefficients ($r^2 > 0.999$).

The analysis was carried out in negative ionization mode with following conditions: laser pattern programming of 1 s ramp from 0% to 25%, held for 3 s in 25% before shutting off the laser.; carrier gas flow rate (3 L/min); deposition volume (2 µL); sheath gas pressure (0 arbitrary units); auxiliary gas pressure (0 arbitrary units); skimmer offset (6 V); capillary temperature (270 °C); collision pressure (1.5 mTorr) and scan time (0.25 s).

2.6. Yeast Estrogen Screen (YES) assay

The YES assay described by Routledge and Sumpter (1996) was used to measure estrogenic activity of pre-treated (photocatalyzed) wastewater samples. All samples were sterilized at 121 ± 1 °C for 30 min prior to YES assay. Standard stock solutions of CBZ were made in ethanol and all serial dilutions were carried out in ethanol. The procedure for measurement of the total estrogenic activity was carried out by serial dilution of each sample concentrate (100 µL) in ethanol across 12 wells in a 96-well plate (Costar Brand, NY, USA). Each dilution series was initiated by placing 200 µL of the sample concentrate in the first well of the single row. The volume of 100 µL was transferred to the second column and mixed with 100 µL of ethanol. The process was repeated across the row. Aliquots of 5 µL of each concentration were transferred to a 96-well plate. Further, aliquots (195 µL) of the seeded assay medium containing the yeast (hER-transfected recombinant yeast) and chlorophenol red-β-d-galactopyranoside (CPRG) were then dispensed into each sample well. Each plate contained at least one row of blanks (assay medium and solvent ethanol) and a standard curve for 17β-estradiol. The plates were sealed with parafilm and shaken at 150 rpm (to promote suspension of the yeast cells and constant mixing with the assay medium) for a 3-d incubation period at 32 ± 1 °C. The color development of the medium was checked periodically at an absorbance of 540 nm using a multireader microplate spectrophotometer (Epoch, BioTek, USA). Prior to beginning of YES assay, each photocatalyzed (using TiO_2 and ZnO nanoparticles) WW samples (spiked and unspiked) were filtered through a 0.45 μm glass-fiber (Fisherbrand G6 filter circles, Fischer Scientific, Ontario, Canada) and autoclaved at 121 ±1 °C for 30 min to inactivate bacterial growth.

3. RESULTS AND DISCUSSION

3.1. Characterization of TiO_2 and ZnO nanoparticles

The scanning electron micrographs of the TiO_2 and ZnO nanoparticles prepared in the absence (a and c) and presence (b and d) of whey stabilizer is presented in Fig. 2. It was observed that in the absence of whey stabilizer, the resultant TiO_2 and ZnO nanoparticles do not appear as discrete nanoscale particles and form much bulk dendritic floc like structures with varying density. This type of aggregation was mainly due to the high surface energy of TiO_2 and ZnO nanoparticles (Hong et al., 2006; Nikaido et al., 2009) leading to formation of bulk structures with lower reactivity. However, the whey stabilized TiO_2 and ZnO nanoparticles (Fig. 2b and d) appear to be more discrete and well dispersed. The presence of whey fulfilled the steric hindrance between TiO_2 and ZnO nanoparticles and prevented their aggregation and thus maintained the high surface area and reactivity of the particle.

The particle size distribution of TiO_2 and ZnO nanoparticles prepared in the absence and presence of whey stabilizer is presented in Fig. 3 and 4, respectively. The mean particle size of TiO_2 nanoparticles (Fig 3a and b) was calculated as 340 nm (with standard deviation of 15.91) and 23 nm (with standard deviation of 10.53) prepared in the absence and presence of whey stabilizer, respectively. The mean particle size of ZnO nanoparticles (Fig. 4a and b) was calculated as 390 nm (with standard deviation of 26.81) and 80 nm (with standard deviation of 32.75) prepared in the absence and presence of whey stabilizer, respectively.

It was observed that the mean particle size of TiO_2 and ZnO nanoparticles prepared in the presence of whey stabilizer were smaller than the ones prepared in the absence of whey. There was aggregation of TiO_2 and ZnO nanoparticles prepared without whey, so that the mean size reflected the aggregation of nanoparticles. Whey permeate used in the experiment typically contains carbohydrate as lactose (>82%). The titanium-carbohydrate and zinc-carbohydrate interactions and formation of titanium-carbohydrate clusters (Duthaler et al., 1990) and zinc carbohydrate clusters (Mizutani et al., 1997) may play a fundamental role in dispersing and stabilizing the TiO_2 and ZnO nanoparticles prepared in the presence of whey.

3.2. Photocatalytic degradation of carbamazepine

To test the photodegradation of CBZ by commercial TiO_2 nano, TiO_2 nano, whey-TiO_2 nano, commercial ZnO nano, ZnO nano and whey-ZnO nano, each nanoparticle immobilized thin films was introduced into the reactor and photoirradiated using a mercury lamp. At periodic interval of time, aliquots of the sample were withdrawn and the concentration of CBZ was measured. Fig. 5 presents the degradation of CBZ in WW by using different types of TiO_2 and ZnO nanoparticles. The initial concentration of CBZ was 30 ng L^{-1} in all cases. All the results reported in Figure represent the mean of triplicates. The maximum standard deviation observed was 4.5% for commercial TiO_2 nano, 0.5% for TiO_2 nano and 0.7% for whey-TiO_2 nano. In the case of ZnO nanoparticles, the maximum standard deviation observed was 1.7% for commercial ZnO nano, 1.1% for ZnO nano and 0.3% for whey-ZnO nano.

It was observed that for 120 min of irradiation time, the CBZ degradation was 65%, and 46% with commercial TiO_2 nano and TiO_2 nano, respectively. A complete degradation of CBZ was observed in WW with whey-TiO_2 nano. In case of whey-TiO_2 nano, complete degradation of CBZ was achieved with 55 min irradiation time. Further, in case of ZnO nanoparticles, it was observed that for 120 min of irradiation time, the CBZ degradation was 60%, 41% and 92% with commercial ZnO nano, ZnO nano, whey-ZnO nano, respectively. The higher degradation of CBZ with whey stabilized TiO_2 and ZnO nanoparticles was due to the formation of more dispersed TiO_2 and ZnO nanoparticles in the medium. This can be attributed as oxidative degradation of CBZ by TiO_2 and ZnO as direct bandgap excitation of the semiconductor results in electron-hole separation. Photogenerated holes oxidize CBZ at the TiO_2 and ZnO surface. However, the higher degradation of CBZ in wastewater by using TiO2 nanoparticles as compared to ZnO nanoparticles was due to formation of higher photo-generated holes with high oxidizing power of TiO_2. The photocatalytic capacity of ZnO anticipated as similar to that of TiO_2 as it has the same band gap energy (3.2 eV) as TiO_2. However, in the case of ZnO, photocorrosion frequently occurs with the illumination of UV light and this phenomenon is considered as one of the main reasons for the decrease of ZnO photocatalytic activity in aqueous solutions (Tang et al., 2006).

The percentage of degradation of CBZ was also determined by effective adsorption of contaminant onto the TiO_2 and ZnO nano. The following pseudo-first-order reaction kinetics may be used to describe the initial rate of CBZ degradation (Eq. 3 and 4):

$$-\frac{\mathrm{d[C]}}{\mathrm{dt}} = k\,[\mathrm{C}] \qquad (3)$$

$$ln[C] = -kt + ln\,C_0 \qquad (4)$$

Where C_0 is the initial CBZ concentration (ng L^{-1}) and C is the CBZ concentration (ng L^{-1}) at time t (h), k (h^{-1}) is the pseudo-first-order rate constant. Higher value of k (0.92 h^{-1}) was observed for whey-TiO2 nanoparticles as compared to whey-ZnO (0.84 h^{-1}) nanoparticles.

3.3. Analysis of by-products during photocatalytic degradation of carbamazepine

Samples were taken at different irradiation times and CBZ photo by-products were analyzed by using LDTD-APCI-MS/MS run in both positive and negative electrospray modes. To identify different by-products formed during CBZ photo degradation by using TiO_2 and ZnO nanoparticles, the experiments were carried out in samples with; i) CBZ spiked in milli-Q water, ii) CBZ spiked in WW, and iii) real WW sample (without spiking). A comparison was done in each case to identify the intermediates.

