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Développement d'un module membranaire imprégné par des enzymes ligninolytiques et de biochar pour la dégradation de composés pharmaceutiques

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DEDICACE

This thesis is dedicated to my parents and my wife for their endless love and support.

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RÉSUMÉ

Chlorotétracycline (CTC) est un antibiotique de la famille des tétracyclines qui est couramment utilisé comme médicament pour la prévention, le contrôle et le traitement des infections chez les animaux. Au cours des 60 dernières années, la libération constante de CTC dans l'environnement a entraîné l'exposition des bactéries à la concentration élevée d'antibiotiques par conséquent, les bactéries ont développé la résistance aux antibiotiques principalement à la CTC. La CTC se retrouve dans les stations d'épuration des eaux usées (Wastewater treatment plant: WWTP) à travers le système de collecte des eaux usées, ce qui rend le processus de traitement conventionnel des stations d'épuration inefficace pour la dégradation de la CTC. L'exposition à la lumière de CTC résiduel dans l'environnement transforme la CTC en sous-produits comme indiqué dans les différentes études consultées, les produits photo-dégradés de la CTC sont plus toxiques que la CTC. La plupart des procédés d'oxydation développés pour la dégradation des antibiotiques sont coûteux, nécessitent beaucoup d'énergie ou l'utilisation de produits chimiques dangereux. De plus, la plupart des études de dégradation de la CTC ont été réalisées dans des solutions aqueuses enrichies où la concentration de CTC n'était pas pertinente aux concentrations réelles dans l'environnement, ce qui rend l'extrapolation des résultats aux conditions réelles difficiles.

Le développement de nouvelles techniques de dégradation des antibiotiques, a l'instar de la CTC, est intéressant. Dans la présente étude, après avoir examiné les méthodes d'élimination actuelles, un nouveau système basé sur l'intégration de la dégradation enzymatique et de l'adsorption dans des membranes d'ultrafiltration a été développé. D'une part, les enzymes, en particulier les laccases, sont bien connus pour transformer les composés organiques en sous-produits moins nocifs par rapport aux processus d'oxydation conventionnels, cependant ces enzymes sont lents et sensibles aux changements de processus. D'autre part, le biocharbon issu de la pyrolyse de la biomasse des déchets est capable d'adsorber et de retenir efficacement les micropolluants, mais il ne dégrade pas les composés. L'incorporation de biocharbon et l'immobilisation de laccase sur la membrane d'ultrafiltration peuvent simultanément augmenter la stabilité de l'enzyme et fournir

suffisamment de temps pour la dégradation enzymatique en intégrant les avantages de l'association entre la membrane, enzymes et biocharbon.

Premièrement, la capacité d'adsorption de biocharbon et la capacité de dégradation de CTC par laccase ont été étudiées par l'incorporation du biocharbon dans la membrane par électrofilage et l'immobilisation de la laccase sur la membrane. Enfin, la performance de la laccase immobilisé sur l'ensemble membrane-biocharbon pour la dégradation dans l'eau de la CTC ainsi que d'autres produits pharmaceutiques étudiée en mode continu.

L'étude du comportement de l'adsorption de la CTC sur le biocharbon à différents pH (1, 5 et 9) a révélé que l'adsorption est dominée par les forces électrostatiques puisque la CTC montre une charge électrostatique variée (+1, 0 et -1, pour les pH 1, 5 et 9, respectivement). De plus, les résultats indiquent que CTC a suivi l'isotherme de Langmuir et que l'adsorption maximale s'est produite à pH 1. De plus, l'activation du biocharbon par traitement alcalin a augmenté la surface spécifique du biocharbon de 14,86 m^2/g à 852,95 m^2/g. La capacité d'adsorption maximale (q_m) du biocharbon synthétisé et activé (434 mg/g) était considérablement plus élevée en comparaison avec les différentes recherches (57-303 mg/g).

Pour étudier la biodégradation de CTC en utilisant la laccase libre, la méthodologie réponse de surface (RSM) avec un modèle composite central a été utilisée pour étudier l'effet de différents paramètres tels que le pH, la température, la concentration du médiateur et la concentration de laccase. Un modèle quadratique a été adopté pour exprimer l'effet de chaque paramètre, y compris les termes d'interaction quadratiques et linéaires. Les valeurs pour R^2 et R^2 ajusté étaient de 0,85 et 0,70 respectivement indiquant que le modèle est raisonnablement bon pour des applications pratiques. Parmi les paramètres examinés, les termes linéaires de température et de pH ont eu un effet important. Il a été observé que l'efficacité de dégradation maximale d'environ 95% peut être atteinte au pH, à la température, à la concentration d'enzyme et de médiateur de 5,2, 35,5 ° C, 62,3 U/l et 10,9 µM respectivement.

L'incorporation de microparticules de biocharbon dans la membrane par électrofilage afin d'obtenir des membranes adsorbantes a été réalisée à différentes charges de biocharbon (0-2 % p), les résultats ont montré qu'à 1,5 % p de charge de biocharbon, la surface maximale a été atteinte en raison de l'agrégation des particules à des concentrations plus élevées ainsi que de la formation d'agrégats,

deux facteurs qui réduisent le rapport surface/volume. Le test d'adsorption en mode continu a montré que la membrane fabriquée peut éliminer efficacement la CTC des milieux aqueux à un flux d'ultrafiltration normal (30 $L/m^2.h$). Les observations préliminaires sur l'immobilisation de l'enzyme sur la membrane adsorbante ont montré une efficacité d'immobilisation (> 70%) et une stabilité au stockage élevée (90% après un mois) et une haute réutilisabilité (95% après 5 cycles).

La dégradation de CTC en mode continu en utilisant l'enzyme immobilisé sur la membrane adsorbante présentait 58,3%, 40,7% et 22,6% d'efficacité d'élimination de la chlorotétracycline à des vitesses de flux de 1, 2 et 3 $mL/h.cm^2$ respectivement. L'utilisation de laccase immobilisée pour la dégradation de trois composés pharmaceutiques dans des expériences discontinues présentait 72,7%, 63,3% et 48,6% d'efficacité de dégradation pour Diclofénac (DCF), CTC et Carbamazépine (CBZ) respectivement après 8 heures de réaction. L'eau traitée n'a montré aucune toxicité selon l'essai de criblage d'œstrogène de levure (YES). L'incorporation de chitosane dans la membrane a entraîné une réduction de 99,9% du nombre de bactéries viables et de 72,7%, 63,3% et 48,6% d'efficacité de dégradation pour le DCF, la CTC et le CBZ respectivement dans l'effluent de l'usine de traitement des eaux usées (WWTP).

ABSTRACT

Chlortetracycline (CTC) is a broad-spectrum antibiotic from the family of tetracyclines that is commonly used as veterinary medicine for preventing, controlling, and treating animal infections. In recent 60 years, constant release of CTC into the environment has resulted in the exposure of bacteria to the high concentration of antibiotics and consequently the development of antibiotic resistance. CTC finds its way through sewage collection system into wastewater treatment plants (WWTPs) and unfortunately the treatment process as of the date in conventional WWTPs is not effective for degradation of CTC. The residual CTC in environment nevertheless may be exposed to light and as reported in studies, photodegraded products of CTC are more toxic than CTC itself. Most of the oxidation processes developed for degradation of antibiotics are costly, energy intensive or involve the use of hazardous chemicals. Furthermore, most of the CTC degradation studies were performed in spiked aqueous solutions where the concentration of CTC was not relevant to real environmental concentrations and therefore it was not possible to extrapolate the results to real conditions.

Thus, developing new techniques for degradation of antibiotics, such as CTC is of interest. In the present study, after reviewing the current removal methods, a new system based on the integration of enzymatic degradation and adsorption into ultrafiltration membranes is proposed. On one hand, enzymes, specifically laccases, are well known to transform organic compounds to less harmful by-products compared to oxidation processes, however, they are slow and sensitive to process changes. On the other hand, biochar, a product of waste biomass pyrolysis, is able to adsorb and retain micropollutants efficiently however it does not degrade the compounds. Incorporation of biochar and immobilization of laccase into an ultrafiltration membrane can simultaneously increase the stability of enzyme and provide enough time for enzymatic degradation, thus integrating the ternary advantages

Firstly, the capacity of biochar for adsorption and capability of the enzyme for degradation of CTC was studied. Later, the possibility of incorporation of biochar into the membrane through electrospinning and immobilization of laccase onto membrane was studied. Finally, the performance of laccase immobilized on

membrane-biochar for degradation of CTC in water and also CTC along with other pharmaceuticals will be investigated in continuous mode.

Study of the adsorption behavior of CTC onto biochar at three different pHs (1, 5 and 9) revealed that adsorption is dominated by electrostatic forces since CTC shows varied electrostatic charge (+1, 0 and -1, at pH 1, 5 and 9, respectively). Also, the results indicated that CTC followed Langmuir isotherm and the maximum adsorption occurred at pH 1. Moreover, activation of biochar through alkali treatment increased the specific surface area of biochar from 14.86 m^2/g to 852.95 m^2/g. The maximum adsorption capacity (q_m) of the synthesized activated biochar (434 mg/g) was considerably higher than previously reported investigations (57-303 mg/g).

For studying the biodegradation of CTC using free laccase, response surface methodology (RSM) with a central composite design was utilized to investigate the effects of different parameters including pH, temperature, mediator concentration and laccase concentration on biodegradation of CTC in the aqueous phase. A quadratic model was fitted to express the effects of each parameter including quadratic, linear and interaction terms. The values for R^2 and adjusted R^2 were 0.85 and 0.70, respectively indicating a reasonably good model for practical applications. Among the examined parameters, linear terms of temperature and pH had the largest effects. It was observed that the maximum degradation efficiency of around 95% can be achieved at pH, temperature, enzyme concentration and mediator concentrations of 5.2, 35.5 °C, 62.3 U/l and 10.9 µM.

Incorporation of biochar microparticles into membrane through electrospinning in order to obtain adsorptive membranes was performed at different biochar loading (0-2wt%) and the results showed that at 1.5% biochar loading, maximum surface area occurred which is due to aggregation of particles at higher concentrations and also formation of large beads which reduce surface to volume ratio. Adsorption test in the continuous mode indicated that the fabricated membrane can efficiently remove CTC from aqueous media at normal ultrafiltration flux (30 $L/m^2.h$). The preliminary observations on immobilization of enzyme onto the adsorptive membrane showed high immobilization efficiency (>70 %), high storage stability (90 % after one month) and high reusability (95 % after 5 cycles).

The degradation of CTC in continuous mode using the immobilized enzyme onto adsorptive membrane exhibited 58.3%, 40.7% and 22.6% chlortetracycline removal

efficiency at flux rates of 1, 2 and 3 mL/h.cm^2. Using immobilized laccase for degradation of three pharmaceutical compounds in batch experiments exhibited 72.7%, 63.3%, and 48.6% degradation efficiency for DCF, CTC, and CBZ, respectively, after 8 hours of reaction. The treated water showed no toxicity according to Yeast estrogen screen (YES) assay. The incorporation of chitosan into membrane caused up to 99.9% reduction in the number of viable bacteria and 72.7%, 63.3%, and 48.6% degradation efficiency for DCF, CTC, and CBZ, respectively in the effluent of wastewater treatment plant.

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LISTE DES ABRÉVIATIONS

ABTS	2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)
AOPs	Advanced oxidation processes
CBZ	Carbamazepine
CCD	Central composite design
CTC	Chlortetracycline
CTN	Chitosan
COD	Chemical Oxygen Demand
Da	Dalton
DCF	Diclofenac
FO	Forward Osmosis
GAC	Granular activated carbon
MBR	Membrane bioreactor
MF	Microfiltration
MLSS	Mixed Liquid Suspended Solids
MW	Molecular Weight
MWCO	Molecular weight Cut Off
NF	Nanofiltration
NFM	Nanofibrous membrane
NOM	Natural organic matters
PAC	Powdered activated carbon
PAN	Polyacrylonitrile
PhACs	Pharmaceutically active compounds
UF	Ultrafiltration
UV	Ultraviolet
RO	Reverse osmosis
WHO	World Health Organization
WW	Wastewater
WWS	Wastewater sludge
WWTPs	Wastewater treatment plants

LISTE DES ÉQUATIONS

$$q_e = \frac{(C_{0-} C_e) * V}{M}$$

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{b q_m}$$

$$q_e = k C_e^{\frac{1}{n}}$$

$$Degradation\ Efficiency\ (\%) = 100 * \frac{C_i - C_r}{C_i}$$

CHAPITRE 1

SYNTHÈSE

Partie 1. Introduction

Les résidus des composés pharmaceutiquement actifs (PhAC) constituent une classe importante de contaminants émergents (CE) dans l'environnement. Après ingestion par les humains et les animaux, un grand nombre de produits pharmaceutiques intacts et de leurs métabolites sont rejetés dans les eaux usées. En termes de charge de la décharge dans les eaux usées (WW), les antibiotiques sont parmi les composés en haut de la liste, ils sont même trouvés à des concentrations relativement plus élevées avant et après le traitement WW. La décharge continue des antibiotiques bioactifs pourrait avoir des effets néfastes sur les organismes aquatiques et terrestres. Considérant de la consommation actuelle des antibiotiques et de la tendance passée du marché, la demande mondiale des antibiotiques devrait augmenter à un taux de croissance annuel composé de 0,8% entre 2013 et 2018 [1].

La famille des tétracyclines figure parmi les antibiotiques inscrits dans l'élevage de bétail comme étant l'utilisation la plus élevée (15,8%) [2]. Parmi les membres de cette famille, la chlortétracycline (CTC) est l'antibiotique le plus utilisé dans le monde dans les applications vétérinaires. En 1945, la CTC a été découverte par Benjamin Minge Duggar qui l'a isolé à partir d'un microorganisme du sol, *Streptomyces aureofaciens* et en 1951, la FDA a approuvé l'application de la CTC à des fins prophylactiques chez le bétail [3]. Cet antibiotique largement utilisé peut inhiber le transfert de "aminoacyl tRNA" au complexe "mRNA-ribosome" sur le site accepteur, par conséquent empêcher la reproduction des cellules bactériennes chez les animaux. La solubilité de la CTC dans l'eau est de 0,8 mg/mL et son absorption dans le tractus gastro-intestinal est de 25 à 30% de la dose initiale, ce qui est inférieur à celle des autres membres de la famille des tétracyclines.

Au cours des 60 dernières années, la consommation de CTC n'a cessé de croître en raison de l'augmentation de la population et de la demande alimentaire. Cette tendance a entraîné une augmentation constante de la CTC dans l'environnement, ce qui s'est traduit par une plus grande résistance aux antibiotiques qui à son tour a compromis l'efficacité des antibiotiques chez l'homme. L'enfouissement des déchets et l'épandage de déchets municipaux et de fumier animal comme engrais entraîne également l'accumulation de la CTC dans le sol, par conséquent, la CTC peut s'accumuler dans les plantes ou s'infiltrer dans les eaux souterraines ou ruisseler

dans les eaux de surface pour retourner dans la chaîne alimentaire et atteindre indirectement les humains.

Le transport de la CTC à travers les procédés mentionnés ainsi que ses propriétés physicochimiques ont conduit à des concentrations résiduelles variées de CTC dans les sédiments, les sols, les eaux de surface et les eaux souterraines.. Des études ont montré que la détection de la CTC est entre (0,02-0,8 mg/L) dans les eaux usées (Waste Water : WW) municipales et des boues d'épuration (Waste Water Sludge: WWS) [4, 5]. Pour le cas des eaux usées provenant des industries pharmaceutiques et des eaux souterraines près des fermes d'élevage, la concentration de CTC a été rapportée jusqu'à plusieurs mg/L [6-8].

Les procédés de traitement classiques par voie humide ont montré une dégradation faible ou nulle de la CTC, car le procédé de traitement principal est effectué par des bactéries sensibles à la CTC. Une photodégradation, une chloration, une ozonation ou une irradiation UV plus poussées ont abouti à des produits plus toxiques que la CTC. De nos jours, des techniques, telles que la séparation sur membrane, des systèmes adsorbants et des enzymes ont été utilisées pour une dégradation ou une élimination efficace de tels contaminants récalcitrants. Ces processus peuvent être considérés comme un système complémentaire dans la phase de traitement tertiaire des stations d'épuration (WWTPs). Parmi ces procédés de séparation physique : la filtration sur membrane et les systèmes d'adsorption sont modulaires et efficaces pour l'élimination des contaminants, mais ils ne sont pas capables de dégrader les contaminants. D'autre part, la dégradation des micropolluants utilisant des enzymes oxydoréductases s'est avérée efficace dans la dégradation et même la minéralisation, mais le processus est lent et sensible aux conditions opérationnelles.

La présente étude vise à développer un système hybride robuste pour une dégradation efficace de la CTC à une concentration dans les WW appropriée pour l'environnement, sur la base d'une combinaison de séparation / filtration sur membrane, de système adsorbant et de dégradation enzymatique.

Partie 2. Revue de littérature

2.1 Contaminants émergents – Produits pharmaceutiques

La détection des contaminants émergents (ECs) dans l'ensemble de la biosphère au cours des dernières décennies est devenu un problème environnemental [9, 10]. Ces composés sont utilisés dans les produits de la vie quotidienne, tels que des composés pharmaceutiques, les produits de soins personnels, les plastifiants, les pesticides, les tensioactifs, etc. alors que leur élimination semble être très difficile à court terme [11]. Ces produits sont libérés continuellement dans l'environnement à un taux légèrement croissant; ils n'ont donc pas besoin d'être persistants pour avoir des effets néfastes sur différents organismes vivants.

Les produits pharmaceutiques sont conçus pour avoir des fonctions biologiques spécifiques dans les organismes récepteurs à leur niveau thérapeutique. Les antibiotiques occupent la troisième place sur le marché des produits pharmaceutiques [12]. Ces composés jouent un rôle dans le traitement, la prévention et la protection des humains et des animaux contre les infections. Outre les humains, l'application préventive des antibiotiques chez le bétail a été autorisée à des niveaux sous-thérapeutiques pour la prévention des maladies, la promotion de la croissance et l'amélioration de l'efficacité alimentaire. La demande mondiale croissante de protéines animales a entraîné l'utilisation régulière des antibiotiques dans les élevages pour augmenter la productivité. Le taux de consommation d'antibiotiques chez le bétail a été estimé à 63 151 tonnes en 2010, soit une augmentation de 36% par rapport à l’an 2000. Le taux de consommation devrait augmenter de 67% (105 596 tonnes/an) d'ici 2030 [13].