LDTD-MS/MS chromatography of CBZ photo by-products formed is presented in Fig. 6. Two similar by-products, such as epoxycarbamazepine (retention time 2.9 min) and hydroxycarbamazepine (retention time 5.1 min) were observed during photocatalytic degradation (due to the reaction of $OH^{\bullet}$ radicals) of CBZ by using TiO_2 and ZnO nanoparticles. However, one more by-product, named dihydroxycarbamazepine (retention time 2.3 min) was observed during photocatalytic degradation of CBZ using TiO_2 nanoparticles. In order to confirm that these by-products were formed during oxidative treatments, a comparison was made between LDTD-MS/MS chromatography with and without treatment of WW. A proposed possible pathway of degradation of CBZ during photocatalytic treatment processes is presented in Fig. 7.

3.4. Estrogenic activity of photocatalyzed wastewater

Estrogenic activity was carried out in synthetic wastewater. The YES test is an assay system in which the human estrogen receptor (hER) is expressed in yeast in a form capable of activating transcription of a promoter carrying estrogen-responsive sequences (ERE), in an estrogen-dependent manner (Routledge et al., 1998). The sensitivity and reproducibility of the estrogen screen were assessed by measuring the response of the yeast to solution with 17β-estradiol and different photocatalyzed WW samples spiked with CBZ. The estrogenicity appeared with 17β-estradiol, as the wells are red in color after 3 d incubation. However, none of the photocatalyzed WW samples spiked and unspiked with CBZ showed any change in color.

The response of the yeast to photocatalyzed WW samples spiked and unspiked with CBZ is presented in Fig. 8. The blank remained within the absorbance range of 0.73 to 0.9 (yellow to light orange) for a 3 d incubation. None of the photocatalyzed WW samples tested were estrogenic (absorbance range 0.8 to 1.2). The results proved that photocatalyzed WW samples with CBZ and its different by-products (epoxycarbamazepine and hydroxycarbamazepine) are not estrogenic. Hence, these pre-treatments may be important and essential for safe disposal of wastewater, however more studies are recommended on toxicity test (battery of assays) to confirm results obtained from testing procedures.

4. CONCLUSIONS

The study on photocatalytic degradation of carbamazepine in wastewater by using a new class of whey-stabilized nanocrystalline TiO_2 and ZnO led to the following conclusions:

(1) The size distribution analysis and SEM images of TiO_2 and ZnO nanoparticles indicated that nanoparticles prepared in the presence of whey stabilizer agglomerated less and dispersed better than that prepared in the absence of whey.

(2) Complete photocatalytic degradation of carbamazepine was achived by using whey stabilized TiO_2 nanoparticles with 55 min irradiation time as compared to ZnO nanoparticles (92%).

(3) Similar photo by-products of carbamazepine degradation, epoxycarbamazepine and hydroxycarbamazepine were observed during photocatalytic degradation in wastewater using TiO_2 and ZnO nanoparticles.

(4) The Yeast Estrogen Screen (YES) assay results showed that photocatalyzed wastewater containing carbamazepine was not toxic to the yeast (not estrogenic).

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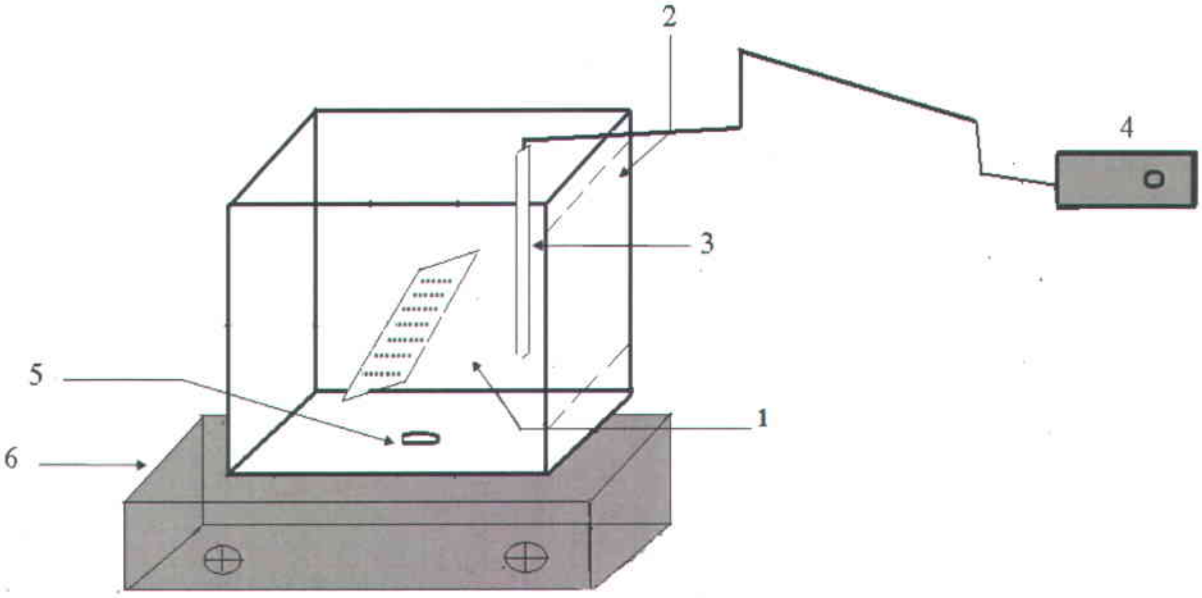


Figure 1. Schematic diagram of the photocatalytic reactor: (1) Photocatalyst (2) quartz window (3) UV lamp (4) photometer (5) stirring bar (6) magnetic stirrer

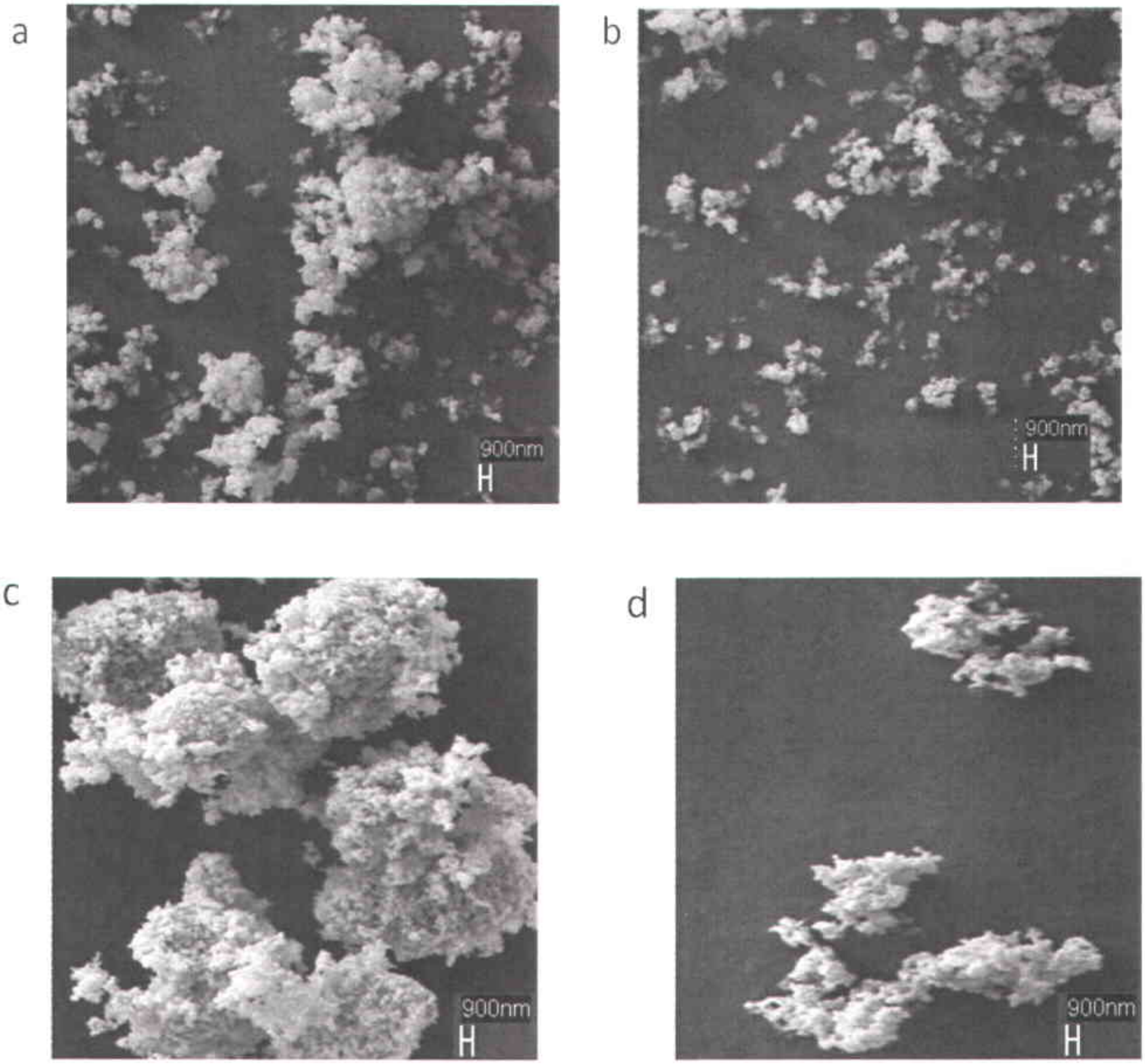


Figure 2. SEM image of TiO_2 and ZnO nanoparticles prepared: (a) TiO_2 nanoparticles without whey; (b) TiO_2 nanoparticles with whey; (c) ZnO nanoparticles without whey; (d) ZnO nanoparticles with whey

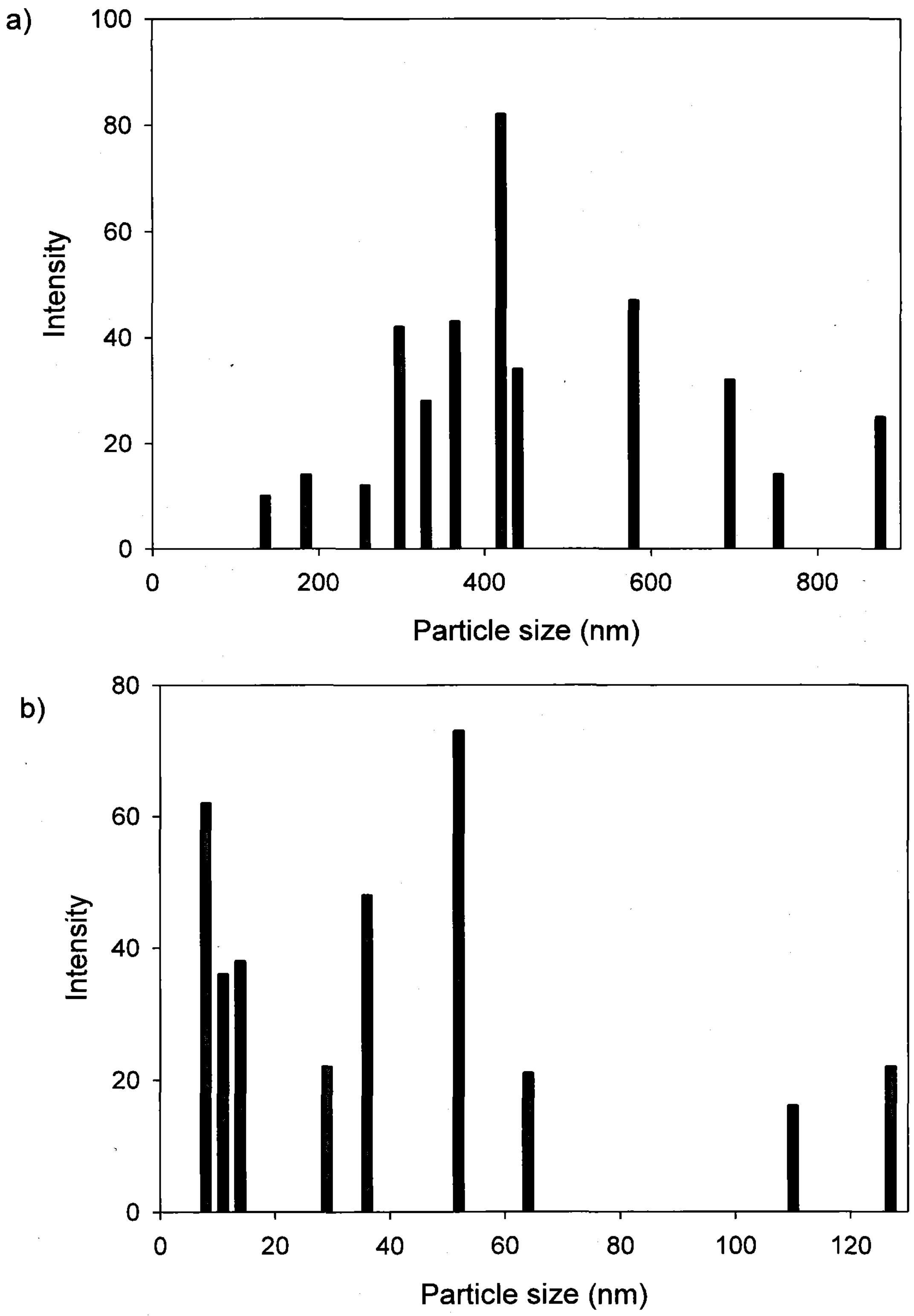


Figure 3. Particle size distribution of TiO2 nanoparticles prepared: (a) without whey; (b) with whey

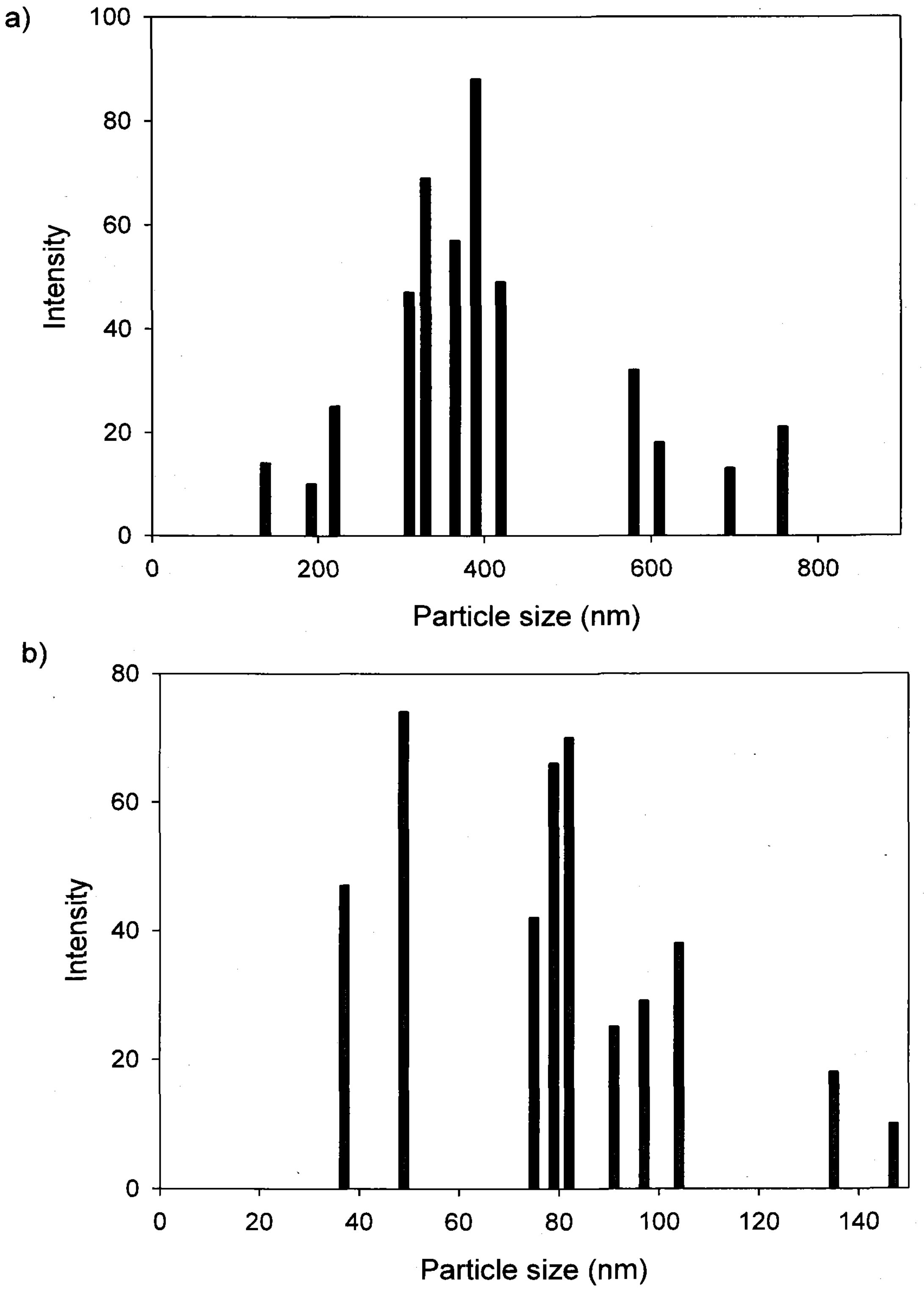


Figure 4. Particle size distribution of ZnO nanoparticles prepared: (a) without whey; (b) with whey