Les parties non métabolisées des antibiotiques sont libérés dans les eaux usées (WW), ils se retrouvent dans les stations d'épuration des eaux usées (WWTPs). L'effluent des usines de traitement des eaux usées est l'une des principales sources de rejets dans les eaux de surface, qui se retrouvent ensuite dans les sédiments, le sol, les eaux souterraines et les mers [14, 15]. Au cours des deux dernières décennies, la détection des composés antibiotiques dans différentes matrices environnementales, a attiré l'attention en raison de leurs effets indésirables potentiels sur les différents milieux récepteurs.

2.1.1 Tétracyclines– chlorotétracycline

La famille des tétracyclines (TC) figure parmi les antibiotiques inscrits dont l'utilisation est la plus répandue (15,8%) dans l'élevage [2]. En 2011, les TC ont représenté 42% des ventes totales des antibiotiques aux États-Unis, ce qui les classe parmi les produits pharmaceutiques les plus vendus [16]. Les TC sont également les antibiotiques les plus largement utilisés dans d'autres pays, notamment au Royaume-Uni, au Canada, en Nouvelle-Zélande et en Chine [2]. Parmi les membres de cette famille, la chlorotétracycline (CTC, IUPAC: 2- [amino (hydroxy) méthylène] -7-chloro-4- (diméthylamino) -6, 10, 11, 12a-tétrahydroxy-6-méthyl-4, 4a, 5,5a-tétrahydrotétracène-1,3,12-trione, $C_{22}H_{23}ClN_2O_8$) est l'antibiotique le plus commun à travers le monde pour l'application vétérinaire. En 1945, la CTC a été découverte par Benjamin Minge Duggar qui l'a isolé à partir d'un micro-organisme du sol, *Streptomyces aureofaciens* et en 1951, la FDA a approuvé l'application de la CTC à des fins prophylactiques chez le bétail [3]. Ce composé antibiotique est largement utilisé pour inhiber le transfert de “aminoacyl tRNA” au complexe “mRNA-ribosome” sur le site accepteur et par conséquent empêcher la reproduction des cellules bactériennes [17].

2.1.2 Application et propriétés de la CTC

La CTC a été approuvée en 1951 par la Food and Drug Administration pour être utilisée comme additif dans les fermes d'élevage [18]. La CTC a été un composé efficace pour le traitement des infections chez les humains et les animaux en raison de ses faibles effets secondaires, de son rapport coût-efficacité et de son effet antibactérien à large spectre. Actuellement, la CTC est largement utilisée à des niveaux thérapeutiques et sous-thérapeutiques (10-500 g/tonne de nourriture) pour l’élevage de bétail, de poulet et du porc dans la plupart des pays du monde [2La solubilité de la CTC dans l'eau est de 8,6 mg/mL et son absorption dans le tractus gastro-intestinal est de 25 à 30% de la dose qui est inférieure à celle des autres membres de la famille de tétracycline. Les propriétés physicochimiques de la CTC sont présentées dans le tableau 1.2.1.

Tableau 1.2.1 Propriétés physicochimiques de la chlorotétracycline

Propriété	Détail
Formule moléculaire	$C_{22}H_{23}ClN_2O_8$
Masse moléculaire	478,2
Densité	1,7±0,1 g/cm^3
Solubilité dans l'eau	8,6 mg/mL
Point de fusion	210–215 °C
Point d'ébullition	821,1±65,0 °C (760 mmHg)
La pression de vapeur	0,0±3,1 mmHg (25 °C)
log Ko/w	-0,53
pK_a	3,3 7,55 9,33

Parmi les membres de la famille des tétracyclines, la CTC a une faible solubilité dans l'eau et présente l'absorption la plus faible (25-30% de la dose) dans le tractus gastro-intestinal. La CTC est excrétée dans l'urine et les fèces en quantités comparables, inchangées ou microbiologiquement inactives [19]. La demi-vie de la CTC est de 6 à 8 h dans le corps humain et le 6-iso-CTC et le 4-épi-6-iso-CTC sont les principaux métabolites de la CTC chez l'humain et du 4-épi-CTC chez le bétail. Bien que ces métabolites soient inactifs sur le plan microbiologique, leur profil toxicologique n'est pas connu.

2.1.3 Structure de la CTC - activité antibactérienne

La CTC est un acide organique et sa structure moléculaire est représentée dans la figure 1.2.1. La molécule de la CTC comprend un noyau tétracyclique fusionné linéaire. Tous les changements structurels dans ces groupes fonctionnels ont entraîné la perte de l'activité antibactérienne des TC [17].

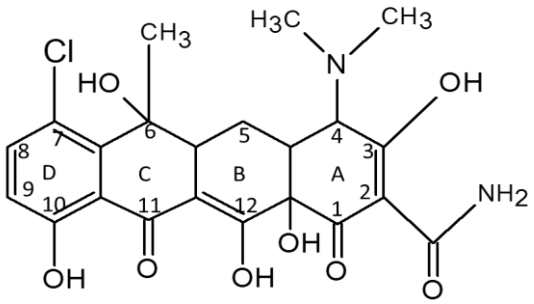

Figure 1.2.1 Structure de la chlorotétracycline

Les atomes de carbone, C-4 est une exigence essentielle pour l'action pharmacologique et le groupement amide en C-2 est considéré comme une caractéristique structurale requise pour l'action biologique des tétracyclines [20]. CTC inhibe le transfert de “aminoacyl tRNA” au complexe “mRNA-ribosome” au site accepteur (sous-unité ribosomique 30S), qui affecte la synthèse des protéines. Ce qui implique que la CTC ne tue pas le microorganisme, mais empêche la reproduction bactérienne, donc le microorganisme est éliminé par le système de défense du patient [20]. Aucune preuve n'a été trouvée pour la cancérogénicité et la génotoxicité de la CTC, mais elle a eu des effets toxiques aigus plus faibles à des concentrations très élevées (2150 à 5000 mg/kg de poids corporel) chez les rats et les souris [21].

Trois valeurs de constante de dissociation (pKa) distinctes proviennent de trois groupes acides qui subissent différents équilibres acide-base en fonction du pH. Les valeurs de pKa de la CTC sont importantes pour déterminer les propriétés d'absorption, de distribution, de métabolisme et d'excrétion de la CTC, comme indiqué dans le tableau 1.2.2.

Tableau 1.2.2 Propriétés pharmacodynamiques et cinétiques de la chlorotétracycline

Propriété	**Détail**
Absorption	20–30%
Temps pour atteindre la concentration maximale	3–4h
Demi-vie	5,6h
Liaison protéique	50–55
Élimination urinaire %	30–40
Élimination fécale %	>50

La CTC existe sous plusieurs formes de protonation en fonction du pH, par conséquent, les valeurs de pKa peuvent être utiles pour estimer les espèces chargées dans l'environnement. Sur la base des valeurs de pKa, nous pouvons observer que la CTC existe sous la forme d'un zwitterion neutre (1 charge négative et charge positive) entre pH 4 et 7. La figure 1.2.2 présente, les groupes fonctionnels correspondants à chaque pKa. Les valeurs de pKa de la CTC conduisent l'absorption, la solubilité et la distribution dans différentes phases, ainsi aident à la

compréhension des phénomènes, tels que l'absorption biologique, la liaison aux matrices environnementales et la formation de chélates avec des cations métalliques.

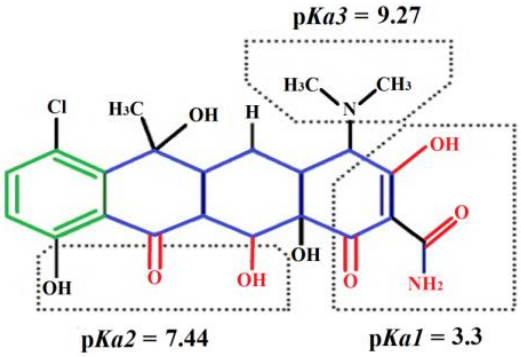

Figure 1.2.2 Groupes fonctionnels de la chlortétracycline et valeurs de pKa

La demi-vie de la CTC dans les milieux aqueux est de 6-8 h et la majorité des métabolites sont le 6-iso-CTC et le 4-epi-6-iso-CTC. De plus, le 4-epi-CTC et l'iso-CTC ont été détectés en faibles concentrations sous forme de métabolites de la CTC [22]. La figure 1.2.3 montre les principaux métabolites de la CTC. Bien que ces métabolites soient inactifs sur le plan microbiologique, leur profil toxicologique n'est pas connu. La présence de ces métabolites est un défi majeur dans la quantification des CTC par chromatographie liquide-spectrométrie de masse en tandem puisqu'ils sont apparus à différents temps dans le chromatogramme et qu'ils peuvent se transformer selon plusieurs facteurs, comme le pH et la présence de Mg^{2+} [23].

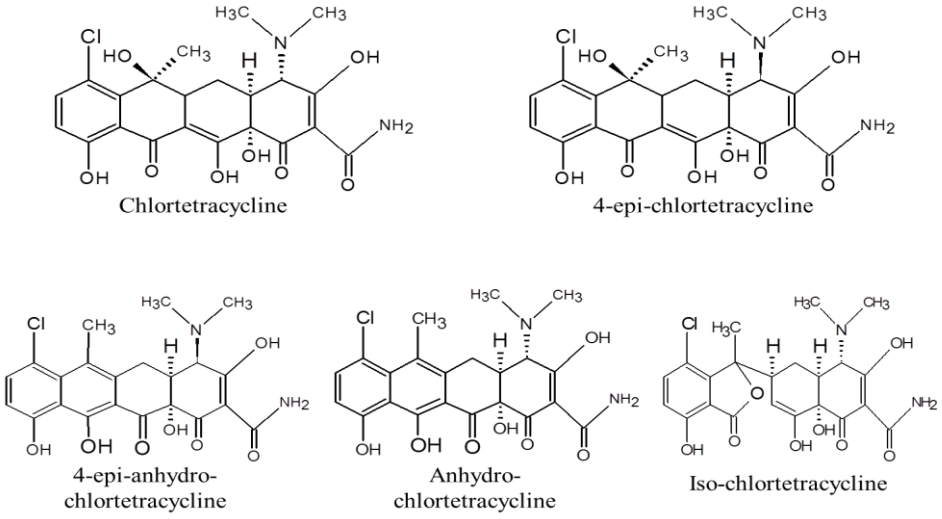

Figure 1.2.3 Chlorotétracycline et ses métabolites

2.1.4 Complexation avec des métaux et résistance aux antibiotiques

Généralement, les ions métalliques tendent à se complexer avec les composés organiques dans les eaux usées à une large gamme de pH, également à modifier les propriétés chimiques des composés associés. Les métaux peuvent induire diverses réactions, y compris l'hydrolyse, la substitution, les réactions d'oxydoréduction, la photodégradation, la biodégradation et l'adsorption. La CTC peut former des complexes avec des métaux, tels que le magnésium, le calcium et le fer qui se trouvent dans l'eau et les WW. Cette complexation peut affecter les propriétés antibactériennes et la cinétique de transformation de la CTC. Les principaux sites de chélation comprennent le système dicétone (C11 et C12) et les groupes énol (C1 et C3) et carboxamide (C2) du cycle A (Figure 1.2.1). Des groupes fonctionnels multiples riches en électrons de la CTC, tels que les acétamides, les fragments de β-dicétone phénolique et les groupes diméthylammonium sont stabilisés par complexation. La charge négative des atomes donneurs d'électrons présents dans la CTC est partagée avec une charge positive des métaux. On pense que, la liaison des ions métalliques stabilise la grande densité électronique de la CTC et facilite sa liaison aux ribosomes et aux protéines, auxquels ils ne peuvent pas se lier par eux-mêmes et donc cette chélation peut augmenter la toxicité de la CTC [24, 25].

2.2 Présence et devenir de la CTC dans l'environnement

Le sort de chaque composé antibiotique dans l'environnement est une situation unique qui nécessite une enquête. Selon la loi de conservation de la masse, les contaminants émergents ne disparaissent pas mais ils sont transformés en différents composés qui peuvent être moins ou plus toxiques pour l'environnement. Cette transformation peut être due à divers processus, tels que la sorption, les processus abiotiques et biotiques. Le devenir de ces contaminants émergents dépend des propriétés physicochimiques du composé et des conditions de l'environnement dans lequel il existe. Les stations d'épuration municipales sont une source importante de gènes de résistance aux antibiotiques dans l'environnement. Comme les procédés de traitement dans les WWTP entraînent le mélange de diverses bactéries avec des antibiotiques à des concentrations sub-inhibitrices, ils provoquent une résistance chez les bactéries [26, 27]. Des concentrations variables de CTC, de 0,02 mg/kg à

0,8 mg/kg, ont été détectées dans des eaux usées et des boues d'épuration en tant que principaux milieux de transport de la CTC dans l'environnement. Environ 90% des CTC dédiées aux animaux se retrouvent dans les excréments et dans l'urine, de sorte que la concentration de la CTC dans les effluents d'élevage peut atteindre 300 mg/kg [28]. Dans le tableau 1.2.3, les concentrations environnementales de la CTC dans plusieurs pays sont indiquées.

Tableau 1.2.3 Concentrations environnementales de chlorotétracycline dans différents pays

Matrice	Concentration moyenne (µg/L)	Pays
Les eaux de surface	0,15	Etats-Unis
Eaux usées	1,2	Etats-Unis
Les eaux de surface	0,42	Etats-Unis
Eaux usées	0,97	Canada
Sol	55,3	Canada
Les eaux de surface	0,69	Royaume-Uni
Les eaux de surface	0,9	Royaume-Uni
Sol	4,6-7,3	Allemagne
Sol	41.8	Royaume-Uni
Le fumier de porc	<46	Autriche
Ref: [20, 29, 30]		

Lorsque le fumier contenant de la CTC est épandu sur les champs agricoles, l'engrais peut répandre la CTC dans l'environnement par ruissellement de surface et peut être considéré comme une source non ponctuelle. Par conséquent, la CTC et d'autres antibiotiques sont largement répandus dans l'environnement en raison des sources ponctuelles et non ponctuelles de pollution. La mise en décharge des déchets et l'épandage des déchets municipaux comme engrais sont d'autres sources de pollution par la CTC qui conduisent à l'accumulation de CTC dans le sol puis à la lixiviation vers les eaux de surface et souterraines. Par la suite, la CTC peut pénétrer dans les plantes et revenir dans la chaîne alimentaire humaine. La figure 1.2.4 montre le cycle de vie des antibiotiques dans l'environnement.

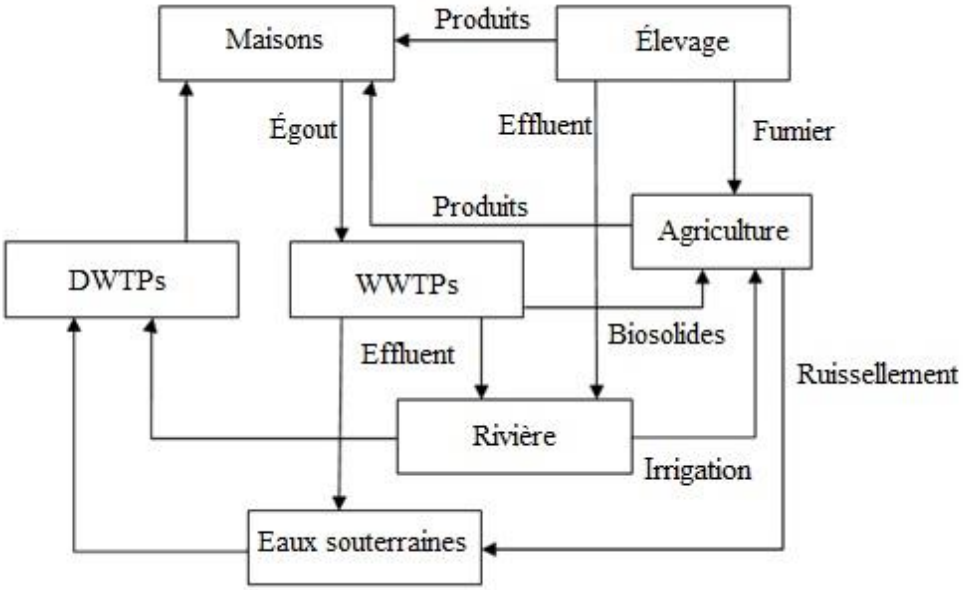

Figure 1.2.4 Cycle de vie de la chlorotétracycline

Lorsque la CTC présente dans l'eau de surface est exposée à la lumière du soleil, la photolyse est considérée comme un processus de dégradation important dans l'environnement aquatique naturel. La photolyse peut se produire soit par absorption directe du rayonnement par la molécule du médicament, soit par la photosensibilisation de la molécule par des composants naturels, tels que les nitrates et les acides humiques [31]. Par conséquent, la matière organique dissoute et le nitrate dans les milieux aquatiques naturels peuvent influencer la réaction photocatalytique [32].

2.3 La CTC dans une station d'épuration

Les WWTPs sont considérées comme une source ponctuelle significative pour les antibiotiques et leurs gènes de résistance aux antibiotiques apparentés dans l'environnement. Lorsque les antibiotiques entrent dans les WWs, leur sort dépend de leurs propriétés physico-chimiques. Ils peuvent être soit minéralisés en CO_2 et en eau ou soit transformés en molécules plus hydrophiles et passer à travers la WWTP avec des composés intacts. L'efficacité de dégradation de chaque stratégie de traitement dépend principalement de la composition de la matrice, par exemple la concentration, le type d'ions et de matières organiques. Dans le cas des tétracyclines, lors de leur passage à travers les WWTPs, la modification chimique

peut se produire par hydrolyse, biodégradation ou adsorption sur les boues. De nombreux auteurs ont rapporté que l'adsorption est le principal facteur d'élimination des antibiotiques, ce qui signifie que l'accumulation prédomine plutôt que l'élimination [33, 34]. Différents taux d'élimination des antibiotiques dans les WWTPs de 20% à 90% ont été rapportés dans la littérature [35, 36], mais ils sont principalement retenus dans les boues ou transformés en une autre forme de pollution plus toxique que la molécule mère. Par conséquent, les produits et/ou voies de transformation devraient être inclus dans les études futures afin d'obtenir des données précises sur les stratégies d'élimination. Les antibiotiques ont un effet négatif sur la performance des processus biologiques dans les stations d'épuration des eaux usées. Par exemple, la présence de CT >5 mg/L a montré une diminution significative de l'efficacité totale d'élimination de l'azote [37].