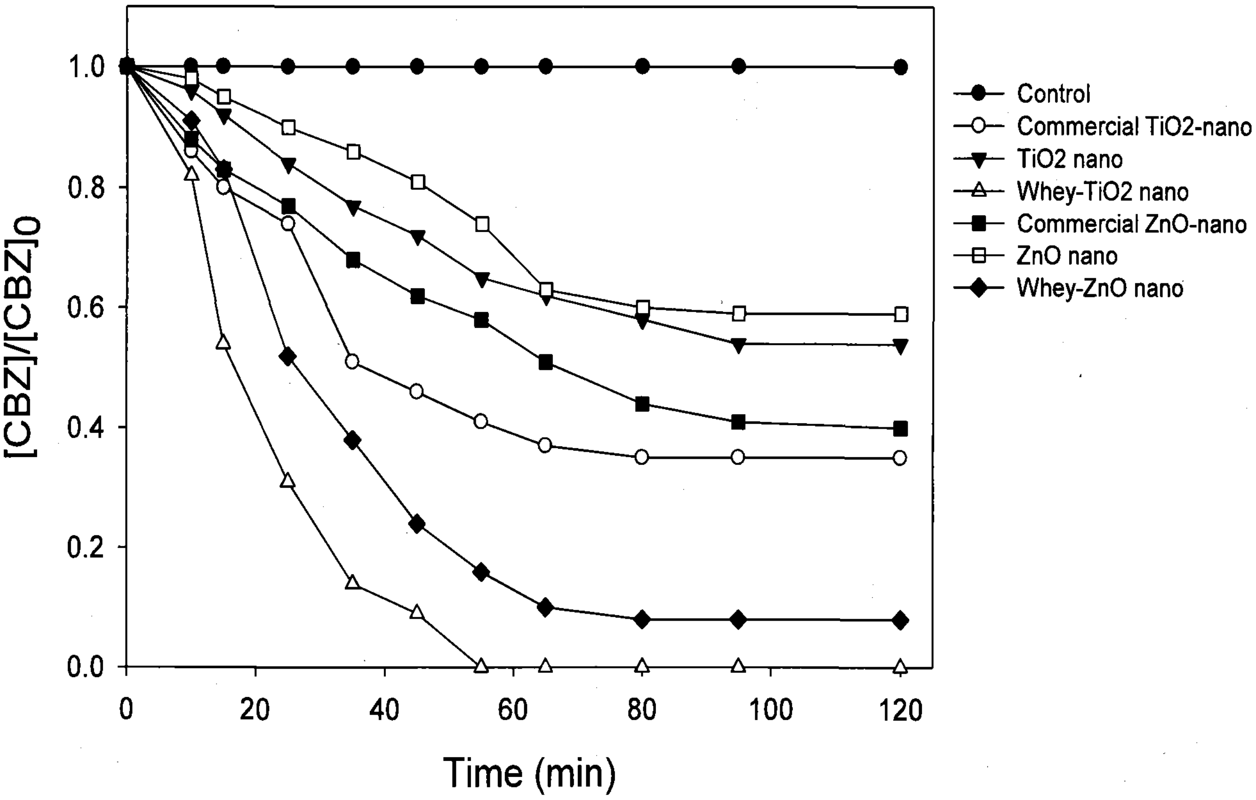


Figure 5. Photocatalytic degradation of carbamazepine by using different types of TiO_2 and ZnO nanoparticles

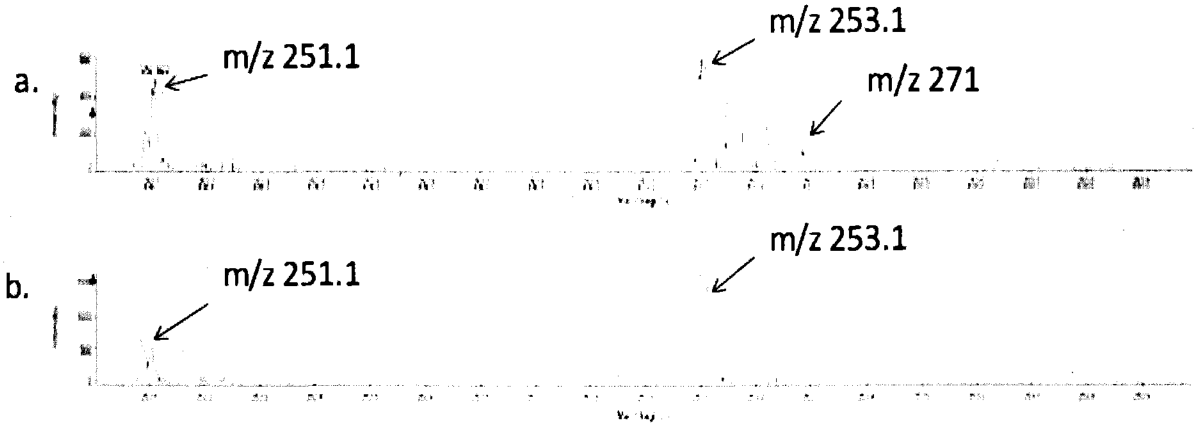


Figure 6. Mass spectra of oxidation products formed in wastewater during photocatalytic degradation of carbamazepine with: (a) TiO2 nanoparticles; (b) ZnO nanoparticles

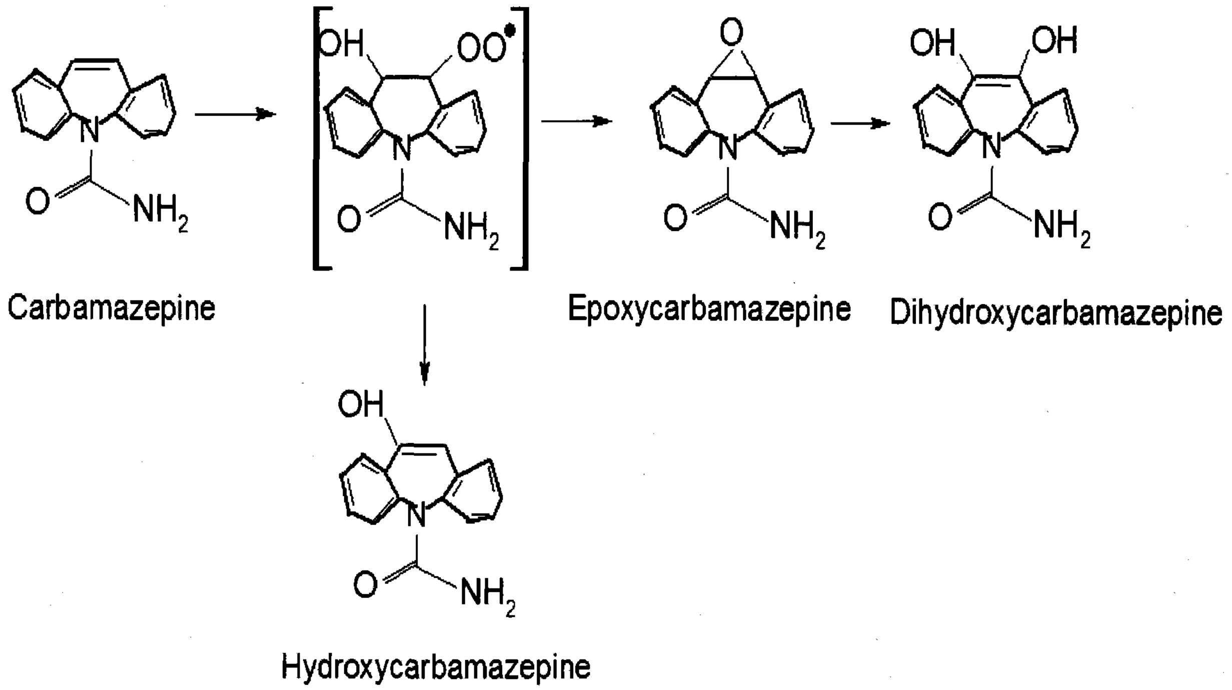


Figure 7. Carbamazepine degradation pathways induced by reaction with hydroxyl radical produced during photocatalytic degradation

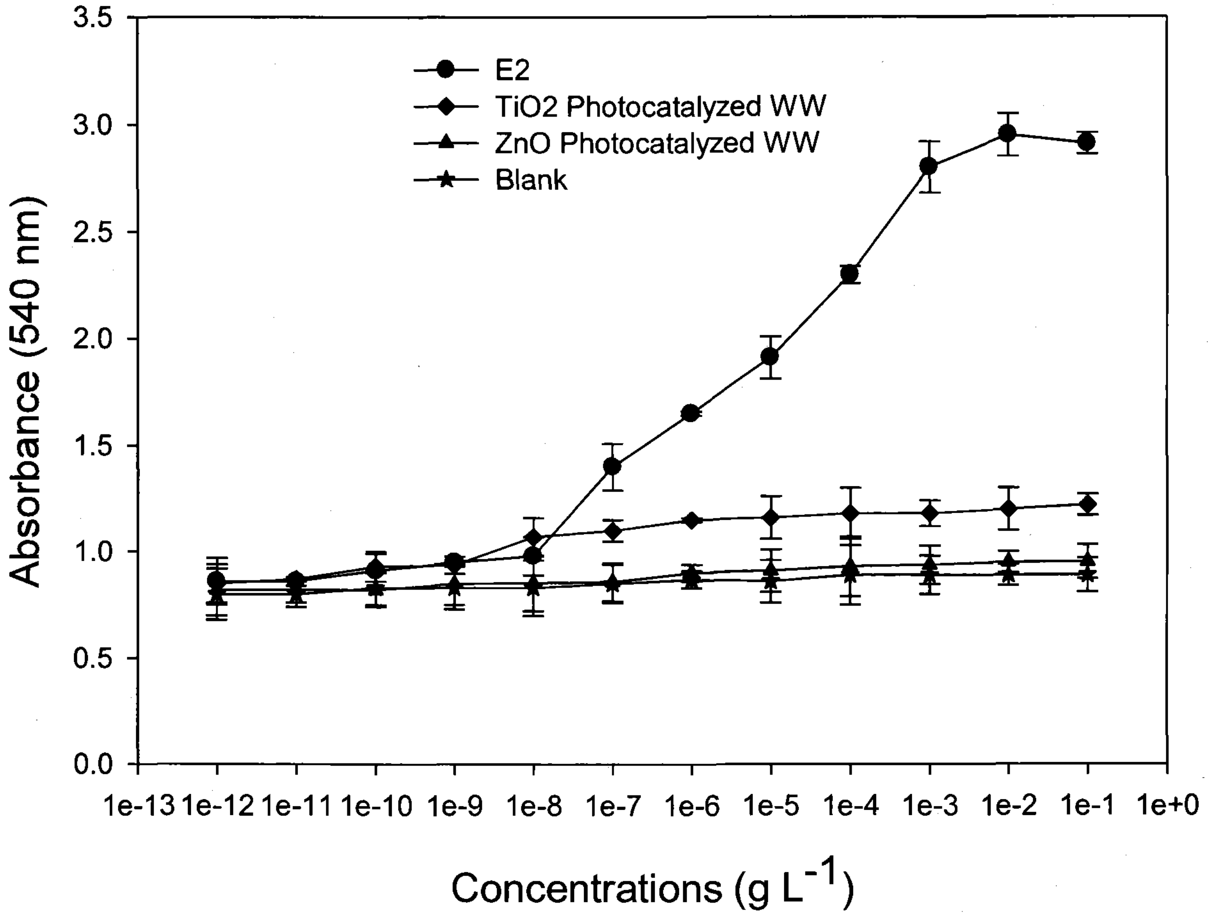


Figure 8. YES assay curves for E2 and pre-treated wastewater spiked with carbamazepine

LES ANNEXES

ANNEXE 1

Table A.1. Standard curve preparation for analysis of BPA by LDTD-APCI-MS/MS method

STD_20:	20uL of stock solution BPA (1005 mg/L) 1000uL of MeOH Concentration: 20 mg/L	STD_20 with IS:	100 uL of STD_20 10 uL of IS solution*
STD_2:	20uL of STD_20 180uL of MeOH Concentration: 2 mg/L	STD_2 with IS:	100 uL of STD_2 10 uL of IS solution*
STD_0.2:	20uL of STD_2, 180uL of MeOH Concentration: 0.2 mg/L	STD_0.2 with IS:	100 uL of STD_0.2 10 uL of IS solution*
STD_0.02:	20uL of STD_0.2 180uL of MeOH Concentration: 0.02 mg/L	STD_0.02 with IS:	100 uL of STD_0.02 10 uL of IS solution*
		BLK with IS: (ZS)	100 uL of MeOH 10 uL of IS solution*
Dilution:	20 uL of sample or STD 180uL of MeOH:Water (75:25)		

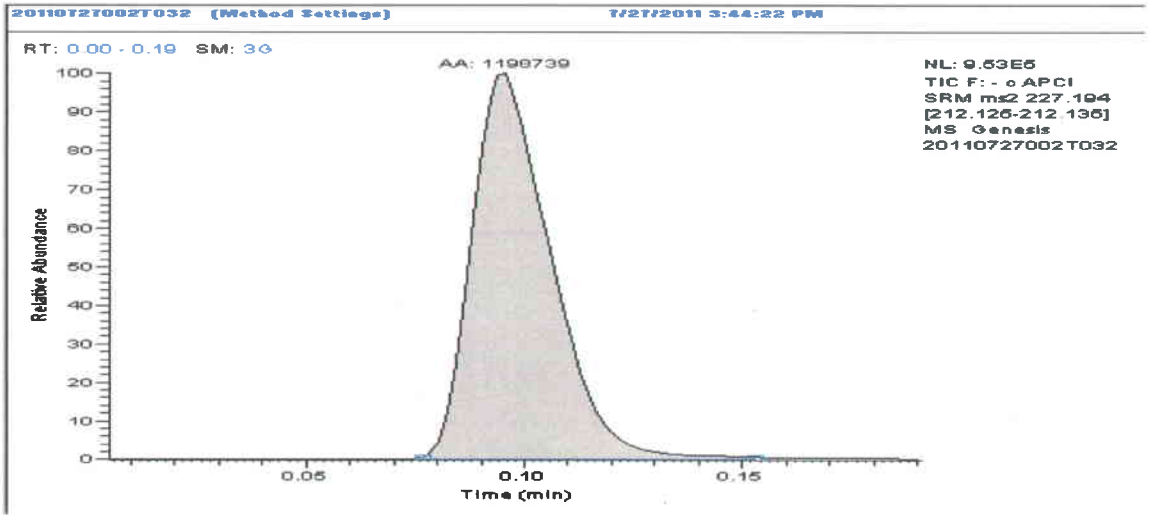
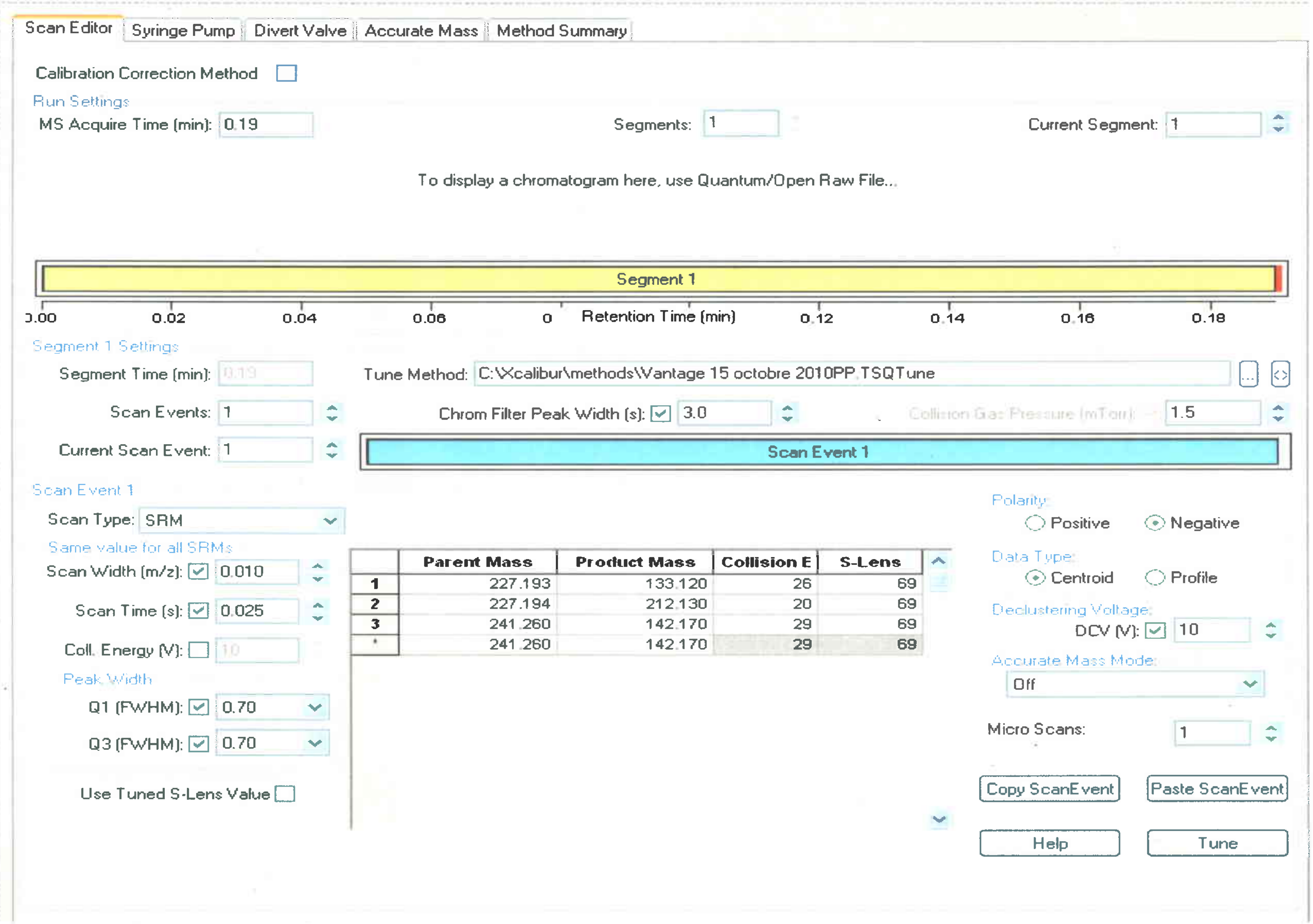