2.4 Méthodes de traitement pour l'élimination des produits pharmaceutiques

Pour surmonter les inconvénients du traitement biologique actuel dans les WWTPs conventionnelles, la recherche a été orientée vers la conception de technologies de traitement pour l'élimination efficace des antibiotiques résiduels de l'eau et de WW. Jusqu'à présent, de nombreuses méthodes ont été développées qui peuvent être divisées en séparation physique, transformation chimique et aussi leurs processus combinatoires.

2.4.1 Procédés d'oxydation avancée

Les procédés d'oxydation avancée (AOPs), tels que l'ozonation, la lumière ultraviolette (UV) et électrochimique, permettent d'obtenir des taux d'élimination plus élevés des antibiotiques et de réduire la toxicité des sous-produits [38, 39]. Par exemple, plus de 98% d'élimination de l'oxytétracycline et de la doxycycline ont été obtenues en utilisant UV/H_2O_2 [40]. Le principe des AOPs est de produire du radical hydroxyle qui est un oxydant très puissant capable d'oxyder une large gamme de composés organiques, dans l'eau [41, 42]. Les AOPs ne peuvent pas être totalement efficace en mode autonome pour la dégradation complète et la minéralisation des contaminants organiques, dans certains cas, ils conduisent également à la formation de sous-produits plus toxiques. De plus, la performance des AOPs en mode

autonome est affectée par plusieurs paramètres tels que la turbidité, les matières organiques naturelles et les composés inorganiques qui compromettent leur efficacité [43]. Dans ce cas, une combinaison de processus peut être utile, mais elle augmente les coûts d'installation et d'exploitation. Les inconvénients mentionnés, ainsi que l'utilisation de produits chimiques dangereux, qui consomment beaucoup d'énergie ou qui sont sensibles à la matrice, indiquent que les études approfondies sont nécessaires pour mettre au point un système AOP robuste et rentable.

2.4.2 Processus enzymatiques

Le traitement de l'eau polluée à l'aide d'enzymes oxydoréductases a suscité l'intérêt des chercheurs pour le traitement de WW en raison de leur faible empreinte environnementale et du fait qu'elles ne sont pas spécifiques aux composés cibles [44]. La laccase est un membre des enzymes ligninolytiques connue pour son activité catalytique dans l'oxydation de divers composés, elle est facilement produite par fermentation de plusieurs microorganismes, tels que les champignons de pourriture blanche sur biomasse lignocellulosique, tels que les copeaux de bois. De plus, elle a été rapportée pour son excellente capacité de dégradation des contaminants émergents, tels que les produits pharmaceutiques [45]. Les laccases ont quatre atomes de cuivre par monomère situés au site catalytique. Le cuivre de type 1 est responsable de l'oxydation du substrat [46]. Dans une réaction typique de la laccase (Figure 1.2.5), la laccase se transforme en forme oxydée en transformant l'oxygène en eau dans la première étape et en seconde étape la laccase oxydée peut attaquer les groupes donneurs d'hydrogène dans le substrat, comme les groupes phénoliques et les retourner en radicaux. Les radicaux produits peuvent en outre subir des réactions catalysées par la laccase et/ou des réactions non enzymatiques, telles que la polymérisation, l'hydratation ou l'extraction d'hydrogène, ce qui contribuera à la dégradation [46].

la laccase est bien connue pour l'oxydation des composés phénoliques et en particulier la laccase obtenue à partir de champignons de la pourriture blanche est connue pour son pouvoir à oxyder les hydrocarbures aromatiques polycycliques (PAHs) en leurs quinones correspondantes et ensuite dégrader le composé en CO_2 [47]. La capacité de la laccase peut être améliorée en utilisant des médiateurs enzymatiques tels que le 1-hydroxybenzotriazole (HBT), car ils facilitent le transfert d'électrons. D'après Suda et al. l'utilisation de la laccase seule ne pouvait pas

dégrader la CTC de plus de 45% même après 4 h, mais la laccase avec HBT dégradait 98% de la CTC en moins de 1 h [48].

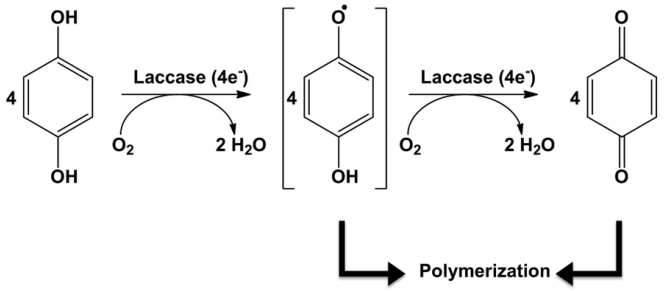

Figure 1.2.5 Dégradation de composés organiques en utilisant la laccase

L'un des défis d'utilisation de la laccase à grande échelle est le coût de production élevé par les méthodes conventionnelles [46]. Alors, l'utilisation des déchets, tels que les déchets agricoles comme substrats pour la production de la laccase peut réduire les coûts de production. Les déchets de l'industrie de la pomme, appelés marc de pomme, ont été utilisés pour produire différents enzymes [49, 50]. Au Canada, la production totale de pommes commercialisée était de 390 362 tonnes métriques en 2011, ce qui signifie que des milliers de tonnes de marc de pomme sont produites comme déchets au Canada [51]. La plupart des marcs sont maintenant utilisés comme aliments pour les animaux, mais dans une mesure limitée en raison de leur faible digestibilité et de leur faible teneur en nutriments, comme les protéines [52, 53]. Par conséquent, le marc de pomme est un substrat facultatif rentable pour la production de la laccase. La faible stabilité et la possibilité de séparation du milieu sont parmi les défis des enzymes. La stabilité de la laccase dépend du microorganisme et des conditions de stockage. Par exemple, la demi-vie de la laccase purifiée à 50 °C est de quelques minutes pour l'enzyme produite à partir de *Botrytis cinerea* et de plusieurs jours pour l'enzyme produite à partir de *Trametes sp.* [54, 55]. Pour surmonter ces problèmes, l'immobilisation ou l'encapsulation des biocatalyseurs ont été développées pour éviter le lessivage et faciliter leur réutilisation [56]. Selon la littérature, la stabilité et l'activité des enzymes immobilisés sont supérieures à celles de l'enzyme libre. Mais, les taux de dégradation étaient plus élevés pour les enzymes libres en raison des limitations de transfert de masse pour les enzymes immobilisées [57, 58]. Des matériaux inorganiques poreux, en particulier des nanoparticules de silice et d'alumine, ont été largement utilisés comme matériau porteur d'enzyme [58]. En outre, des polymères

synthétiques, tels que le polyacrylonitrile (PAN) et les biopolymères, tels que le chitosane ont été étudiés en tant que support pour l'immobilisation des enzymes [59, 60].

2.4.3 Systèmes d'adsorption

Les adsorbants classiques, tels que le charbon actif, ont été testés avec succès pour l'élimination des contaminants émergents [61]. L'efficacité d'élimination de ces adsorbants dépend des propriétés physicochimiques des adsorbants et des adsorbats, tels que l'hydrophobie, les groupes fonctionnels, la taille des pores et la surface externe ainsi que les conditions opérationnelles telles que le pH et la température [61, 62]. Par conséquent, tous les adsorbants ne peuvent pas être des adsorbants idéaux pour tous les contaminants. Par exemple, la plupart des charbons actifs ont des pores plus petits que 2 nm et ne peuvent donc pas adsorber des molécules de plus de 3000 Da [63]. Le biocharbon qui est un matériau carboné produit par pyrolyse de différente biomasse, de la sciure de bois et des boues de WWTP a montré ses capacités en tant que produit à valeur ajoutée pour plusieurs applications environnementales [64-66]. Le biocharbon utilisé dans la séquestration du carbone, la production d'énergie et l'augmentation du rendement des cultures a stimulé sa production à travers le monde [65, 66]. La structure poreuse et la chimie de surface du biocharbon en font un excellent adsorbant pour une large gamme de contaminants [67]. Il y a des facteurs incluant le pH, la température, les charges de surface et les groupes fonctionnels d'adsorbat qui affectent directement la capacité d'adsorption et le mécanisme d'adsorption. De plus, si le biocharbon est activé en utilisant une méthode chimique ou physique, sa capacité pour les composés organiques peut être augmentée jusqu'à cent fois. Par conséquent, la compréhension de l'interaction entre le biocharbon et le contaminant est nécessaire pour concevoir une technique d'élimination efficace. Dans le cas de la CTC, étant donné qu'il a différentes charges électrostatiques à différents pH, les interactions électrostatiques avec le biocharbon sont significativement importantes dans la capacité d'adsorption.

2.4.4 Séparations membranaires

Les processus membranaires sont intéressants en raison du taux élevé d'élimination des polluants organiques de faible poids moléculaire, l'excellente qualité de l'effluent, la modularité et la capacité d'intégration avec d'autres systèmes [68]. Parmi les

quatre catégories de séparation membranaire incluant l'osmose inverse (RO), la nanofiltration (NF), l'ultrafiltration (UF) et la microfiltration (MF), seuls RO et NF sont capables de les éliminer directement de l'eau car les composés pharmaceutiques sont solubles dans l'eau et sont présents à leur taille moléculaire. L'efficacité d'élimination des membranes RO et NF pour différents composés pharmaceutiques est rapportée jusqu'à 99,6% [69].

Dans le cas des antibiotiques tétracyclines, étant donné que leurs poids moléculaires (> 450 Da) sont supérieurs à un seuil de coupure (Masse molaire critique pour laquelle 90 % des solutés sont retenus par la membrane) des membranes NF et RO (<200 Da), des taux de rejet élevés (> 85%) sont attendus pour ces membranes [70]. Cependant, malgré les avantages et la haute performance mentionnée, un inconvénient majeur est que les procédés de filtration sont conçus pour concentrer mais pas pour dégrader les polluants et par la suite exiger l'élimination du flux de déchets qui représente normalement 5 à 30% du flux entrant. De plus, si les PhACs ne sont pas dégradés, la concentration des solutés sur le côté perméat des membranes augmente en augmentant la récupération du perméat et par la suite, la performance globale sera négativement affectée [68]. Par conséquent, les polluants dans le flux de retentât doivent être dégradés ou transformés en composés chimiques inoffensifs par l'une des méthodes chimiques. Outre ces avantages et inconvénients, il existe plusieurs paramètres non clairement détaillés qui influencent l'efficacité d'élimination des procédés de séparation par membrane [71].

2.4.4 Méthodes de traitement innovantes

Les chercheurs ont toujours travaillé sur de nouvelles méthodes pour améliorer la performance des processus de traitement. La combinaison ou l'intégration de méthodes actuelles présentent un intérêt puisque différentes méthodes peuvent se compléter et montrer des effets synergiques. Dans le tableau 1.2.4, plusieurs exemples de combinaison de systèmes membranaires avec d'autres technologies ont été répertoriés. Selon ce tableau, dans la plupart des cas, les systèmes combinatoires/hybrides ont atteint des taux globaux d'enlèvement plus élevés. Dans les paragraphes suivants, certaines méthodes innovantes bien connues sont décrites.

Tableau 1.2.4 Efficacité d'élimination des composés actifs pharmaceutiques par des traitements combinatoires

Composants	Type	Composé	Enlèvement (%)	Référence
Osmose inverse + photolyse UV	Combiné	Naproxéne	>99	[72]
Nanofiltration / Osmose inverse + procédé photo-fenton	Intégré	Diclofénac Ranitidine Sulfaméthoxazole	>80 >99 >99	[73]
Nanofiltration + UV / Ozone		Roxithromycine	>98	[74]
Bioréacteur à membrane + photolyse UV	Combiné	Carbamazépine	>96	[75]
Bioréacteur à membrane + Ozonation	Intégré	Acyclovir	>99	[76]
Nanofiltration + charbon actif granulaire	Combiné	Atnolol	>99	[77]
Bioréacteur à membrane + charbon actif granulaire	Combiné	Diclofénac Carbamazépine	>98 >98	[78]
Bioréacteur à membrane + charbon actif granulaire	Combiné	Sulfaméthoxazole Carbamazépine	>82 >92	[79]

2.4.4.1 Séparation membranaire + procédés d'oxydation avancés

L'intégration de processus membranaires avec des procédés d'oxydation avancés présente un grand intérêt car les membranes ne sont pas capables de dégrader les contaminants, même si elles peuvent efficacement les séparer de l'eau. Par exemple, Kim et al. ont combiné nanofiltration (NF) avec oxydation catalytique pour une décomposition efficace de perturbateur endocrinien (EDC) de telle sorte que l'influent entre dans la réaction avant de passer à travers la membrane NF. Ils ont rapporté que l'oxydation catalytique homogène, utilisant le fer (III) - tétrasulfophtalocyanine (FeTsPc) comme catalyseur et le peroxyde d'hydrogène comme oxydant, entraînait une décomposition de 90% du BPA et du diclofénac dans des conditions faiblement acides [68]. En raison du problème de séparation du catalyseur de l'eau dans les systèmes homogènes, ces procédés n'ont pas été utilisés à des fins environnementales. Par conséquent, Kim et al. ont immobilisé catalyseur de FeTsPc sur une résine échangeuse d'ions Amberlite pour l'utiliser en mode hétérogène pour l'élimination des PhACs et observer une amélioration remarquable de la stabilité de FeTsPc due à l'immobilisation avec un taux d'élimination plus élevé pour l'ibuprofène (89%) et le diclofénac (99%) [80, 81].

2.4.4.2 Séparation membranaire + traitement biologique

Osmose inverse (RO) et la NF pourraient éliminer les composés pharmaceutiques directement de l'eau, les membranes d'ultrafiltration (UF) et de microfiltration (MF) peuvent aider à éliminer ces composés en combinaison avec d'autres systèmes. Le bioréacteur à membrane (MBR) est la combinaison d'une membrane (principalement UF et MF) avec un bioréacteur de croissance en suspension. Dans ce système, trois mécanismes d'adsorption, de biodégradation et de séparation membranaire sont combinés pour permettre la production d'un effluent avec de très faibles quantités de solides en suspension, de turbidité, de demande biologique en oxygène et de pathogènes [82-85]. L'efficacité d'élimination du système pour différents composés pharmaceutiques tels que le diclofénac et l'ibuprofène a été rapportée jusqu'à 99,9% [69] et jusqu'à 83% pour la CTC [86].

Le réacteur à membrane enzymatique (EMR) est un autre exemple de combinaison de membrane UF avec le processus biologique dans lequel l'enzyme libre ou l'enzyme immobilisée sur les particules solides est utilisée pour dégrader les composés et la membrane UF laisse l'eau et les sous-produits de transformation quitter le réacteur mais ne retient que l'enzyme, ce qui rend l'immobilisation des enzymes sur la membrane UF ou MF applicable. Ces systèmes intégrés présentent des avantages, tels qu'une capacité enzymatique élevée, une activité enzymatique prolongée, des débits plus élevés, un fonctionnement et un contrôle réalisables réduisant les coûts d'investissement, d'exploitation avec des rendements élevés [87]. De nombreuses études ont incorporé la lipase, la cellulase, la catalase et la laccase dans des membranes polymères et céramiques pour intégrer la capacité de rétention des membranes avec caractère oxydatif des enzymes pour la dégradation de diverses matières organiques [88-99]. Par exemple, Cazes et al. ont immobilisé la laccase sur une membrane UF alumine et obtenu 56% de dégradation de la tétracycline après 24 h [100].

2.4.4.3 Processus membranaires + systèmes d'adsorption

La combinaison de systèmes d'adsorption et la séparation par membrane a été importante pour l'élimination des composés pharmaceutiques. Nam et al. ont étudié l'adsorption de produits pharmaceutiques hydrophiles et hydrophobes sur carbone activé en poudre (PAC), ils ont observé que l'augmentation du dosage et du temps

de contact augmente l'élimination par l'adsorption, mais une analyse de rentabilité de ce système est nécessaire avant l'étape de l'application dans les WWTPs. D'après leur expérience, Ils ont constaté que chaque PhAC présentait une efficacité d'élimination optimale (49,6-79,4%) dans un certain pH en raison de la variation de la charge de soluté et de la charge de surface de PAC mené par le changement de pH [101]. Snyder et al. ont utilisé le PAC et le charbon actif granulaire (GAC) comme prétraitement pour les systèmes de séparation membranaire, ils ont observé que le GAC et le PAC peuvent éliminer efficacement les produits pharmaceutiques de l'eau (élimination de plus de 90%), mais l'efficacité de GAC est influencée par la matière organique naturelle (NOM) qui entre en compétition pour les sites de liaison du GAC alors que le PAC a plus d'avantages vu qu'il ajouté en continu ce qui élimine son recyclage [72]. Par conséquent, une analyse plus approfondie est nécessaire pour prendre en considération le coût de la récupération du GAC et la consommation de PAC ainsi de leur performance.

Les membranes adsorbantes sont une autre combinaison de membrane et de système d'adsorption. Ils ont de nombreuses applications dans les procédés de clarification, de concentration et de purification; ils offrent plusieurs avantages par rapport aux systèmes conventionnels à lit emballé, notamment une faible contre-pression, l'absence de canalisation, des temps de séjour courts et un débit volumétrique élevé [97]. Les membranes adsorbantes peuvent être fabriquées en utilisant des précurseurs de membrane ayant une affinité avec des composés ciblés, en modifiant la surface de la membrane avec des groupes fonctionnels ou en incorporant des adsorbants dans les matrices de membrane. [102-107]. Après une démonstration des fibres submicroniques produites par les techniques de filature dans les années 1990, de nouveaux horizons sont apparus pour différents domaines, en particulier les processus membranaires [108]. Les membranes nanofibreuses (NFM) produites par électrofilage peuvent avoir un impact sur les performances des technologies de séparation en raison de leur rapport surface/volume élevé, d'une taille de pore ajustable et de la facilité de fonctionnalisation [109]. Les NMF adsorbants peuvent être utilisés pour éliminer les métaux lourds, les composés organiques, les micro-organismes et les biomolécules, ce qui en fait des candidats potentiels pour les applications environnementales. Il existe de nombreux rapports récents sur la fonctionnalisation des NMF pour l'élimination des composés préoccupants pour l'environnement. Par exemple,

Vanraes et al. ont utilisé le NMF polyamide en combinaison avec une décharge électrique pour adsorber et dégrader l'atrazine dans l'eau [110].

2.4.4.4 Procédés membranaires + procédés biologiques + systèmes d'adsorption

Bien que le MBR ne soit pas efficace pour l'élimination des produits pharmaceutiques persistants et hydrophiles tels que la carbamazépine et les tétracyclines, alors l'ajout d'adsorbant dans le bassin MBR peut compléter le système de traitement en augmentant la capacité d'élimination des composés hydrophobes et hydrophiles. Selon les résultats de Nguyen et al, les composés persistants, tels que le diclofénac et la carbamazépine présentant des efficacités d'élimination inférieures à 40%, par le traitement MBR ont atteint des taux d'élimination globaux de 98% ou plus après le traitement par GAC. Cependant, ils ont noté que ce système intégratif nécessite une surveillance stricte pour détecter la percée de composés hydrophiles et persistants entrainés par la saturation du lit de GAC [78]. Aussi, Urase et al. a ajouté le PAC directement au système MBR et observé que le coefficient de partage boue-eau de la carbamazépine augmentait de 0,057 L/g de solides en suspension mélangés liquides (MLSS) avant PAC à 0,597 L/g SSML après PAC, ce qui augmente la capacité d'adsorption de la carbamazépine par boue activée. [111].

Dans une nouvelle étude, Wang et al. ont immobilisé la laccase sur une membrane composite électrofilée faite de PAN, d'oxyde de graphène (GO) et de montmorillonite (MMT) pour dégrader le catéchol, ils ont eu une dégradation d'environ 40% [108]. Cependant, ils ont utilisé un procédé d'adsorption simple pour immobiliser la laccase, ce qui a entraîné une faible réutilisation des biocatalyseurs. Finalement, d'autres études sont nécessaires dans ce domaine, en particulier l'essai de la liaison covalente comme méthode d'immobilisation et l'utilisation de matériaux adsorbants économiques.

En conclusion, la combinaison et l'intégration de systèmes d'adsorption de membranes et de procédés biologiques pourraient permettre aux opérateurs de tirer parti de tous les composants, y compris la production de produits de transformation non toxiques, la haute qualité des effluents et la possibilité d'automatisation.

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Partie 3. Problématique

En se basant sur les différents travaux de recherche qui porte sur le devenir des tétracyclines dans les WWTPs et l'environnement, les problèmes suivants ont été identifiés:

- La CTC est utilisée comme additif alimentaire pour la promotion de la croissance et la prévention des maladies depuis plus de 60 ans. L'ajout continu de la CTC à forte dose à l'alimentation du bétail au cours de la période mentionnée a conduit à la libération constante de la CTC dans l'environnement et a contribué à l'augmentation de la résistance bactérienne dans les écosystèmes. Bien que la CTC soit utilisée depuis longtemps dans le traitement des infections humaines et animales, le développement de bactéries résistantes aux antibiotiques compromettra l'efficacité de tous les antibiotiques chez le corps humain

- La CTC a une faible solubilité dans l'eau (8,6 mg / mL) et une faible absorption (25-30% de la dose) dans le tractus gastro-intestinal. Une concentration élevée de la CTC non métabolisée dans le fumier a confirmé que plus de 70% des CTC quittent le corps du bétail sans perdre leur activité. L'épandage de fumier et de bio-solides (déchets municipaux) comme engrais dans les champs agricoles entraîne une accumulation de CTC dans le sol. À partir du sol, la CTC peut s'infiltrer dans les eaux souterraines et ruisseler dans les eaux de surface et se retrouver dans les plantes et retourner par la suite à la chaîne alimentaire.

- En considérant la consommation actuelle des antibiotiques et de la tendance du marché, la demande mondiale d'antibiotiques devrait augmenter à un taux de croissance annuel de 0,8%. La libération constante de CTC bioactif pourrait avoir un impact négatif sur tous les organismes terrestres. En fait, nous avons montré que la CTC induisait des radicaux hydroxyles chez les plantes à des concentrations supérieures à 25 mg/L. Ces radicaux ont des effets génotoxiques potentiels sur la germination et la croissance.

- La CTC trouve son chemin vers les WWTPs à travers le système de collecte des eaux usées et forme des complexes avec différents métaux dans des boues d'épuration. Les complexes CTC-métal peuvent présenter une toxicité encore plus élevée que celle de la CTC envers les bactéries et une résistance accrue aux antibiotiques.

- Les WWTPs ne sont pas en mesure d'éliminer complètement les antibiotiques de WW, entre 20 et 70% de la CTC restent dans l'effluent qui subit une photolyse en se transformant à des sous-produits plus toxiques que la CTC elle-même.

- La dégradation des micropolluants à l'aide d'enzymes est un domaine de recherche émergent. La laccase présente une excellente capacité de dégradation vis-à-vis des micropolluants, tels que les produits pharmaceutiques. Diverses études de dégradation ont été effectuées en utilisant des concentrations de la CTC surchargées qui n'étaient pas pertinentes pour le milieu naturel réel ou le WW. Par conséquent, il est difficile d'extrapoler les résultats aux conditions réelles.

- Dans les traitements enzymatiques, la réutilisabilité et la stabilité d'enzymes libres sont les deux principaux obstacles qui peuvent être surmontés par l'immobilisation de l'enzyme sur une variété de supports. L'immobilisation des enzymes augmentera sa stabilité et fournira une protection supplémentaire contre la dénaturation par une gamme de co-solvants organiques. Cependant, l'immobilisation peut conduire à une diminution de l'activité de l'enzyme nécessitant une optimisation et une conception améliorée.

- De grandes quantités d'enzymes sont nécessaires pour un traitement qui entraîne des coûts en utilisant des méthodes de production conventionnelles. Par conséquent, des substrats rentables pour la production d'enzymes doivent être étudiés.

- L'adsorption et l'élimination des résidus pharmaceutiques par des adsorbants classiques, tels que le charbon activé, ont été largement étudiées au cours de la

dernière décennie. Cependant, ces études ont été réalisées à des concentrations plus élevées, telles que des dizaines de mg.L-1 , il est donc difficile d'extrapoler les résultats aux conditions réelles.

- Le biocharbon, en tant qu'alternative à la gestion des déchets a démontré son potentiel de durabilité et son utilité pour plusieurs applications environnementales. Ses propriétés supérieures, telles que sa structure poreuse et sa chimie de surface en font un excellent adsorbant pour plusieurs contaminants environnementaux. Cependant, en raison de l'interaction des polluants avec la surface du biocharbon, les conditions d'adsorption doivent être étudiées.

- Le traitement enzymatique comme d'autres méthodes de traitement produit des composés de transformation. Donc, le devenir et la toxicité des produits de transformation de la CTC doivent être étudiés.

- Les membranes d'adsorption fabriquées par incorporation d'adsorbants, tels que la silice, le charbon actif et le biocharbon dans des matrices, telles que le polyvinylidène difluorure, le polysulfone et le polyacrylonitrile, constituent une autre combinaison de membrane et de système d'adsorption. Ils offrent plusieurs avantages par rapport aux systèmes à lit tassé conventionnels, tels qu'une contre-pression inférieure et un débit volumétrique élevé. Cependant, un temps de résidence court peut affecter l'efficacité et la possibilité d'adsorption pendant un temps de séjour normal des membranes doit être vérifié. L'utilisation de membranes adsorbantes comme support pour l'immobilisation de l'enzyme peut intégrer les fonctions d'adsorption et de dégradation de sorte que l'adsorption fournira suffisamment de temps pour l'action des enzymes.

Partie 4. Hypothèse

"Le développement de membranes au biocharbon imprégnées d'enzymes pour la dégradation des contaminants à base de chlorotétracycline" est basé sur les hypothèses suivantes:

1- D'après la littérature, la CTC est rejetée dans l'environnement en raison de sa grande consommation, ce qui implique que sa concentration devrait augmenter dans le futur. De plus, les mesures législatives visant à contrôler la présence de la CTC dans l'environnement sont faibles. La CTC n'est pas complètement éliminée dans les WWTPs, par conséquent, la modification des méthodes actuelles ou le développement de nouvelles méthodes d'élimination peuvent constituer un progrès.

2- Le biocharbon produit de la pyrolyse de la biomasse a des propriétés supérieures, notamment une structure fortement condensée et une densité de surface qui lui permet de concurrencer les charbons actifs conventionnels dans l'élimination des micropolluants. L'étude d'adsorption de la CTC sur le biocharbon pourrait révéler les interactions entre la CTC et la surface du biocharbon, en testant l'efficacité d'adsorption rapide de la CTC par le biocharbon.

3- Les membranes adsorbantes sont produites par l'incorporation d'adsorbants, tels que le biocharbon dans des matrices polymères qui peuvent surmonter les problèmes actuels des systèmes à lit tassé y compris la haute contre-pression et la canalisation. L'étude de l'efficacité de la membrane adsorbante pour l'adsorption de la CTC pourrait être utile dans le traitement des eaux.

4- L'utilisation de la technique d'électrofilature pour la fabrication de la membrane adsorbante pourrait avoir un impact sur les performances du procédé de séparation en raison de leur porosité élevée, de leur rapport surface / volume élevé et de la facilité de les fonctionnaliser.

5- Les enzymes ligninolytiques, en particulier la laccase, sont capables de dégrader de manière non spécifique les composés organiques. L'utilisation de médiateurs, tels que l'ABTS peut augmenter l'efficacité de la laccase. L'étude de la dégradation de la CTC en utilisant le système laccase-ABTS dans différentes conditions de pH et de température pourrait aider à concevoir une méthode de traitement efficace.

6- L'immobilisation de la laccase sur des membranes adsorbantes pourrait être avantageuse pour le système d'adsorption et le traitement enzymatique. De plus, l'immobilisation pourrait augmenter la stabilité de l'enzyme. **Un tel système fournit le temps nécessaire pour la dégradation des composés organiques par l'enzyme**. Cette approche pourrait être utilisée comme une approche conviviale et efficace pour le traitement de la CTC dans les eaux usées.

7- **Le traitement de la CTC par l'intermédiaire d'une enzyme immobilisée sur la membrane contenant le biocharbon produira des quinones qui peuvent être minéralisées avec une oxydation supplémentaire**. Ce type de bioréacteur peut être mis à l'échelle dans une WWTP particulièrement au niveau du traitement tertiaire. La production de biocharbon et d'enzymes à partir de déchets peut effectivement réduire le coût.

Partie 5. Objectifs

L'objectif global de ce travail est de "développer un système membrane-biocharbon imprégné d'enzyme pour l'élimination de la CTC de WW". Le présent sujet de recherche comprend les objectifs de recherche spécifiques suivants:

1- Étude de l'adsorption de la CTC sur le biocharbon brut et activé (Chapitre 2, Partie 3)

2- Fabrication de la membrane adsorbante en utilisant le biocharbon et le polyacrylonitrile (PAN) par électrofilature en étudiant sa capacité à adsorber la CTC à un temps de séjour normal pour les membranes (Chapitre 2, Partie 4)

3- Production de la laccase à partir de substrats à faible coût, l'étude de la dégradation de la CTC à l'aide de la laccase libre et l'estimation de la toxicité des sous-produits (Chapitre 3, Partie 1)

4- Immobilisation physique de la laccase sur la membrane adsorbante, l'étude de l'efficacité de son immobilisation, de sa réutilisabilité et de sa stabilité thermique (Chapitre 3, Partie 2)

5- Immobilisation covalente de la laccase sur la membrane adsorbante, l'étude de la dégradation de la CTC dans l'eau pure (Chapitre 3, Partie 3)

6- Fabrication de membranes multifonctionnelles en utilisant le chitosane et le PAN, l'étude de son activité antibactérienne et l'utilisation de la laccase immobilisée sur une membrane PAN-chitosane pour la dégradation de la CTC avec deux autres composés des autres groupes pharmaceutiques présentes dans le WW (Chapitre 4, Partie 1-2)

Partie 6. Originalité

À partir des hypothèses et objectifs qui précèdent, cette étude englobe l'originalité due aux points suivants :

1. Activation du biocharbon de pin et l’étude du comportement d'adsorption de la CTC sur le biochar activé à différentes valeurs de pH.
2. Fabrication d'une membrane adsorbante par électrofilage de PAN-biocharbon en testant son efficacité à éliminer des résidus pharmaceutiques de l'eau.

Partie 7. Sommaire des différents volets de recherche effectués dans cette étude

1. Fabrication d'une membrane adsorbante et étude de sa capacité à adsorber la CTC

Titre: Étude d'adsorption de concentrations de chlorotétracycline pertinentes pour l'environnement sur le biocharbon de pin

Le comportement de l'adsorption de CTC à des concentrations qui se trouvent dans l'environnement sur le biocharbon de pin activé et non a été étudié à 298 K et à différents pH (1, 5 et 9). Les résultats ont montré que l'activation du biocharbon a augmenté la surface spécifique de 14,86 m^2/g à 852,95 m^2/g, ce qui a abouti à une plus grande capacité d'adsorption du biocharbon. De plus, les résultats ont montré que la CTC suivait l'isotherme de Langmuir pour l'adsorption sur les deux biocharbons et que l'adsorption maximale se produisait à pH égale 1. L'interaction électrostatique est le facteur le plus important qui domine l'adsorption. En outre, il a été constaté que la capacité d'adsorption maximale (q_m) du biocharbon synthétisé et activé dans cette étude (434 mg/g) était beaucoup plus élevée que les précédentes études (57-303 mg/g). Ainsi, le bois de pin biocharbon est un bon candidat pour la production d'une nouvelle génération d'adsorbants à haute performance à partir de ressources renouvelables avec de méthodes non polluantes, notamment pour les contaminants émergents tels que la CTC à des concentrations pertinentes pour l'environnement qui seront par la suite utilisés pour une éventuelle application dans les WWTPs.

Titre: Développement d'une membrane adsorbante par confinement de biocharbon activé dans des nanofibres électrofilées

Les membranes nanofibreuses adsorbantes ont été fabriquées par électrofilature à partir d'une solution de PAN qui contient 0-2% de biocharbon activé. Les résultats du test d'adsorption BET ont montré qu'à une charge de 1,5% de biocharbon, la surface maximale a été atteinte en raison de l'agrégation des particules à des concentrations plus élevées ainsi que de la formation d'agrégats qui réduisent le rapport surface/volume. Le test d'adsorption en mode continu a indiqué que la membrane

fabriquée peut éliminer efficacement la CTC des milieux aqueux à un flux d'ultrafiltration normal (30 L/m^2.h). Cela indique que ce système est prometteur et efficace pour l'élimination des contaminants émergents des flux environnementaux aqueux.

2. Application de BIMeMS pour l'enlèvement de CTC.

Titre: Biodégradation de la chlorotétracycline par la laccase produite par *Trametes Versicolor* : Identification des sous-produits

Les effets de pH, de la température, de la concentration enzymatique et de la concentration du médiateur sur l'efficacité de la dégradation ont été étudiés en utilisant la méthodologie réponse de surface. Les paramètres d'ajustement R^2 et R^2 étaient ajustés à 0,85 et 0,70 respectivement, ce qui indique que ce modèle est raisonnablement bon pour des applications pratiques, telles que les systèmes de traitement des eaux usées pharmaceutiques. Selon l'équation obtenue, la température et le pH ont eu un effet très important sur la biodégradation de la chlorotétracycline. Selon l'optimisation réalisée, les conditions optimales de pH, température, concentration de l'enzyme et de médiateur ont été de 5,2, 35,5 °C, 62,3 unités/L et 10,9 µM respectivement, qui entraînent une biodégradation maximale d'environ 95% pour la chlorotétracycline après 8 heures. En outre, il a été montré qu'en utilisant la laccase libre, nous ne pouvons pas dégrader plus de 43% de la CTC après 24 heures de réaction.

Titre: Dégradation de la chlorotétracycline à l'aide de laccase immobilisée sur membrane nanofibreuse composite polyacrylonitrile-biocharbon

La laccase a été immobilisée sur une membrane nanofibreuse composite fabriquée à partir de Polyacrylonitrile-biocharbon, le biocatalyseur obtenu a été utilisé pour l'élimination de chlorotétracycline à partir de milieux aqueux en mode continu. L'utilisation d'une liaison amidoxime a entraîné une charge en laccase de 10,1 unités/g de membrane sèche. Les résultats ont montré que la laccase immobilisée a une meilleure stabilité au stockage, à la température et au pH par rapport à la laccase libre. De plus, elle a conservé plus de 50% de son activité initiale après 7 cycles d'oxydation ABTS, ce qui indique une meilleure réutilisation de l'enzyme.

L'utilisation de la laccase immobilisée pour la dégradation de chlorotétracycline en mode continu présentait 58,3%, 40,7% et 22,6% d'efficacité d'élimination de la chlorotétracycline à des vitesses de flux de 1, 2 et 3 mL/h.cm^2 respectivement. L'incorporation de particules adsorbantes à l'intérieur de la membrane a réduit significativement le temps de traitement. L'augmentation de flux diminue l'efficacité de l'élimination en réduisant le temps de contact.

Titre: Immobilisation covalente de laccase sur une membrane nanofibreuse pour la dégradation des résidus pharmaceutiques dans l'eau

La laccase a été immobilisée par covalence sur une membrane composite polyacrylonitrile/biocharbon et les paramètres d'immobilisation ont été optimisés. Le biocatalyseur obtenu a été utilisé pour l'élimination de la chlorotétracycline (CTC), la carbamazépine (CBZ) et le diclofénac (DCF) en mode discontinu. Les résultats ont montré que la laccase immobilisée avait une stabilité au stockage, à la température et au pH améliorée par rapport à la laccase libre. De plus, elle a maintenu plus de 17% de son activité initiale après 10 cycles d'oxydation ABTS, ce qui indique une meilleure réutilisabilité de l'enzyme. En utilisant la laccase immobilisée pour la dégradation de trois composés pharmaceutiques en mode discontinu présentaient 72,7%, 63,3% et 48,6% d'efficacité de dégradation pour le DCF, la CTC et la CBZ, respectivement, après 8 h de réaction. La tendance à la baisse de l'adsorption au cours du temps de réaction pour tous les composés a confirmé l'effet régénérateur de la laccase sur les sites d'adsorption du biocharbon.