Table A.2. MS/MS method used for analysis of BPA by LDTD-APCI-MS/MS method



Calibration Correction Method ☐

Run Settings

MS Acquire Time (min): 0.19

Segments: 1

Current Segment: 1

To display a chromatogram here, use Quantum/Open Raw File...

Segment 1 Settings

Segment Time (min): 0.19

Tune Method: C:\Xcalibur\methods\Vantage 15 octobre 2010PP.TSQTune

Scan Events: 1

Chrom Filter Peak Width (s): ☑ 3.0

Collision Gas Pressure (mTorr): 1.5

Current Scan Event: 1

Scan Event 1

Scan Type: SRM

Same value for all SRMs

Scan Width (m/z): ☑ 0.010

Scan Time (s): ☑ 0.025

Coll. Energy (V): ☐ 10

Peak Width

Q1 (FWHM): ☑ 0.70

Q3 (FWHM): ☑ 0.70

Use Tuned S-Lens Value ☐

	Parent Mass	Product Mass	Collision E	S-Lens
1	227.193	133.120	26	69
2	227.194	212.130	20	69
3	241.260	142.170	29	69
*	241.260	142.170	29	69

Polarity:

○ Positive ● Negative

Data Type:

● Centroid ○ Profile

Declustering Voltage:

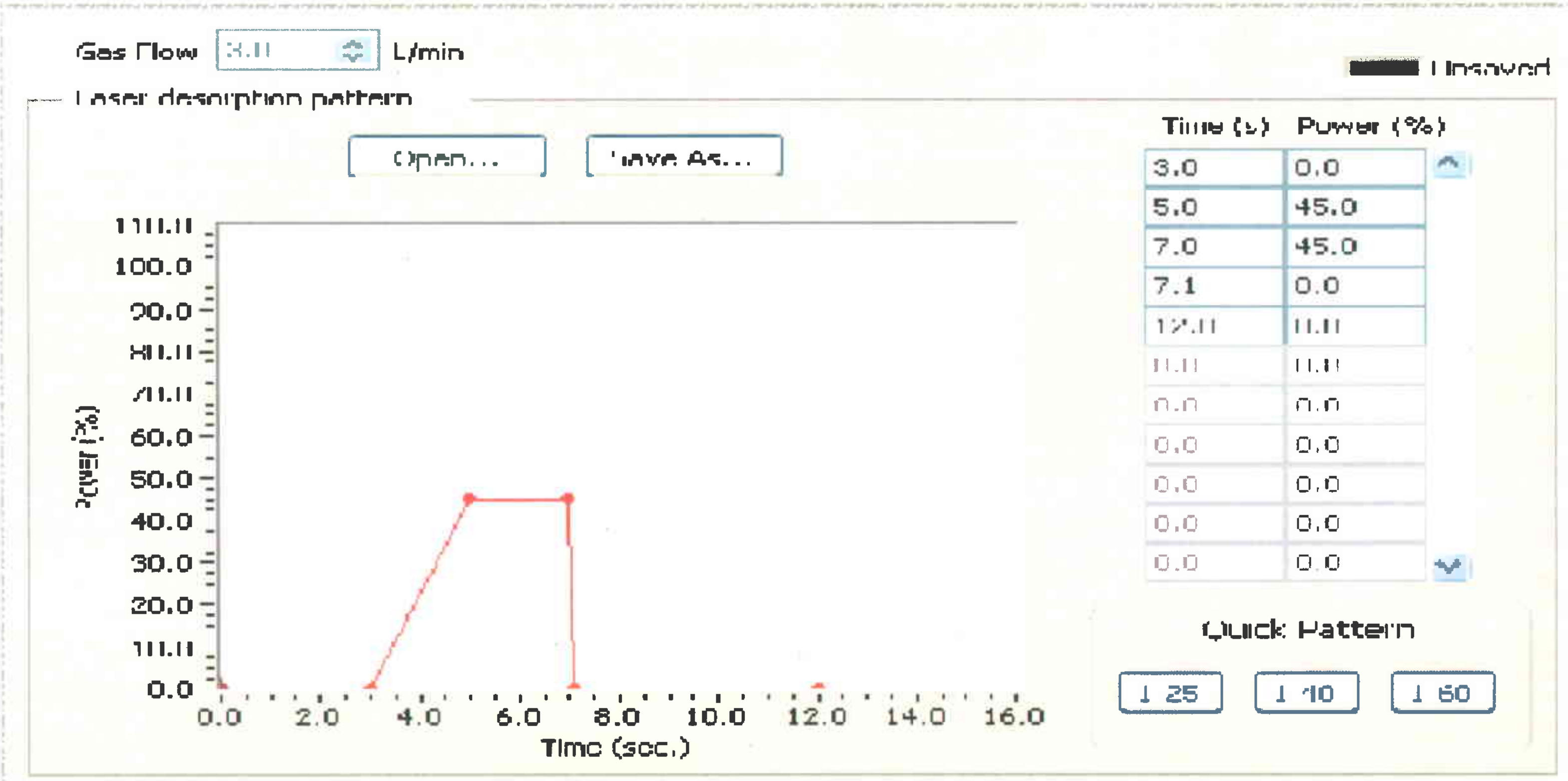
DCV (V): ☑ 10

Accurate Mass Mode:

Off

Micro Scans: 1

Table A.3. LDTD method used for analysis of BPA



Gas Flow 3.0 L/min

Unsaved

Laser desorption pattern

Time (s)	Power (%)
3.0	0.0
5.0	45.0
7.0	45.0
7.1	0.0
12.0	0.0
0.0	0.0
0.0	0.0
0.0	0.0
0.0	0.0
0.0	0.0
0.0	0.0