3. Application de BiMeMS pour l'élimination des résidus pharmaceutiques des effluents d'eaux usées et d'eaux.

Titre: Développement d'une membrane multifonctionnelle pour l'élimination des résidus pharmaceutiques dans l'eau

La membrane composite de polyacrylonitrile-chitosane (PAN-CTN) a été produite par le procédé d'inversion de phase et la laccase a été immobilisée sur cette dernière par une liaison covalente. Le biocatalyseur à membrane d'ultrafiltration fabriquée a été utilisé pour l'élimination de trois composés pharmaceutiques à savoir le diclofénac (DCF), la carbamazépine (CBZ) et la chlorotétracycline (CTC) à une

concentration pertinente pour l'environnement en mode discontinu. Les résultats ont montré que la laccase immobilisée sur la membrane PAN-CTN avait une stabilité à la température et au pH améliorée par rapport à la laccase libre. En outre, il n'a pas eu de perte d'activité qu'après 15 cycles d'oxydation d'ABTS, ce qui indique que la stabilité opérationnelle de l'enzyme a été améliorée. À température ambiante, la laccase immobilisée a montré une efficacité de dégradation de 61%, 56% et 48% pour le DCF, la CTC et la CBZ dans l'eau pure et 75%, 68% et 57% respectivement dans l'effluent secondaire de la WWTP.

Titre: Développement d'un purificateur d'eau portable multifonction avancé

Une conception de purificateur d'eau portable a été proposée. Il s'est avéré efficace pour l'élimination des microorganismes, de la turbidité et de micropolluants de quatre eaux lacustres au Québec, Canada. Ce système comprend trois systèmes de membranes constitués d'une combinaison différente de polyacrylonitrile, de chitosane, de biocharbon et de la laccase. Dans les essais préliminaires, ce système a permis d'éliminer environ 99% des micro-organismes, jusqu'à 77% de réduction de la turbidité et 83% d'élimination des micropolluants en moins de 5 minutes de contact. Il reste encore des essais en laboratoire à effectuer pour quantifier la capacité d'épuration ainsi que les performances sur différentes sources d'eaux de surface et souterraines. Ce purificateur portatif présente plusieurs avantages par rapport aux autres dispositifs traditionnels, tels que l'absence de source d'énergie ou de produits chimiques et l'élimination simultanée des bactéries et des micropolluants.

CHAPTER 2

Fabrication of an adsorbent membrane and study of its ability to adsorb the CTC

Part 1

Emerging contaminants: Here Today, There Tomorrow!

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Résumé

Le sujet des contaminants émergents a attiré l'attention des chercheurs et de la société mondiale en raison de leurs impacts négatifs émergents et potentiels sur l'environnement. De nombreuses tentatives ont été faites pour faire lumière sur l'importance de la libération de ces contaminants dans divers environnements et pour pousser en outre les politiciens à prendre les mesures requises. Néanmoins, la stratégie efficace à long terme pour traiter ce problème est latente dans la modification des technologies actuelles des usines de traitement des eaux potables / eaux usées et leur intégration dans un système de gestion de l'eau holistique et circulaire. La recherche scientifique sur les nouvelles technologies de traitement de l'eau doit être réalignée pour prendre en compte les conditions réelles et répondre à certaines exigences définies selon des perspectives techniques et environnementales.

Mots clés

Contaminants émergents, Traitement des eaux usées, Toxicité, Gestion des ressources en eau

Abstract

The subject of emerging contaminants has attracted wide attention from researchers and society world over due to their emerging as well as potential adverse impacts on the environment. Many attempts have been made to shed light on the importance of release of these contaminants into various environments and further urge policymakers to take required measures. Nevertheless, the effective long-term strategy for addressing this issue is latent in the modification of current technologies of wastewater/water treatment plants and integrating them into a holistic and circular water management system. The research on the new technologies of water treatment must be realigned to consider the real conditions and meet certain requirements set according to technical and environmental perspectives.

Keywords

Emerging contaminants, Wastewater treatment, Toxicity, Water resource management

Importance of Emerging Contaminants

The detection of so-called emerging contaminants (ECs) throughout the biosphere in recent decades has emerged as an environmental issue [1, 2]. These compounds are used in everyday products, such as personal care products, plasticizers, pharmaceuticals, pesticides, surfactants, etc. and removing them from products seem to be very difficult in short-term [3]. However, most of the researchers are of firm opinion that legislative intervention by governments can help to control this contamination. At the present time, ECs are not regulated around the world but there are some attempts in EU and North America to make a priority list and reduction of their release into environment. For example, in Canada and Switzerland several projects were performed to develop strategies for reduction of ECs release due to wastewater treatment plants (WWTPs) [4-6]. These compounds are released continuously into the environment with a slightly increasing rate (causing, so-called, "pseudo-persistence"); hence they do not need to be persistent to have adverse effects on different organisms. The effluent from WWTP is one of the main sources of ECs which normally are released into surface waters and then they end up into sediment, soil, groundwater and seas [7, 8]. The reason for residual ECs in the effluent is that most of the WWTPs are designed for partial purification of wastewater (reduction of biological oxygen demand, pathogens, N- and P- compounds) and therefore they are not capable of treating such substances at very low concentrations (normally in micro-or nano-gram per liter) [9]. ECs have diverse chemical properties and are present in complex matrices at very low concentrations. Despite known chemical properties, it is difficult for many compounds to assess if they will go into the solid phase or remain in the aqueous phase and this behavior can bog the suggested treatment technologies. Furthermore, the complexity of matrices and being present at very low concentrations has led to the lack of efficient and standard methods for determination of ECs such as pesticides. This big analytical challenge has hindered the acquisition of data on occurrence, pathways, ecotoxicology and risk assessment of ECs [10-12]. As a result, the spatial-temporal prediction of the fate of ECs in the environment is still a conundrum.

The society at large may assume that there is no need to care about ECs' adverse effects since their concentrations are very low, albeit their toxicity is chronic and often translated across generations. Moreover, due to the world population increase,

the release rate of these compounds is gradually increasing. Furthermore, among all the ECs, antibiotics are of the biggest concern due to their role in formation of resistant bacteria at very low concentrations of these ubiquitous compounds [13]. At very low concentrations, apparently the antibiotic cannot kill the bacteria, but the bacteria can enter a mutagenic reaction coercing them to develop genes to protect them against antibiotics. Furthermore, they can propagate these genes across other bacterial strains [14, 15]. Similarly, other ECs especially acidic pharmaceuticals e.g. naproxen, ketoprofen, etc. and pesticides e.g. clopyralid, picloram, etc. need attention since their chronic effects have not still been studied. Therefore, ECs should be considered as today's problem since in coming years the environment may encounter with plenty of resistant bacteria without certain effective treatment. According to the prediction of World Bank, these resistant bacteria can kill 10 million people per year by 2050 and push 28 millions of people into poverty [16]. Antibiotics account only for a small proportion of ECs and they can be destructive to humans [17]. It would be worth estimating the collective effects of all ECs, but how to do it? Do we need a one by one study for each of these ECs and then just try to introduce their monitoring as a normal standard procedure in the drinking/wastewater treatment plants? Or should we make a priority list like EU commission [5] and each year add or remove several compounds to update the list? Apparently, the former is not possible and the latter is reasonable until we deal with a limited number of components. Thus, age-old source control has importance as this would nip the bud at its renaissance.

Circular or linear model? The decision of the society.

Akin to other conventional manufacturing lines, our current drinking water and wastewater treatment plants (Figure 2.1.1 A) follow the old linear strategy in which there is a "lifetime" for the products [18, 19]. After reaching this end-of-life time, the product should be rejected somewhere in unseen space so that they do not pose any acute threat to the nearby habitations. In this strategy, the economy of the process dictates all the details and paying attention to the creation of pollution point sources and their adverse chronic effects on the environment has the least priority [20].

Switching to the circular strategy, in which the products are completely recycled and the processes with least waste production are preferred, has been successfully implemented for many metallic and plastic products. This attitude should be extended to water distribution and municipal wastewater collection systems which currently release different pollutants and these contaminants of emerging concern along with the wastewater effluent into the environment. This approach will force the authorities to allocate funds and attempts to find greener and more efficient processes for water and wastewater treatment systems. As illustrated in Figure 2.1.1 B, the product of such new treatment processes can either come back indirectly to drinking water network or can be used for irrigation if it meets the standards. The closed cycle concept is necessary to achieve the sustainable urban water management to mitigate the effects of vicious competition between the humans and wildlife over limited sources of fresh water and foods [21-23]. And beyond the circular approach, in order to address the growing list of ECs, is single technology a solution or an integrated one.

Single or hybrid technologies?

Technologies for water/wastewater treatment can be divided into physical removal (sedimentation, precipitation, adsorption, filtration, ion exchange, etc.), chemical oxidation/disinfection (chlorination, ozonation, ultraviolet irradiation, etc.) and biological transformation (activated sludge, enzymatic reactors, etc.) [24]. Each of these technologies can be appropriate and cost-effective for a specific purpose [25]. For example, chlorination is an economical method for disinfection of drinking water through the distribution network. Likewise, reverse osmosis is an efficient method for desalination of seawater. However, a wastewater treatment which involves a very complex matrix containing natural organic matter, metals, microorganisms, organic compounds, pharmaceuticals, monomers, etc., a single technology will not be capable of achieving the required quality for a circular model of water management [26]. Conventional activated sludge is the most popular and cost-effective method for reducing carbonaceous biological mater, nitrogenous matters and phosphorus compounds at large scale. However the quality of effluent cannot even meet the criteria of agricultural farms or other industries. For example, some constituents of WWTP effluents such as salts, heavy metals, suspended solids, nutrients and

contaminants can degrade the quality of water as well as the infiltration properties of soil required for crop production [27, 28]. In this case, hybridizing or coupling different technologies can result in higher quality and even better controllability [29].

Membrane bioreactor is a very good example of such hybrid system in which addition of a microfiltration/ultrafiltration membrane to a conventional activated sludge system can enhance the flexibility of the treatment system and the quality of effluent [30]. It is achieved through expansion of the operational range of mixed liquor suspended solids and eliminating the necessity of formation of big flocs which consequently reduce the mass transfer resistance [31]. Although coupling these two systems increase the capital and operational costs (38-53%) due to higher constriction and energy consumption costs, the higher quality of effluent and less consumption of chemicals can justify its implementation in real world applications [32]. Likewise, for an efficient removal of emerging contaminants from municipal wastewater, we need to hybridize several technologies to be able to remove emerging contaminants at related conditions [33]. In this hybrid technology, conventional activated sludge or membrane bioreactors still can be responsible for removing most nutrients and suspended particles. As illustrated in Figure 2.1.2, after reaching an intermediate quality, the process can be complemented with a combination of physical, chemical or other biological methods (e.g. wetlands, enzymatic reactors, etc.) [34, 35]. Degradation of the target compounds should also be considered in the separated sludge since it may be utilized as fertilizer which is a pathway for the contaminants to the soil [36, 37], in turn intoxicating our farms and hence the produce.

Many hybrid configurations were developed at bench scale to investigate their performance for removal of emerging contaminants and several of them have been tested at pilot scale in EU countries. These techniques still suffer from being costly which hinders their wide application [38, 39]. Therefore, streamlined research is needed to find greener and economical methods. Likewise, the society and decision-makers should be convinced to pay the expenses of a new generation of wastewater treatment plants based on hybrid technologies.

Research Lacunae

Several hundreds of research publications have been brought out in recent 20 years on detection, removal, and degradation of different categories of emerging contaminants from water and wastewater [38, 40, 41]. Key stumbling blocks persist in attitude, experimental design and interpretation of results through this pile of literature. The following context reviews these problems to suggest more realistic outlook for future research works.

Attitude

The sole use of physical removal methods, such as membrane separation, failed to consider the challenge of creating a concentrated stream which will be rejected somewhere in the environment [42]. For example, nanofiltration and reverse osmosis membranes can remove pharmaceutical compounds such as carbamazepine and ibuprofen from water with more than 95% efficiency but their recovery rate (the ratio of product flow rate to feed flow rate) under normal conditions is around 30-70%. It means that, at highest recovery rate, they produce a stream with around one third in flow rate and three times more concentration (respect to feed flow) [43, 44]. Therefore, a stalemate will be reached through incurring high capital costs. The same discussion can be applied to the processes involve adsorption columns in which an adsorbent, such as activated carbon is used to remove the target compounds from water. After saturation of the adsorbent, an eluent is used to strip the adsorbent from target compounds and this concentrated stream should be treated [45]. Thus, any approach without considering a system to degrade the residues of target compounds will not be applicable in real-world treatment schemes and may not be considered holistic. Investigating the implementation of these technologies in combination with oxidative processes such as enzymatic treatment and electro-oxidation will be an interesting research topic.

Experimental design

In experimental designs, the matrices have been most of the times spiked with high concentrations of the target compounds (up to 100 mg/L) for investigating the performance of selected degradation systems, while the environmentally related concentrations can barely reach up to one mg/L. This leads to an overestimation of degradation efficiency and the results cannot be extrapolated to real conditions [46]. Especially in the case of enzymatic degradation which follows Michaelis-Menten

mechanism, spiking compounds such as tetracyclines at high concentrations (>50 mg/L) results in rapid zero-order kinetics instead of the relatively slow first-order kinetics which is more compatible with the environmentally-relevant low concentrations of substrates [47]. Also working with mineralized or ultrapure water for the experiments brings a source of uncertainty into consideration since in real effluents of wastewater treatment plant (WWTP) and surface waters there are many compounds that play different roles in degradation reactions [48]. For example, performing photodegradation experiments in pure water cannot be related to conditions in WWTP effluent since the high turbidity does not allow the photons to reach deeper into the matrix [49]. Similarly, the presence of different surfactants, metals and natural organic matters can influence the efficiency of enzymatic and advanced oxidation processes by acting as cofactors, inhibitors, radical scavengers, etc. [50-53]. Lack of realistic data due to overlooking of the effects of these constituents hinders performing a techno-economic analysis for feasibility evaluation of the developed treatment methods. Therefore it is required to consider maximum similarity between test conditions and real world conditions including concentration, pH, temperature, flux rate, microorganisms and mineral/organic load.

Data Interpretation

As for data interpretation, it has been largely interpreted that the “chemical transformation of target compounds into some other products” is degradation or removal of the parent compounds from the matrix. Although this interpretation is literally true, the goals of designing a treatment system have not been met, if these transformation products can pose equal or even more toxicity to the environment. This phenomenon frequently happens in advanced oxidation processes which are based on reactive hydroxyl radicals [54]. For example, ozonation is a robust method for disinfection of drinking water, but it can transform bromide into bromate which has been found to be a genotoxic carcinogen in rats [55]. To overcome this knowledge gap, some researchers managed to assay the toxicity of their treated effluent through available test methods, such as Yeast Estrogenicity/Androgenicity Screen (YE/AS) assay [56] and Microtox bioassay [57]. However, considering a limited number of organisms, such as yeast and bacteria makes it difficult to generalize the results of toxicity tests to different categories of terrestrial and aquatic organisms. Also, restricting the bioassays to common ecotoxicological endpoints,

such as mortality may lead to generation of illusive data due to very low concentration of target compounds. In this case, observation of other endpoints, such as reproductive status, enzyme production, gene expression and changes in behavioral, physiological and biochemical properties can be considered. Furthermore, investigating only the acute effects rather than the chronic effects of transformation products is confusing since the concentrations of emerging contaminants are very low and their chronic effects are imminent [58]. Also, there is lack of study on reliability and uncertainty of sampling as well as analysis procedures whereas inappropriate sampling and analysis methods lead to inaccuracies in measurement of ECs [59, 60]. Therefore, further research and effort is required to compare intra-laboratory data and standardization of sampling techniques.

Conclusion

A close collaboration among researchers and decision makers is required to develop a holistic strategy to confront the issue of emerging contaminants and prevent their obvious as well as unpredicted adverse outcomes in coming years. Restricting the consumption of certain products, such as antibiotics to crucial cases and replacement of some others with less harmful compounds, are possible strategies in short-term. Setting new standards for the quality of wastewater treatment plants as well as mandating the authorities of water management systems to integrate the municipal, agricultural and industrial water consumers in a closed cycle can simultaneously solve the problems of freshwater scarcity and environmental pollution in long-term. Research should be focused on the development of hybrid systems for degradation and removal of these contaminants from municipal wastewaters. Conventional activated sludge or membrane bioreactors system can still be responsible for removing the majority of organic loads within the treatment arrangement. This secondary treatment should be integrated with an advanced treatment scheme (combination of physical, chemical or biological methods) to polish the effluent and hence remove emerging contaminants. Also, more research should be devoted to the toxicology of emerging contaminants in a variety of organisms and development of reliable methods for toxicity test at extremely low concentrations.

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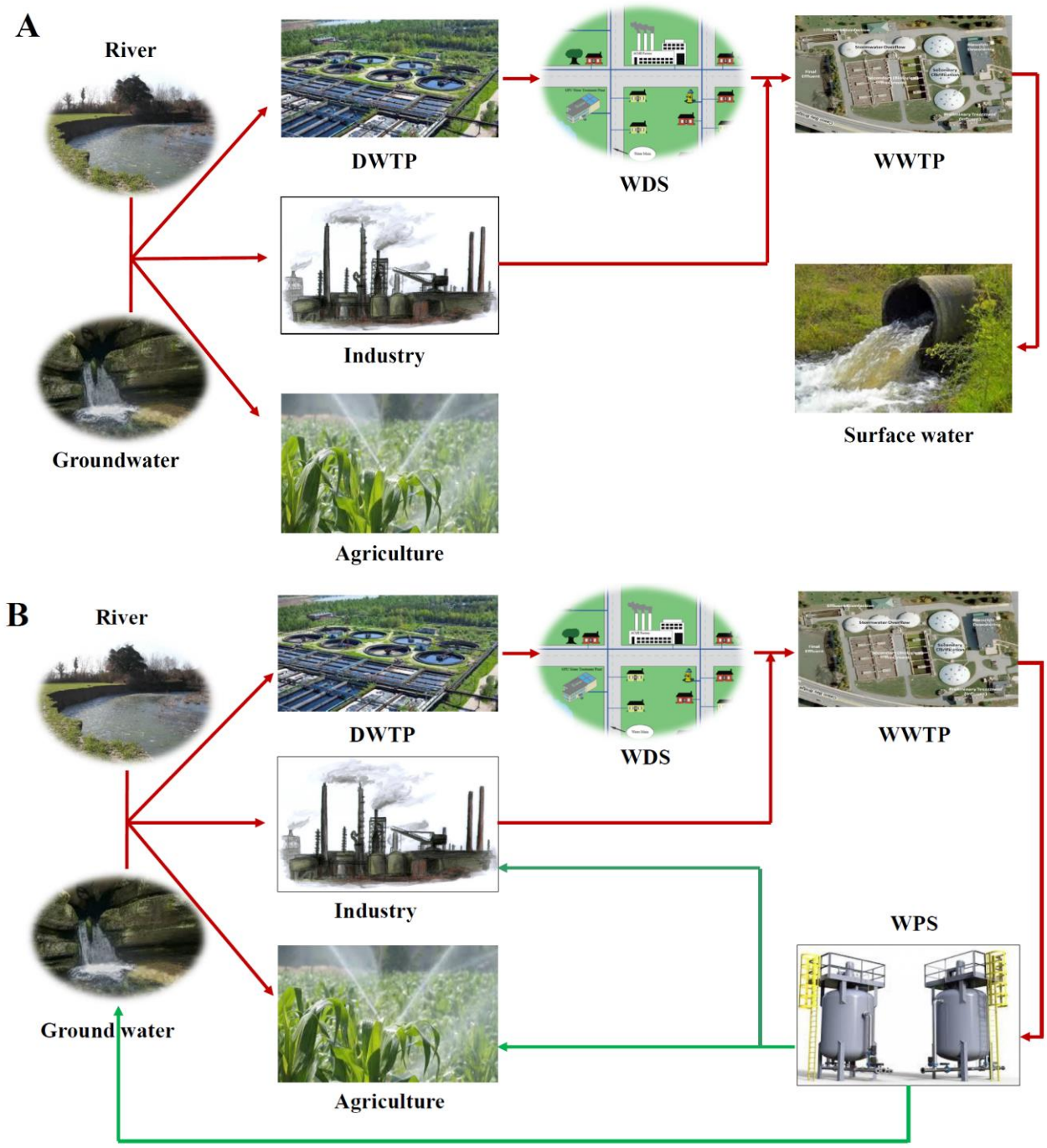

Figure 2.1.1 A) Linear and B) circular approach for management of water resources (DWTP: Drinking water treatment plant, WDS: Water distribution system, WWTP: Wastewater treatment plant, WPS: Water polishing system).

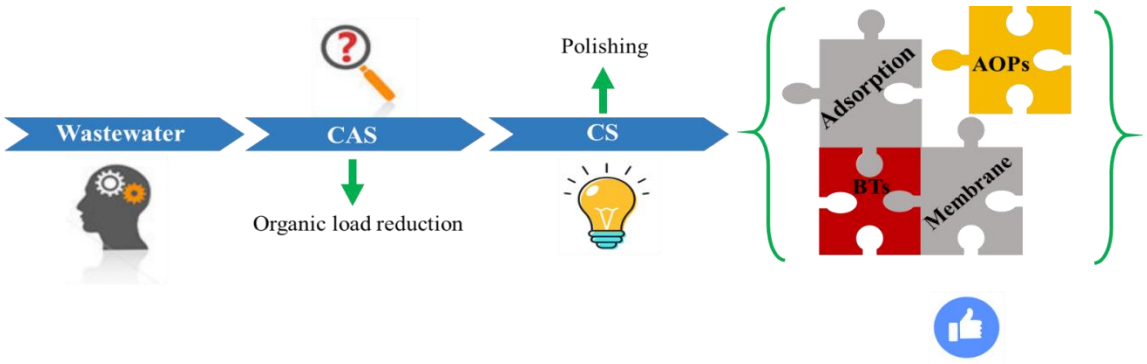

Figure 2.1.2 Proposed approach for addition of complementary systems for future wastewater treatment plants to remove emerging contaminants (CAS: conventional activated sludge, CS: complementary systems, AOPS: Advanced oxidation process, BTs: Biological treatments)

Part 2

Membrane Processes for Removal of Pharmaceutically Active Compounds (PhACs) from Water and Wastewaters

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Résumé

Les composés pharmaceutiquement actifs (PHACs), que nous retrouvons régulièrement dans les sources d'eau, apparaissent comme une préoccupation majeure et les enjeux relatifs pour la qualité de l'eau potable et liés à la vie aquatique. Par conséquent, leur retrait des sources d'eau est une priorité du point de vue environnemental. Au cours de la dernière décennie, différentes méthodes incluant la séparation par membrane, les systèmes d'adsorption et la transformation chimique ont été évaluées pour l'élimination de ces composés. Cet article passe en revue différents aspects de l'élimination des PhAC en utilisant des procédés de séparation par membrane, car ils ont été classiquement connus pour montrer un potentiel élevé dans la production d'eau potable et industrielle de qualité supérieure. En bref, les membranes d'osmose peuvent éliminer efficacement presque tous les PhAC, bien que son coût opérationnel soit relativement élevé et que les membranes de nanofiltration (NF) soient fortement influencées par l'interaction électrostatique et hydrophobe. De plus, l'efficacité des bioréacteurs à membrane (MBR) est difficile à prévoir en raison de l'interaction complexe des composés avec les micro-organismes. Pour améliorer la performance et la robustesse de la technologie membranaire, il est suggéré de combiner les membranes avec d'autres systèmes, tels que le charbon actif et la dégradation enzymatique.

Mots-clés

Composés pharmaceutiquement actifs (PHACs); séparation par membrane; osmose inverse; nanofiltration; bioréacteur à membrane

Abstract

Pharmaceutically active compounds (PhACs), which find their way easily into the water sources, are emerging as a major concern for drinking water quality and aquatic species. Therefore, their removal from water sources is a priority from environmental point of view. During the past decade, different methods including membrane separation, adsorption systems and chemical transformation have been evaluated for removal of these compounds. This paper reviews different aspects of PhACs removal by using membrane separation processes, as they have been conventionally known to show high potential in the production of superior quality drinking and industrial water. In brief, osmosis membranes can efficiently remove almost all PhACs though its operational cost is relatively high and nanofiltration (NF) membranes are highly influenced by electrostatic and hydrophobic interaction. Moreover, the efficiency of membrane bioreactors (MBRs) is difficult to predict due to the complex interaction of compounds with microorganisms. To improve the performance and robustness of membrane technology, it is suggested to combine membranes with other systems, such as activated carbon and enzymatic degradation.

Keywords

Pharmaceutically active compounds (PhACs); membrane separation; reverse osmosis; nanofiltration; membrane bioreactor

Overview

In this review, a brief explanation of health and environmental issues related to the pharmaceutically active compounds (PhACs) and the importance of their removal are presented. This topic is followed by a quick review of different treatment methods and especially the features of membrane processes that need more study to be completely understood. In subsequent section, the performance data of different membrane technologies, their dominant mechanisms and prediction of rejection of compounds are discussed. Likewise, the attempts of researchers to use membranes in combination with other systems are included and finally the outlook of membrane technology is presented suggesting several themes for further research.

The release of PhACs, such as antibiotics, anti-inflammatory drugs, analgesics, lipid regulators, beta-blockers and X-ray contrast media into the environment has been increasing in recent years due to enhanced human health standards and it has become one of the largest environmental problems in the recent decades [1-3]. Despite their very low concentration and lack of standard methods for their quantification, many studies have been carried out to determine the concentrations of PhACs and the causes of their presence in different water sources [4, 5]. As per the investigations performed in developed countries, more than 160 PhACs have been detected at concentrations up to several μg/L in surface, and ground waters and sewage [1, 6]. Due to the prescription of the high amounts of pharmaceuticals in human medical care, higher quantities of persistent PhACs residues are transferred to wastewater treatment plants (WWTPs) through municipal wastewater collection systems. Some of these compounds are removed efficiently, while others are removed partially or not at all. The inefficient removal of some PhACs in conventional WWTPs is almost impossible due to several facts including low volatility, different hydrophobicity, complex structures, extremely low concentration, influencing the microorganisms and interaction with other solutes and the separation medium (membrane, sludge, etc) [7-9]. These materials remain dissolved in wastewater effluents, or may bind to sludge which later release into the neighboring aquatic and soil environment. In addition to WWTPs, using high dosage of medicines in animal farms, in order to protect them from diseases, can form other point sources since animal manure is used in agriculture [4]. The occurrence of these compounds

in soil and water resources at higher concentrations causes adverse impacts on humans and environment. For example:

1- PhACs may return to human body through water cycle, and food chain. According to World Health Organization (WHO), reports of trace amounts of PhACs in the water cycle have raised concerns over potential human health risks [10]. As an instance, the persistence of *Escherichia coli* increases due to gradual rise in the concentration of antibiotics in water sources [11]. In fact, a kind of mutation occurs which necessitates the prescription of higher dosage of antibiotics and finally invention of new compounds. Moreover, this problem exacerbates every day since population density and consumption of pharmaceuticals continue to increase.

2- Releasing estrogenic drugs into rivers can interfere with reproductive hormones of aquatic vertebrates and cause sex differentiation, reduction of fertility or increase eggs production [12]. Therefore, in long term, the population of affected aquatic species decreases and subsequently the food chain will be affected.

Until now, the concentration of many PhACs has been lower than that of being toxic for human and environment but, according to their increasing trend, restricting their usage and release into environment through legislation or public training may be desirable in the future [8, 13]. Meanwhile, as regard to the precautionary principle, assessment of current status of purification methods and also developing new treatment technologies to remove organic contaminants from any kind of water source is necessary.

Generally, there are physical separations, chemical transformations and also their combinational processes for removal of micropollutants and each of these processes can be divided into other subcategories that are listed in Table. 2.2.1. Among the treatment options described in Table 2.2.1, membrane processes are of great interest because of higher removal rate of low molecular weight organic pollutants, excellent quality of effluent, modularity and ability to integrate with other systems [20, 21]. Despite their advantages, a major drawback is that filtration processes are basically designed to concentrate but not to degrade pollutants and require the disposal of wastes stream. Furthermore, if the PhACs are not degraded, the concentration of solutes on the permeate side of membranes increases by increasing permeate recovery and subsequently the overall performance would not

be as high as expected [20]. Therefore, pollutants in the retentate stream must be degraded or transformed into harmless chemical compounds by one of the chemical methods. Besides these advantages and drawbacks, there are several not clearly understood parameters that influence the removal efficiency of membrane separation processes [18], and therefore more studies are needed to shed light on all the aspects of PhACs removal by membrane separation processes.

Several comprehensive review papers were published on removal of emerging contaminants through membrane and other processes between the years 2002 and 2009 [14, 22, 23]. Since then, new data on the removal of PhACs from water sources have been published. The objective of this review is to summarize the current status of PhACs removal by membrane separation processes in single and combination modes, the effectiveness of these methods, related challenges and future outlook. For these purposes, all possible data on removal of PhACs by membrane separation technologies was considered. We also referred to many papers published prior to 2009 to complete tables and emphasize our discussion and conclusions. In Table 2.2.2 and Table 2.2.3, the related information about physicochemical properties of studied compounds that are helpful in prediction of the efficiencies of different membrane technologies have also been pooled together.

Membrane technology and PhACs removal

Membrane separation processes including NF, RO and FO show higher efficiency in removal of organic and inorganic pollutants and they can overcome the drawbacks of the traditional methods in removing PhACs [30]. Membranes can remove micropollutants either by size exclusion, electrostatic repulsion or adsorption [18]. Many researchers have tried to evaluate the performance of different membranes in various operational conditions and for variety of PhACs and these results are summarized in Tables 2.2.4, 2.2.5 and 2.2.7. However, prediction of compounds removal is quite difficult since it is dependent on physico-chemical properties of the compound (Table 2.2.3), membrane properties (Table 2.2.6), membrane-solute interactions and also influent matrix. In following sections the influencing parameters on each kind of membrane technology are discussed to give a basis for selecting the appropriate removal system for specific or general range of PhACs.

FO and RO Membranes

In forward osmosis, pure water is drawn from its mixture with contaminants to a concentrated solution (of for example mineral salts) through a semipermeable membrane as a result of concentration difference and then the diluted solution should be purified through another method like distillation. In reverse osmosis, no concentrated solution is used and pure water is forced to pass through the semipermeable membrane as a result of pressure difference. The removal mechanism of PhACs in FO and RO systems is complex and poorly understood. Generally, the rejection of solutes by osmosis processes is influenced by the dipole moment, hydrophobicity and molecular size of compounds [31, 32]. However, within the actual processes, it is difficult to determine which rejection mechanism is dominant due to solute-solute and solute-membrane interactions [33].

Kimura *et al.* studied the rejection of different PhACs using two RO membranes made of different materials (polyamide and cellulose acetate). They concluded that the molecular weight cut-off (MWCO) of RO membranes would be more useful than "salt rejection" (see Table 2.2.6) for evaluating PhACs rejection, although it cannot be used for precise prediction since properties of compounds used for standard determination of MWCO and those of target PhACs are considerably different. They also found that rejection of polar PhACs by cellulose acetate membranes was more than non-polar PhACs and therefore the dominant rejection mechanism for RO membranes depended on the membrane material and the physico-chemical properties of target compounds [32]. Xie *et al.* studied the rejection of selected PhACs by RO and FO processes and found that because the molecular width of triclosan (0.75 nm) was larger than the estimated mean effective pore size of membrane (0.74 nm), it was completely removed in both RO and FO processes. On the other hand, the molecular width of diclofenac (0.70 nm)was slightly smaller than the membrane pore size and consequently it had different rejection rate in RO and FO processes (90 % and 98 % respectively) [26]. In another study, they investigated the effect of solution pH on the rejection of carbamazepine and sulfamethoxazole in a FO process and found that rejection of the neutral carbamazepine was independent of solution pH, while rejection of sulfamethoxazole was enhanced when pH increased. In fact, carbamazepine (pKa=9.73) is a neutral compound with pH < 9.73, and therefore the steric hindrance is the dominant mechanism for its rejection. However, sulfamethoxazole (pKa=5.83) can transform to a negatively charged anion at pH values above 5.8 and therefore a repulsive interaction occurred between this

anion and the negatively charged membrane surface [36]. Jin *et al.* studied the rejection of four selected PhACs by two FO membranes made of cellulose triacetate (CTA) and thin film composite (TFC) polyamide and concluded that polyamide membranes have excellent performance due to the coupled effects of size exclusion, electrostatic repulsion and adsorption of solutes to the membrane surface. In comparison, cellulose triacetate membrane cannot show electrostatic interaction and therefore its rejection of PhACs is considerably lower than polyamide ones [37].

Heberer *et al.* performed a field trial for producing drinking water from surface sources using RO membranes and found that all the detected contaminants in the surface water, such as PhACs, pesticides, flame retardants, heavy metals, anions, and cations were effectively reduced by the RO system. They also found that the application of the RO in double-pass mode was not necessary for the detected compounds, except for nuclear contaminations [8].

NF Membranes

Separation using NF membranes has been increasingly considered as a reliable and affordable technology for the production of high quality water from unconventional sources such as brackish water, contaminated surface water, and secondary treated effluent of wastewater treatment plants (WWTPs) where micropollutants should be removed. The required pressure in NF systems is considerably lower than RO membranes which subsequently reduce the capital and operating costs. Table 2.2.5 presents the results for different commercial NF membranes to remove PhACs from water. Although many researchers have focused on the mechanisms of solute transport in NF membranes including electrostatic interaction, hydrophobic interaction and size exclusion, still further studies are required to understand the mechanism which is affected by solute properties, membrane parameters, feed water composition and operating parameters [27, 40-44]. The mechanisms and influencing parameters are discussed in the subsequent sections.

Microfiltration (MF) and Ultrafiltration (UF)

MF and UF membranes are used for tertiary treatment stages of WWTPs to obtain a high-quality effluent for groundwater recharge or reuse for irrigation. These types of membranes ensure an efficient removal of suspended solids and disinfection [52]. However, they cannot generally retain PhACs because the MWs of the most of the

PhACs range between 200 and 800 Da (Table 2.2.2) while typical MWCO of MF and UF membranes are well above several thousand Daltons. Therefore, size exclusion of PhACs in MF and UF membranes cannot occur. However, the initial adsorption of PhACs to membrane surface may occur which cannot be interpreted as removal rate since the concentration of solute in permeate will gradually increase after a short time.

Effect of Hydrophobicity

Hydrophobicity of PhACs plays an important role in their rejection by NF membranes. Hydrophobic compounds can be adsorbed onto the membrane surface and pores and diffuse into its matrix. Adsorption onto the membrane causes a high initial rejection of the solute but eventually, the transport of solutes to the permeate and an equilibrium leads to the lower rejection by membranes in comparison to hydrophilic compounds of the same size [42, 53, 54]. After reaching equilibrium, the size exclusion and charge repulsion will be the dominant mechanisms for rejection of PhACs [55]. As many studies were performed over short time frames (<24 h) and using small volumes of water (<10 L), overestimation of membrane removal efficiency occurred in these studies [56-58].

On the other hand, hydrophilic compounds are solvated in water phase and consequently their effective diameter might be larger. Therefore, on a size exclusion basis, hydrophilic compound can be rejected more effectively than hydrophobic ones [59]. The higher hydrophobicity of a compound results in the higher adsorption onto the membrane surface, especially when compounds are electrostatically neutral [18]. Hydrophobicity is expressed as log K_{ow} for non-ionizable compounds and log D for ionizable compounds. The octanol–water partition coefficient, K_{ow}, is defined as the ratio of the concentration of a compound in octanol to its concentration in the water phase in equilibrium state and the pH-dependent octanol–water distribution coefficient, D, is defined as the ratio of the concentrations of all unionized and ionized species of a molecule in octanol to the same species in the water phase in equilibrium [60].