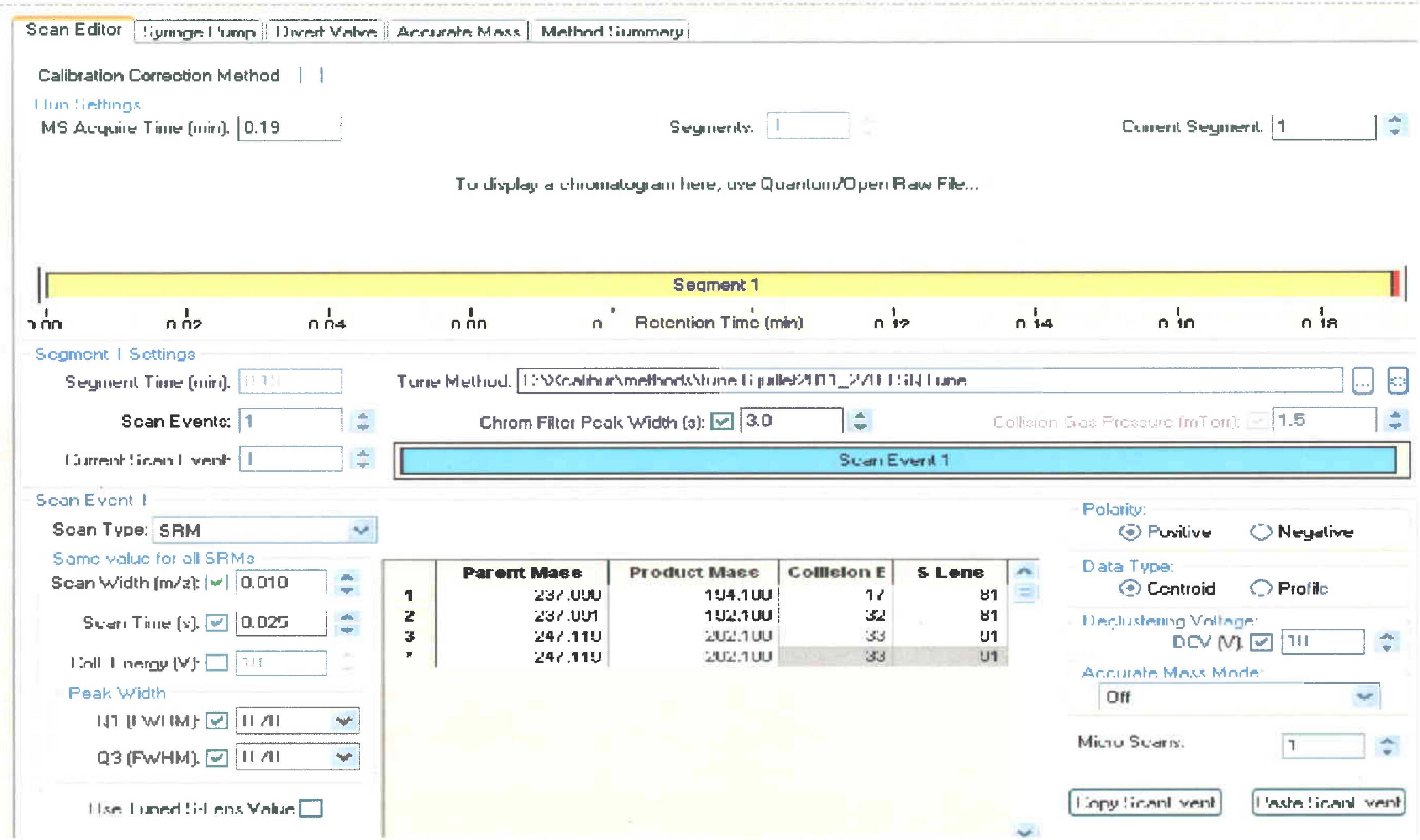
Table A.4. Analysis of BPA in WW and WWS by LDTD-APCI-MS/MS method

Curve Index	Weighting Index	Origin Index	Equation
Linear	1/X	Ignore	Y = 0.00242112+0.433382*X R^2 = 0.9993

Sample ID	Area	ISTD Area	Area Ratio	Specified Amount	Calculated Amount	Units
BLK	NF	0	NF	NA	NF	mg/L
ZS	NF	154572	NF	NA	NF	mg/L
S0.02	1365	137681	0.010	0.020	0.017	mg/L
S0.2	13866	149996	0.092	0.200	0.208	mg/L
S2	138385	156125	0.886	2.000	2.040	mg/L
S20	1340699	157243	8.526	20.000	19.668	mg/L
1	113440	139623	0.812	NA	1.869	mg/L
2	45539	164699	0.276	NA	0.632	mg/L
3	79383	151281	0.525	NA	1.205	mg/L
4	20009	169748	0.118	NA	0.266	mg/L
5	4473	123178	0.036	NA	0.078	mg/L
6	19058	121719	0.157	NA	0.356	mg/L
7	17392	140933	0.123	NA	0.279	mg/L
8	18830	104568	0.180	NA	0.410	mg/L
9	24953	58096	0.430	NA	0.985	mg/L
10	95418	152693	0.625	NA	1.436	mg/L
11	78815	210776	0.374	NA	0.857	mg/L
12	5154	338012	0.015	NA	0.030	mg/L
13	36564	94132	0.388	NA	0.891	mg/L
14	29072	131037	0.222	NA	0.506	mg/L
15	7435	110060	0.068	NA	0.150	mg/L
16	44926	131296	0.342	NA	0.784	mg/L
17	22853	132438	0.173	NA	0.393	mg/L
19	27569	182069	0.151	NA	0.344	mg/L
20	1611	61156	0.026	NA	0.055	mg/L
21	14201	83830	0.169	NA	0.385	mg/L
22	79391	140740	0.564	NA	1.296	mg/L
23	43649	105748	0.413	NA	0.947	mg/L
24	4381	192653	0.023	NA	0.047	mg/L
25	72092	117091	0.616	NA	1.415	mg/L
26	61887	107428	0.576	NA	1.324	mg/L
15gr2	1198739	131064	9.146	NA	21.099	mg/L
25gr2	716527	67809	10.567	NA	24.377	mg/L
30gr2	1529134	129652	11.794	NA	27.209	mg/L
BLK	NF	0	NF	NA	NF	mg/L
ZS	NF	149689	NF	NA	NF	mg/L
S0.02	1158	114094	0.010	0.020	0.018	mg/L
S0.2	13901	143450	0.097	0.200	0.218	mg/L
S2	102659	106489	0.964	2.000	2.219	mg/L
S20	1199372	137973	8.693	20.000	20.052	mg/L

ANNEXE 2

Table A.5. MS/MS method used for analysis of carbamazepine (CBZ) by LDTD-APCI-MS/MS method



Calibration Correction Method

Run Settings

MS Acquire Time (min): 0.19

Segments: 1

Current Segment: 1

To display a chromatogram here, use Quantum/Open Raw File...

Segment 1 Settings

Segment Time (min): 0.19

Tune Method: C:\Xcalibur\methods\tune [illegible]

Scan Events: 1

Chrom Filter Peak Width (s): ☑ 3.0

Collision Gas Pressure (mTorr): ☐ 1.5

Current Scan Event: 1

Scan Event 1

Scan Type: SRM

Same value for all SRMs

Scan Width (m/z): ☑ 0.010

Scan Time (s): ☑ 0.025

Coll Energy (V): ☐ 10

Peak Width

Q1 (FWHM): ☑ 0.70

Q3 (FWHM): ☑ 0.70

Use Tuned S-Lens Value ☐

	Parent Mass	Product Mass	Collision E	S Lens
1	237.000	194.100	17	81
2	237.001	192.100	32	81
3	247.119	202.100	33	91
*	247.119	202.100	33	91

Polarity: ● Positive ○ Negative

Data Type: ● Centroid ○ Profile

Declustering Voltage:

DCV (V): ☑ 10

Accurate Mass Mode: Off

Micro Scans: 1

Table A.6. Standard curve preparation for analysis of CBZ by LDTD-APCI-MS/MS method

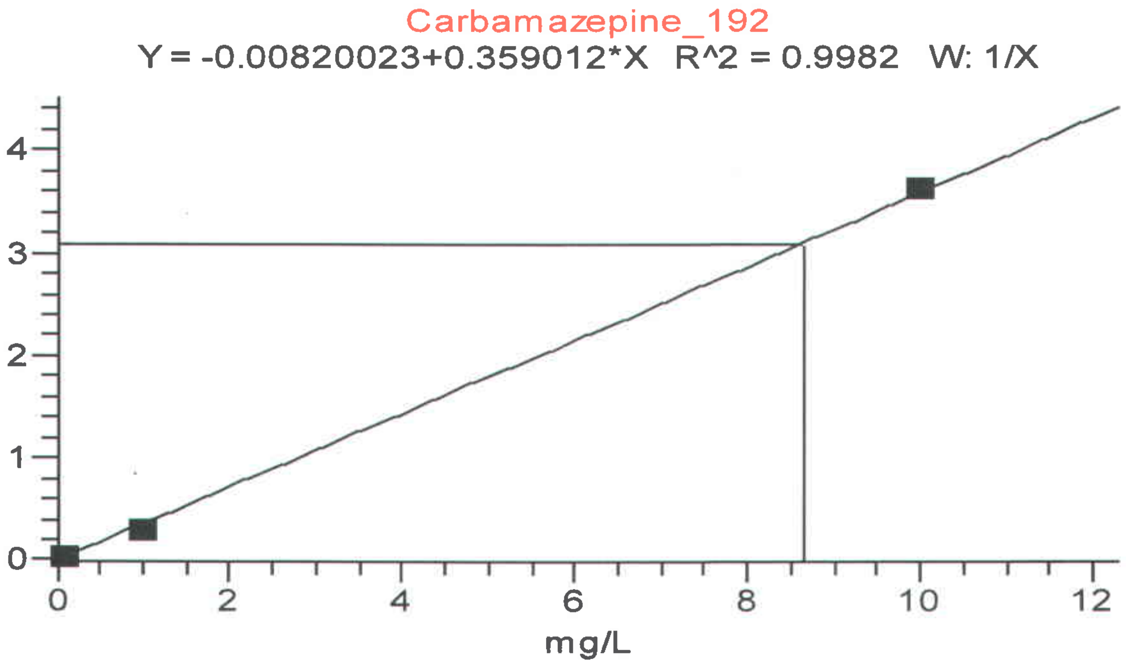


Table A.7. Analysis of BPA in WW and WWS by LDTD-APCI-MS/MS method

Curve Index	Weighting Index	Origin Index	Equation
Linear	1/X	Ignore	Y = -0.00820023+0.359012*X R^2 = 0.9982