Comerton *et al.* found that the effect of hydrophobicity was more obvious for NF membranes with larger pores than NF with smaller ones because larger pores allowed compounds to access adsorption sites in the skin layer, support layer and pores of membrane, in addition to its surface. They also found that apart from

hydrophobicity, increasing pure water permeability of membranes can increase the PhACs adsorption, and reported that the most significant adsorption is observed with the UF membrane followed by NF membranes and RO membrane [45, 56]. However, according to Yoon *et al.*, in some cases, the adsorption onto NF membrane were more than UF membrane [46]. Verliefde *et al*. concluded that in the case of negatively charged NF membrane, rejection of neutral and positively charged PhACs decreased with increasing hydrophobicity due to their enhanced tendency to be adsorbed onto the membrane surface. On the other hand, no clear relationship was observed between the hydrophobicity of negatively charged PhACs and their rejection due to charge repulsion that prevented solutes from approaching the membrane surface [47].

Effect of Electrostatic Interaction

The surface of most of the thin layer composite NF, RO and FO membranes are typically negatively charged in solutions of neutral pH due to the deprotonation of their functional groups. Therefore the charge of PhACs molecules and their electrostatic interaction with membrane surface can be a major contributor to their removal. In the case of positively charged PhACs, attractive forces between the solutes and the negatively charged membrane surface cause an increase in the concentration of solute at the membrane surface, and therefore lead to lower observed rejections. Subsequently, repulsive force between the negatively charged solutes and the negatively charged membrane surface decreases the concentration of solute at the membrane surface and therefore increases rejection [25, 32, 34, 38]. Even uncharged molecules, that have high dipole moment, can be aligned in the direction of the membrane pores, as a result of electrostatic interactions with the membrane charge, and consequently permeate more easily through the membrane [43, 46, 61].

Nghiem *et al.* studied the effect of electrostatic interactions on the rejection of PhACs by a loose NF membrane that was negatively charged at pH values above 5. They found that the retention of ionizable PhACs, such as ibuprofen and sulfamethoxazole increased when the compounds transformed from a neutral to a negatively charged species by increasing pH to above their pKa value. However non-ionizable PhACs, such as carbamazepine were relatively independent of the solution chemistry [43, 48]. In another study, Verliefde *et al*. investigated the removal of pharmaceuticals

using a negatively charged NF system operated at lower (10%) and higher recovery (80%). They concluded that size exclusion was the main mechanism for neutral solute rejection, but higher and lower rejection of positively and negatively charged solutes was attributed to electrostatic repulsions and attractions, respectively. They also found that when operating at low recovery (10%), high removal efficiencies (>95%) can be achieved for all PhACs. However, these efficiencies decreased at higher recovery (80 %) due to the increased average feed concentration of solutes as a result of internal recycle [25, 62]. Bellona *et al.* studied the effect of surface charges of NF membrane on acidic solutes rejection and observed that the presence of calcium in feed water can reduce surface charge but the rejection of negatively charged solutes reduced only for membranes with MWCO larger than the molecular weight of solutes [51]. Their conclusion was in contrast with the result of Comerton *et al.* that reported a decrease in the removal rate of gemfibrozil (MW=250 Da) and unchanged removal rate of acetaminophen (MW=151 Da) using a NF membrane (MWCO = 200 Da) after addition of divalent cations [51]. Also, Dolar *et al.* studied the effect of influent matrix on the rejection of PhACs through NF and RO membrane using four different water sources, namely Milli-Q® water, model water, tap water and real pharmaceutical wastewater and observed that the rejection of PhACs was higher in model and tap water than in Milli-Q water due to ion adsorption inside the membranes pores which strengthened the size exclusion effect [63].

Effect of Size Exclusion

Membrane retention should not be considered as a simple filtration process because it is not solely governed by molecular geometry. Not only sieving property affects the convection transport of solutes through membranes, but also the net sorption at the membrane-solute interface and the transport inside the membrane can play a significant role in removal efficiency [64]. The preferential sorption and transport are influenced by other parameters, such as charge repulsion and hydrophobic interaction. Generally speaking, size exclusion is responsible for rejection of uncharged and hydrophilic solutes and it can bring about very high rejections (i.e., >85%) for solutes that have MW greater than the MWCO of NF/RO membranes. Radjenovic *et al.* studied the rejection of several PhACs by NF/RO membranes in real drinking water treatment plant. They concluded that because the MW of acetaminophen was lower than MWCO of the employed NF and RO membranes and

also it was uncharged in neutral pH, its rejection rate was lowered to 44.8-73 %. On the other hand, diclofenac with its higher MW and negative charge in neutral pH had the highest rejection rate. However, low rejection rate of gemfibrozil despite its high MW and the presence of charge repulsion effect was surprising [34]. Quintanilla *et al*. found that rejection of hydrophilic neutral solutes can be linearly correlated to their molar volume and molecular length, but no correlation was observed between their rejections and equivalent width [65]. However, Agenson *et al.* observed a better correlation between rejection and molecular width [64]. Pronk *et al.* and Rehbun *et al*. explained the higher removal rate of some solutes to their complexation with oxalic acid, uric acid, amino acids and humic acid and consequently increased their size [66, 67]. Kim *et al*. used methacrylic acid, ethylene diamine and succinic acid to modify the surface of commercial NF membrane and concluded that methacrylic acid can increase the hydrophilicity, steric hindrance and negative surface charge of the membrane and therefore increase the rejection of ibuprofen and salicylic acid (by 1 %). Nevertheless, cross-linking with ethylene diamine reduced the surface charge and had negative effect on rejection of charged PhACs [68].

Effect of Membrane Fouling and Deterioration

Wei *et al.* tried to identify the constituents of foulants on NF membranes fed by the bio-treatment effluent of sequencing batch reactor and found that fouling layer was a mixture of natural organic matter (NOM) and inorganic matter and there were two distinct fouling stages: 1) deposition of sulfate and carbonate of calcium due to high content of inorganic ions in NF feed and concentration polarization; and 2) deposition of complex organic foulants containing carboxyl acid, amide, and alkyl halide functional groups due to adsorption of cations to membrane surface. Decreasing the negative charges of both membrane surface and NOM (due to formation of complex with calcium ion) can reduce the charge repulsion between them and accelerate the deposition of NOM onto membrane surface, gradually forming a densely packed fouling layer [30]. Linares *et al.* studied the performance of the FO process for the removal of selected PhACs and concluded that when the FO membrane was fouled, the hydrophilic nature of foulants caused the hydrophilic ionic compounds and hydrophobic neutral compounds to be rejected more effectively due to higher negative charge of the fouled membrane and reduced the passage of hydrophobic compounds respectively. On the other hand, rejection of hydrophilic neutral

compounds decreased within the fouled membrane, due to higher MWCO caused by membrane swelling and the increased adsorption capacity for hydrophilic compounds. In their experiments, fouling did not affect membrane flux in the case of treating inorganic synthetic solution but reduced the membrane flux by 20% in the case of secondary wastewater effluent [24].

Xie *et al.* studied the effect of humic acid fouling on PhACs permeation in forward osmosis and found that the permeation of sulfamethoxazole and carbamazepine decreased from 10% and 23% to 2% and 6%, respectively [73]. Nghiem *et al.* studied the effect of RO and NF membrane fouling with hydrophobic and hydrophilic foulant models and concluded that in the case of hydrophilic colloidal silica as foulant, no change in permeate flux or rejection of triclosan was observed. Hydrophobic foulants, such as alginate considerably reduced the flux and increased triclosan rejection [74]. In their other research, they found that NF membrane fouling can influence the retention of pharmaceuticals via three mechanisms i.e. pore restriction, modification of the membrane surface charge, and cake enhanced polarization concentration effect. According to their results, the effect of pore restriction for NF membrane with larger pore size was more obvious than NF membrane with smaller pore size [49]. In the same way, NF membranes with larger pore size were more sensitive to degradation effect of chlorine [75] in agreement with the results of Urase et al. [76]. In another study, Nghiem *et al.* found that for hydrophilic compounds, fouling causes an enhanced concentration polarization which consequently increased the concentration of the solutes within the fouling layer and resulted in higher passage of solutes through the membrane. But for hydrophobic compounds, fouling layer can interfere with its adsorption to the membrane and reduce its sorption diffusion transport through the membranes [27]. Verliefde *et al.* studied the effect of NF membrane fouling by using river water pretreated with ultrafiltration (that removes colloids) and ion-exchange (that removed negatively charged NOMs) as NF influent and found that colloids caused more flux decline than NOM. They also reported that in the case of ion-exchange pretreatment, the rejection of some positively charged PhACs decreased by more than 40% and rejection of some negatively charged PhACs increased by more than 15%. They also claimed that although the zeta potential of the colloidal fouled membrane was lower than for the membrane fouled with the natural organic matter, charge repulsion played an important role due to the morphology of the fouling layer [47]. In another

study, Comerton *et al.* studied the effect of NOM and cations present on the rejection of PhACs from an MBR effluent by NF membranes and observed that divalent cations did not have significant effect on the rejection of acetaminophen and carbamazepine, but dramatically decreased the rejection of gemfibrozil. On the other hand, NOM increased the rejection of these three PhACs [45]. Reznik *et al.* studied the seasonal effects on the rejection of carbamazepine and found that the rejection of RO membranes was not affected by seasonal change but the rejection of carbamazepine by NF membrane decreased from 92 % in summer to 50 % in winter. They concluded that reducing the temperature can affect metabolic rate which consequently affects organic matter degradation and their interaction with solutes [50].

Foulants type, loading and pretreatment method strongly affect the membrane cleaning period, required chemicals and consequently cleaning cost. Generally, membranes should be cleaned in the case of 10-15% decline in permeate flux, 10-15% increase in permeate solute concentration or 15-20% of pressure drop in a pressure vessel. As a rule of thumb, the acceptable cleaning frequency is once to four times per year and 5-20 % of operational costs are associated with the cleaning procedure [77]. In a case study, the total costs of cleaning RO membrane including labor, chemicals and production loss were calculated in Orange County Water District's groundwater replenishment system. According to provided data, for total capacity of 265000 m^3/day operated at 85 % recovery rate, the total cleaning cost was $15 929 that should be multiplied by the number of cleanings per year [78].

Membrane bioreactors

Membrane bioreactor (MBR) is a combination of adsorption, biodegradation and membrane separation processes that enable production of an effluent with very low amounts of total suspended solids (TSS), turbidity, biological oxygen demand (BOD) and pathogens. MBRs could be robust systems for removal of organic and inorganic contaminants since they provide three basic aspects including: (i) enhanced adsorption through improved sludge characteristics, high concentration of biomass and more effective surface; (ii) better biodegradation with high sludge age (iii) direct separation through membrane with efficient removal of many contaminants bound to rejectable colloids and particulates [13, 79-84] .

The processes in MBR are very similar to conventional activated sludge (CAS) systems except the aeration spargers and membrane modules which can accelerate the biodegradation and separation processes. Therefore, the performance of these two approaches was invariably different. Radjencovic *et al.* and Clara *et al.* compared the removal of different pharmaceuticals in MBR and CAS systems and concluded that for most of the compounds, MBR showed higher performance than CAS (removal rates >80%) [85, 86]. Typically, MF membranes with the pore size of about 0.4 μm are used for MBRs since the size of microorganism flocs are in the range of 10-100 μm. Also, UF and recently, NF membranes have been studied to enhance the performance of MBRs [87, 88]. However, low MWCO membranes (UF and NF) can increase the capital and operational costs since their permeability was lower than MF membranes. Radjencovic *et al.* studied the removal of different PhACs in two MBRs using MF flat sheet membrane (0.4 μm pore size) and UF hollow fiber membrane (0.05 μm pore size) and their results implied that for most PhACs, flat sheet membrane had better removal rate than hollow fiber one [89]. Higher removal rates of flat sheet membrane, despite its bigger pore size, can be attributed to its higher TSS (13090 mg/L) compared to that for hollow fiber membrane (2180 mg/L).

Since biodegradation is enhanced by sorption process, MBRs can effectively eliminate hydrophobic and readily biodegradable compounds and they are less effective in removal of hydrophilic and biologically persistent materials [90, 99]. Adsorption and biodegradation of pollutants in MBR systems are highly influenced by operational conditions, such as hydraulic retention time (HRT), sludge retention time (SRT), biomass concentration, temperature and pH of influent [100]. Therefore, many researchers have been working on different configuration of MBR systems to optimize influencing parameters for efficient removal of emerging contaminants from wastewater. In Table 2.2.7, the results obtained by different researchers in recent years are presented. Shariati *et al.* employed a MBR to remove acetaminophen from wastewater model media and showed that the significant parameters in removal of PhACs were initial concentration, chemical oxygen demand (COD) and mixed liquid suspended solids (MLSS). In their experiments, the acetaminophen concentration in the permeate reached from 1000 mg/L to below detectable limit in 24h [88] since its structure allowed the unrestricted access of bacteria and enzymes to the sterically unprotected molecule [91]. Quintana *et al*. studied the pathways and metabolites of

microbial degradation of five acidic pharmaceuticals, including diclofenac, ketoprofen, bezafibrate, naproxen and ibuprofen in a series of laboratory tests using fresh sludge from a MBR. They performed their test in presence and absence of milk as co-substrate and observed that bezafibrate, naproxen and ibuprofen were degraded only co-metabolically whereas ketoprophen was degraded only in the absence of co-substrate and no degradation was observed for diclofenac [92]. Kimura *et al.* and Cirja *et al.* attributed the lower removal rate of clofibric acid, diclofenac and carbamazepine to the presence of chlorine or multi-aromatic rings in their structure[99, 101].

Effects of HRT and SRT

Solid retention time (SRT) is one of the most important parameters influencing the removal of PhACs in MBR that significantly affects the removal rates of diclofenac, ketoprofen, gemfibrozil, trimethoprim, and erythromycin. For example, Bernhard *et al.* observed that by increasing the SRT from 20 days to 62 days, the removal rate of diclofenac improved from 8% to 59 % [102]. In another study, Kimura *et al.* showed that the MBR with longer SRT of 65 days had better performance than the MBR with a shorter SRT of 15 days so that the removal rates of ketoprofen and diclofenac improved from 82 to 98% and from 50 to 82%, respectively [93]. Likewise, Maeng *et al.* found that when SRT was increased from 20 to 80 days, the removal rates of gemfibrozil and ketoprofen improved from 41 to 88% and from 64 to 90%, respectively [103]. Generally, increasing SRT can improve the biodiversity of microorganisms inside MBR by providing enough time for growth and adaptation of slowly growing bacteria which favors removal of PhACs. However, researchers showed that changing SRT had no significant impact on the removal of ibuprofen, bezafibrate, naproxen, carbamazepine, and sulfamethoxazole [104] For example, Tambosi *et al.* observed that by increasing the SRT from 15 to 30 days, the removal rate of naproxen remained in the range of 85-90%. [91]. Same behavior was observed for sulfamethoxazole and its metabolites, while SRT changed from 16 to 81 days [105]. Also, carbamazepine was shown to be resistant to biodegradation regardless of changing SRT [103].

It seems that hydraulic retention time (HRT), which directly determines the volume of the reactor and impacts capital and operational costs, has no significant effect on removal of PhACs. For example, Reif *et al.*, studied the removal of ibuprofen,

naproxen and erythromycin and observed that changing HRT did not affect the permeate quality of MBR [94]. Also, according to Table 2.2.7, by comparing the results of different researchers, it can be observed that HRT had no effects on removal of acetaminophen, bezafibrate, ofloxacin, gemfibrozil and metronidazole. Furthermore, for other micropollutants, such as phenol, it has been proven that HRT did not impact the removal rate, however reducing HRT can increase membrane fouling [106].

Effects of pH and Temperature

It is expected that lowering the solution pH lead to increase the hydrophobicity of ionizable PhACs and subsequently their tendency of adsorption to the sludge particles. Therefore, the time for biodegradation is increased and removal rates are also expected to increase. In contrast, no changes in the removal rate of non-ionizable compounds were expected when pH varied. This hypothesis was proven by the results of Tadkaew *et al.* for ionizable diclofenac, ibuprofen, sulfamethoxazole and non-ionizable carbamazepine. [98]. Also, Urase *et al.* performed the same experiment and agreed that at low pH, the target compounds attached to the sludge were not accumulated in the reactor, and that they were biologically degraded [107]. However, in the study of Tadkaew *et al.*, ionizable ketoprofen showed higher removal rate at pH=9 and pH=5 than pH=7 [98]. Similarly, there are reports on the sorption of hydrophilic antibiotics to the sludge which is expected to happen [99].

Hai et al. studied the effect of temperature variation on the removal of micropollutants and found that the removal of most hydrophobic compounds was stable under the temperature range of 10–35 ˚C but the removal of less hydrophobic micropollutants (log D < 3.2) was significantly influenced by temperature variation below and above 20 ˚C[108]. The dependence of sorption and metabolic activity on temperature is responsible for the variation of removal efficiencies among micropollutants.

Effects of microorganisms

Typically, a wide range of microorganisms (bacteria and fungi) are used in biological treatment stage of WWTPs while they are not able to degrade some PhACs sufficiently since they have complex structure or toxic effects for these microorganisms [109]. For example, the removal rates of carbamazepine and

diclofenac are reported to be 13.8 % and 15%, respectively [86, 95]. Therefore, in the recent years, researchers have been working on new microorganisms that are able to efficiently oxidize these compounds.

The white rot fungi and their unique extracellular enzymes, namely lignin peroxidase, manganese peroxidase and laccase play an important role in the ecosystem by degrading lignin. Some researchers investigated the elimination of PhACs, such as diclofenac, naproxen, and ketoprofen by these fungi [7, 110, 111]. Nguyen *et al.* used a mixed culture of bacteria and white-rot fungi to remove organic contaminants in MBR system and concluded that this system can achieve better removal than a system containing fungus or bacteria alone. They observed the removal rates increased from 15 % to 50 % for diclofenac and from 65% to 94 % for ketoprofen [95]. In another study, they used a fungal MBR inoculated with white rot fungus, *T. versicolor* and operated under non-sterile conditions and achieved more than 55 % removal for diclofenac [96]. Zhang *et al.* studied the removal of PhACs under non-sterile conditions using *T. versicolor* and reported the high removal rate (60-80 %) for carbamazepine. They found that sufficient supply with nutrients is crucial for an effective removal [110]. Also, Jelic *et al.* used *T. versicolor* for removal of carbamazepine and observed that for low concentration of carbamazepine (50 µg/l), the removal rate is 61% while for higher concentrations (9 mg/L), the removal rate can reach 94 % [112]. Hata *et al.* found that the repeated treatments of carbamazepine with laccase in the presence of redox mediator 1-hydroxybenzotriazole (HBT) can increase the removal rate from 22% to 60% [113]. Also, Murugesan *et al.* confirmed that the presence of HBT can enhance the removal rate of Triclosan from 56% to 90% [114]. Zhang *et al.* used the lignin peroxidase enzyme that was produced from another white rot fungus *P. chrysosporium* for *in vitro* degradation of PhACs with H_2O_2 and observed complete removal of diclofenac and low removal of carbamazepine (10 %) [115]. Other researchers performed same experiments with white rot fungi and their enzymes and their results were in the same range [116-120].