Sample ID	Area	ISTD Area	Area Ratio	Specified Amount	Calculated Amount	Units
blank	3117	2152906	0.001	NA	0.027	mg/L
0.1	61202	1925520	0.032	0.100	0.111	mg/L
1.0	630640	2061649	0.306	1.000	0.875	mg/L
10	6337136	1749257	3.623	10.000	10.114	mg/L
100	24938303	2313634	10.779	100.000	30.046	mg/L
IN	3255738	3728833	0.873	NA	2.455	mg/L
SI	1405086	3497665	0.402	NA	1.142	mg/L
EF	588570	4102408	0.143	NA	0.422	mg/L
PSL	1700530	6816767	0.249	NA	0.718	mg/L
MSL	1564489	6551554	0.239	NA	0.688	mg/L
SSL	1633760	7679936	0.213	NA	0.615	mg/L
PSS	2402135	6942320	0.346	NA	0.987	mg/L
MSS	522435	6267545	0.083	NA	0.255	mg/L
SSS	450225	5513588	0.082	NA	0.250	mg/L
SS	16537248	5347880	3.092	NA	8.636	mg/L
MS	16619498	5440003	3.055	NA	8.532	mg/L
PS	18707157	5684444	3.291	NA	9.189	mg/L

ANNEXE 3

Table A.8. Statistical analysis for the differences in extraction recoveries among USE, MAE and ASE methods

	Methanol						Acetone						Acetonitrile:ethyle acetate (5:1, v/v)					
	USE		**MAE**		**ASE**		**USE**		**MAE**		**ASE**		**USE**		**MAE**		**ASE**	
	F-test	*P* value	F-test	*P* value	F-test	*P* value	F-test	*P* value	F-test	*P* value	F-test	*P* value	F-test	*P* value	F-test	*P* value	F-test	*P* value
P.S	25.708	0.0419*	5.7249	0.029*	1.035	0.0009*	2.831	0.835	0.0867	0.021*	5.028	0.0080*	0.058	0.8115	7.8249	0.0930*	7.025	0.0003*
S.S	0.2565	0.0372*	8.8062	0.035*	0.466	0.0008*	0.659	0.040*	0.2259	0.030*	0.175	0.0009*	0.388	0.5419	15.062	0.0070*	0.966	0.0000*
M.S	0.0658	0.0170*	0.6320	0.043*	0.552	0.0004*	0.068	0.479	24.0698	0.000*	0.564	0.0005*	0.015	0.030*	6.2320	0.0031*	3.552	0.0041*
D.S	0.8092	0.3816	15.7451	0.017*	0.926	0.0037*	0.109	0.038*	2.4157	0.013*	0.016	0.0081*	1.272	0.025*	5.0751	0.0010*	9.951	0.0038*
P.S.S	0.0025	0.9606	8.6523	0.009*	6.828	0.0018*	2.931	0.606	261.954	0.040*	6.172	0.0024*	0.049	0.8269	9.6523	0.0056*	11.82	0.0150*
S.S.S	0.6768	0.4227	5.7525	0.029*	0.021	0.0000*	6.768	0.422	16.8715	0.012*	1.089	0.0021*	0.339	0.040*	0.7525	0.0190*	2.051	0.0090*
M.S.S	5.9640	0.0193*	146.673	0.040*	1.537	0.0002*	5.640	0.019*	0.0867	0.027*	35.08	0.0030*	1.356	0.015*	36.731	0.0040*	0.537	0.0012*

*significant values (*P*< 0.05); P.S:primary sludge;S.S:secondary sludge;M.S:mixed sludge; D.S: dewatered sludge;P.S.S:primary sludge solids;S.S.S:secondary sludge solids;M.S.S:mixed sludge solids

Table A.9. Statistical analysis of CBZ concentration observed in different samples of treatment plant

Sample	F-test	*P* value	Sample	F-test	*P* value
Influent	0.9861	0.0470*	P.S	1.0867	0.0481*
G.L	12.1451	0.0391*	S.S	0.9522	0.0148*
P.S.L	8.2573	0.0175*	M.S	14.0698	0.0374*
S.S.L	0.9703	0.0381*	D.S	1.7723	0.0492*
M.S.L	2.9817	0.0214*	G.R	5.0493	0.0183*
Effluent	1.5371	0.0001*	P.S.S	2.9511	0.0400*
			S.S.S	0.3562	0.0275*
			M.S.S	6.7680	0.0493*

*significant values ($P < 0.05$); G.L: grit liquid; P.S.L:primary sludge liquid; S.S.L:secondary sludge liquid; M.S.L: mixed sludge liquid; P.S:primary sludge;S.S:secondary sludge;M.S:mixed sludge; D.S: dewatered sludge;P.S.S:primary sludge solids;S.S.S:secondary sludge solids;M.S.S:mixed sludge solids

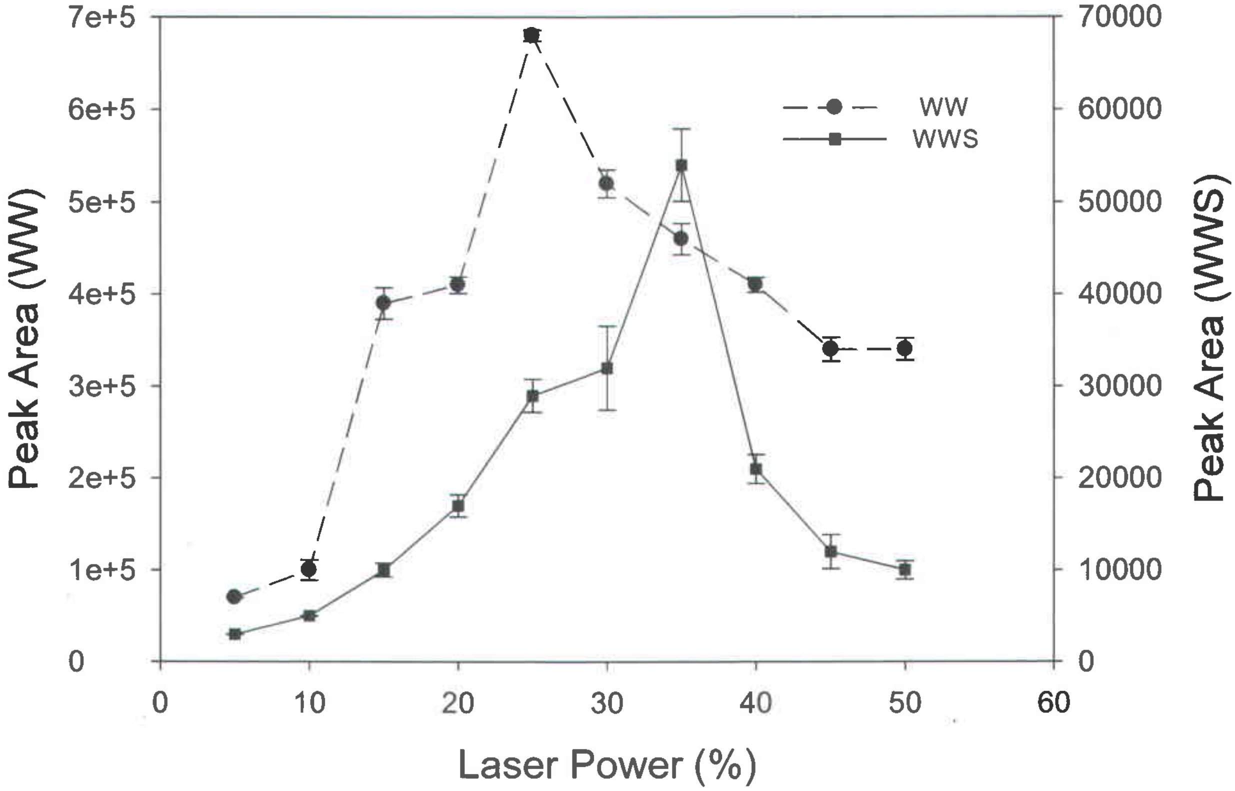


Figure A.1. Laser power (%) optimization for CBZ analysis in WW and WWS samples. The results are the mean of triplicate spikes and the error bar lengths represent the relative standard deviations. WW: Influent, WWS: Secondary sludge