Due to incomplete knowledge of microorganism capabilities, effects of PhACs and their metabolites on microorganisms and other unknown parameters, further study is needed to find an appropriate microorganism and optimum conditions to reach the high removal efficiency for most of the PhACs.

Prediction of PhACs removal during membrane systems

According to the results of researchers, prediction of removal rates of PhACs during treatment by membrane processes including RO, FO, NF and MBR is difficult since there are parameters that influence their rejection mechanisms. The significant parameters for each membrane process are listed in Table. 2.2.8.

Quintanilla *et al.* developed a quantitative structure activity relationship (QSAR) model to predict the removal rates of micropollutants by NF membranes. They tried different models and achieved several linear equations with $R^2 > 0.9$ and therefore the predicted results can be considered reliable. According to their analysis, the most important variables in rejection of organic solutes by NF membranes are log D, salt rejection, equivalent molecular width, depth and length of solutes [121]. A classification of compounds to predict their rejection by different membrane separation processes is presented in Table 2.2.9. Of course this classification is not appropriate for MBR systems. Generally, if a compound is hydrophobic and there are no halogen elements or complex groups, such as multi-aromatic rings in its structure, the removal with MBR will be efficient. Otherwise, the rejection can be qualitatively predicted by using Table 2.2.9 which needs analysis of the physico-chemical properties of compounds and also selected membranes that are listed in Table 2.2.2 and Table 2.2.3.

Apart from rejection rate of each filtration method, it is interesting to compare their capital and operational costs which depend on many parameters, such as feed water quality, required product quality, and cost of chemicals and electricity. In Table 2.2.10, a typical comparison of capital and operational costs are presented based on different case studies. In this table, capital costs include membrane, equipment, construction and buildings and operational cost include membrane replacement, energy, maintenance, chemicals and labor. According to this Table 2.2.10, RO has the highest operational cost due to higher energy requirement. In contrast, MBR has the lowest operational cost because major separation is carried out by microorganisms that require little energy input for aeration. NF membranes resemble RO membranes, but their energy consumption is lower due to larger MWCO and therefore lower operational cost seems logic. For FO membranes, there are still ongoing investigations to develop low cost, non-toxic and readily recoverable draw

solution and also high-flux membranes to reduce the operational and capital costs [122]. Therefore, these data are not reliable and prone to change in coming years.

Innovative Methods

Combination of Membrane Processes with Chemical Transformation

Chemical transformation processes including catalytic oxidation, ozonation, photo-catalysis with semiconductors, photo-Fenton reactions, enzymatic digestion and ultrasonic processes are widely used for water and wastewater treatment [126-128]. Integrating these methods with membrane processes is of high interest because membranes are not able to degrade contaminants even though they can efficiently separate them from water. For example, Kim *et al*. combined NF process with catalytic oxidation for effectively decomposition of EDCs in a way that the influent entered the reaction before passing from NF membrane. They reported that homogenous catalytic oxidation, using iron(III)-tetrasulfophthalocyanine (FeTsPc) as catalyst and hydrogen peroxide as oxidant, resulted in more than 90% decomposition of BPA and diclofenac under weakly acidic conditions [20]. Due to the problem in separation of catalyst from water in homogenous systems, these processes have not been used for environmental purposes. Therefore, Kim *et al*. immobilized FeTsPc catalyst on Amberlite ion-exchange resin to use it in heterogeneous mode for PhACs removal and observed a striking improvement in the stability of FeTsPc due to immobilization and also higher removal rate for ibuprofen (89 %) and diclofenac (99%) [129, 130]. In Table 2.2.11, several examples of combining membrane systems with other technologies have been listed. According to this table, in most cases, the combinational/hybrid systems achieved higher overall removal rates.

Another technique that attracted the attention of researchers in recent years is the immobilization of enzymes on membranes to integrate the membrane separation and oxidation process in one system. In these systems, mass transfer phenomena plays a key role as pollutants must be transported from feed side to the enzymes across the membrane and the products have to diffuse from the reaction site to the permeate side of the membrane. These integrated systems have advantages, such as high enzyme capacity, prolonged enzyme activity, high flow rates, feasible operation and control, reducing capital and operational costs, scale-up capability and

high yields of pure material [135]. There are numerous studies that incorporated lipase, cellulase, catalase and laccase into poly(vinyl alcohol), polysulphone, poly(ethersulphone), poly(acrylonitrile) and other membranes to integrate the retention capability of membranes with oxidative nature of enzymes for degradation of various organic matters [136-147]. However, there is scarce research on the removal of PhACs or other emerging contaminants using this integrative method [148].

Martinez *et al.* tried to degrade the PhACs in the rejected streams of RO and NF membranes by using TiO_2 and Fe_2O_3/SBA-15 in the presence of hydrogen peroxide as photo-Fenton system. They reported that both photo-catalytic treatments can remove sulfamethoxazole, diclofenac and ranitidine at high levels (80-100%) [131]. Mascolo *et al.* integrated an ozonation system in a recirculation stream of their lab-scale MBR system and used it for treatment of a real pharmaceutical wastewater and observed that the removal rates for most of the compounds are comparable to separated treatment systems in series (MBR and then ozonation) but the by-product of ozonation effluent is recirculated and removed in the MBR process while in series mode, it needed more than 60 min of ozonation to reach same results. Therefore, the integration of ozonation in recirculation stream can remarkably reduce the operational costs [133].

Nguyen *et al.* passed the effluent of a MBR through a UV oxidation reactor with 7.5 min contact time and showed that UV oxidation was effective for the degradation of compounds containing chlorine or phenolic group in their molecular structure but less effective for the removal of compounds containing amide group, such as carbamazepine. However, they observed that although MBR and UV oxidation had lower removal rate of carbamazepine in itself, but their combination resulted in more than 96% removal [90].

Combination of Membrane Processes with Adsorption Systems

Adsorption systems are commonly used to remove micropollutants from water because of their feasible operation. The commonly used adsorbents are zeolite, bentonite, granular activated carbon (GAC) and powdered activated carbon (PAC) that have several drawbacks, such as limited adsorption capacity and short life cycles. Nowadays, new adsorbents are being researched based on metal-organic frameworks [149] and biochars [150-154] due to their superior properties including a

highly condensed structure and surface density. Although adsorption systems need more development, combining them with membrane separation has been interesting for several researchers. Nam *et al.* studied the adsorption of hydrophilic and hydrophobic PhACs on PAC and observed that increasing dosage and contact time enhance adsorption removal, but a cost-effectiveness analysis is needed before deploying these systems in real WWTPs. According to their results, the sorption coefficients for hydrophobic and hydrophilic PhACs fit well into the Freundlich and the linear isotherms, respectively. They observed that the sorption coefficient in Log-scale has a positive correlation with log Kow for hydrophobic compounds and a negative correlation for hydrophilic compounds. They also found that each PhAC showed an optimum removal efficiency (49.6-79.4%) in a certain pH due to the variation of solute charge and surface charge of PAC brought out by pH change [155].

Snyder *et al.* used GAC and PAC as a pretreatment to membrane separation systems and they observed that both GAC and PAC can effectively remove PhACs from water (more than 90% removal) but the efficacy of GAC is influenced by NOM which competes for binding sites of GAC whereas PAC has more advantages because its addition to process is continuous and it is not recycled [28]. Therefore, further analysis is needed to consider the cost of GAC recovery and PAC consumption besides their performances. Verliefde *et al.* used a GAC column for the post-treatment of NF effluent and observed that the removal efficiency of the PhACs was very high (>99 %) during the short time-frame experiment (4 days) and since most of the organic matter was removed in the NF step, less competition occurred between organic matter in the feed water and the PhACs for adsorption onto the GAC. Therefore, a short contact time on the GAC was sufficient and the investment costs for GAC column were reduced [25].

Nguyen *et al* used GAC adsorptive system as a post-treatment stage for MBR system and found that although MBR is not efficient for the removal of persistent and hydrophilic PhACs such as carbamazepine, but GAC system can complement it very well with enhancement of the ability of removal of hydrophobic and hydrophilic compounds. According to their results, persistent compounds, such as, diclofenac and carbamazepine showing removal efficiencies lower than 40%, by MBR treatment achieved overall removal efficiencies of 98% or above after GAC treatment. However, they noted that this integrative system needs a strict monitoring should to

detect the breakthrough of hydrophilic and persistent compounds due to the saturation of GAC bed [134]. In another study, Li *et al.* added PAC directly into the MBR to increase the retention time of the soluble PhACs and consequently facilitate their removal in the biological processes. According to their results, after the addition of 1 g/l PAC to the MBR, the removal efficiencies of sulfamethoxazole and carbamazepine increased from 64% and below 1% to 82 % and 92%, respectively [100]. Also, Urase *et al.* added PAC directly to the MBR system and observed that sludge-water partition coefficient of carbamazepine increased from 0.057 L/g MLSS before PAC dosing to 0.597 L/g MLSS after PAC dosing, causing the enhancement of adsorption capacity of activated sludge for carbamazepine [156].

Future outlook

- Although the knowledge of PhACs removal through membrane separation processes has increased dramatically in recent years, there are still gaps that warrant further studies. According to the limited data in Tables 2.2.4, 2.2.5 and 2.2.7, removal rates cannot often be generalized or predicted precisely for a certain PhAC. Therefore, further research may result in better understanding of the processes controlling PhACs removal and the interactions of the various mechanisms.
- There are thousands of pharmaceuticals in the market that can go to the environment, but less than 2 % of them have been detected and investigated for treatment. Therefore, more efforts are needed to work on detection, toxicities, persistence and priorities of other compounds. Also, conducting a risk assessment can help to evaluate the necessity of working on new wastewater treatment processes and modification of current processes.
- Most of the researchers worked on readily available membranes in the market. However, synthesis of inexpensive and more hydrophilic membranes can reduce hydrophobic interactions of membranes with compounds and enhance their rejection. Also, developing fouling resistant membrane can have a major impact on reducing operational costs.
- For MBR systems, there are still unsolved questions including sorption mechanisms, degradation in solid phase and especially the effect of PhACs on microbial activity which consequently affect the fouling and rejection of

compounds by membrane. Therefore, more study is needed to investigate their interactions. Also, identification of the microorganisms that favor removal of pharmaceuticals in MBR seems to be interesting. Furthermore, working on full scale MBRs is another issue that should be considered, since bench scale MBRs is not likely to predict the performance of large scale MBRs.

- FO is a promising technology for water and wastewater treatment since its energy consumption is much lower than RO and NF and therefore future studies should be directed to improve the process efficiency by developing new membranes by integrating with renewable energy, such as wind and solar.
- Conducting a comparative study on treatment of similar water/wastewater sources using different filtration technology and taking many compounds into account can provide more reliable data.
- Combination and integration of methods including separation-oxidation, separation-adsorption and separation-adsorption-oxidation systems may attract the attention of researchers in future due to their potential to overcome the drawbacks of single process methods. In fact, a schematic proposed by authors for separation-adsorption-oxidation system is illustrated in Figure. 2.2.1. In this system, adsorption materials (e.g. carbonaceous materials, zeolites,…) will host living organisms or their biomolecules to form biodegradation sites with adsorption capability for PhACs. These functionalized particles could be dispersed within the membrane matrix to provide appropriate contact surface for water purification. In this system, adsorption material can adsorb PhACs and provide enough time for their biodegradation.

Conclusions

1- For estimation of the PhACs rejection by membrane separation methods, all of the influencing parameters including membrane MWCO, salt rejection, porosity, morphology, charge and hydrophobicity and the MW, molecular size, charge, and hydrophobicity of the PhACs as well as the feed water matrix must be taken into account.

2- RO membranes can remove efficiently almost all kinds of PhACs (with more than 75 % removal rate) and they are less influenced by electrostatic and hydrophobic effects in comparison to NF membranes. However, the capital and operational costs of osmosis systems should be considered carefully since RO units are operated under high pressure.
3- FO membranes showed average to high removal efficiency (40-80 %) for a broad range of PhACs, but their capital and operational costs are important parameters since they need complementary systems, such as distillation columns.
4- NF membranes can show high rejection efficiencies for a wide range of PhACs, although their performance is impacted by size exclusion, electrostatic and hydrophobic interaction especially in the case of loose NF membranes that have larger pore size. Generally, bigger molecules with negative charge and higher hydrophilicity are more efficiently rejected.
5- MBRs show erratic results for removal of PhACs and it can be attributed to the structural complexity of PhACs and also their effects on the different species of microorganisms. As per research, MBRs remove acetaminophen, ibuprofen, ketoprofen, gemfibrozil, bezafibrate and naproxen efficiently (>90 %) while the removal rates of carbamazepine and diclofenac are very low (<40%). For other compounds, the removal rates are in mean range (40-70%) or sometimes there is not sufficient data for judgment.
6- Membrane processes, except MBR, cannot degrade pollutants and concentrated pollutants in the retentate must be degraded or transformed into harmless chemical compounds by one of the chemical methods, otherwise the overall performance of the unit decreases as a result of concentration polarization.
7- Novel hybrid methods which integrate different removal methods (e.g. membrane separation, adsorption and biodegradation) can optimize the processes of PhACs removal in terms of effluent quality, reliability and operational costs.

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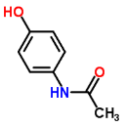
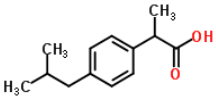
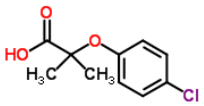
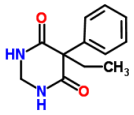
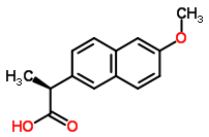
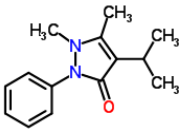
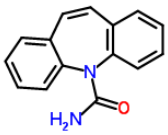
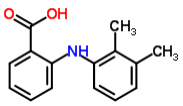
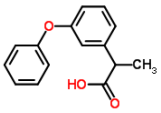
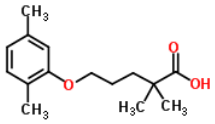
Table 2.2.1 Classification of removal processes for micropollutants from water and wastewater

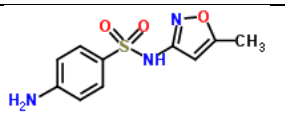
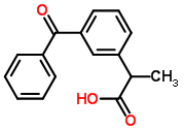
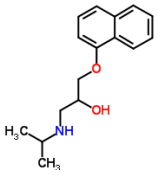
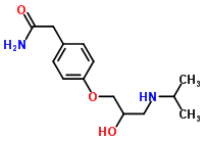
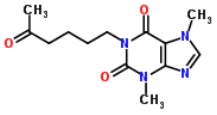
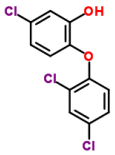
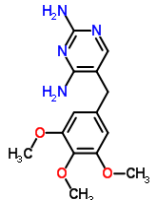
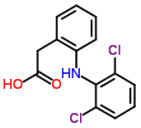
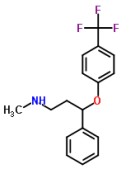
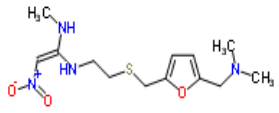
Process[a]	Subcategories	Examples
Physical separation	Membrane separation	Reverse Osmosis (RO)
		Forward osmosis (FO)
		Nanofiltration (NF)
	Adsorption	Zeolite
		Activated carbon
		Metal-Organic Frameworks
	Ion Exchange	Anionic and cationic resin
	Coagulation	Aluminum sulfate
Chemical transformation	Chemical oxidation	Ozonation
		Fenton Process
	Photo-oxidation	Photolysis
		Photocatalysis
		Photo-Fenton reaction
	Electrochemical process	Electro-degradation
		Electro-coagulation
	Biodegradation	Activated sludge
		Biofiltration
		Enzymatic bioreactor
	Ultrasonic treatment	Ultrasonic cavitation
Combinational treatment[b]	Membrane separation + Biodegradation	Membrane Bioreactor (MBR)
		Membrane immobilized enzyme
Integrative treatment[b]	Membrane + photo-oxidation	TiO_2 immobilized on membrane

a References: [13-19]

b Other combinational and integrative processes can be assembled by coupling each set of the subcategories in a series arrangement.

Table 2.2.2 Classification and molecular structure of the selected pharmaceuticals

Compound Name	MW (g/mol)	Molecular Formula	Classification	Structure
Low molecular weight compounds				
Acetaminophen[a]	151	$C_8H_9NO_2$	analgesic antipyretic	
Metronidazole	171	$C_6H_9N_3O_3$	Antimicrobial Agent	
Ibuprofen	206.29	$C_{13}H_{18}O_2$	Anti-inflammatory, Analgesic	
Clofibric acid	214.65	$C_{10}H_{11}ClO_3$	Metabolite of the cholesterol-lowering	
Primidone	218	$C_{12}H_{14}N_2O_2$	Anti-epileptic	
Naproxen	230	$C_{14}H_{14}O_3$	Anti-inflammatory, Analgesic	
Propyphenazone	230.306	$C_{14}H_{18}N_2O$	Anti-pyretic	
Carbamazepine	236.27	$C_{15}H_{12}N_2O$	Anti-epileptic	
Mefenamic acid	241.285	$C_{15}H_{15}NO_2$	anti-inflammatory	
Fenoprofen	242	$C_{15}H_{14}O_3$	anti-inflammatory	
Gemfibrozil	250.34	$C_{15}H_{22}O_3$	Lipid regulator	
Medium molecular weight compounds				

Compound Name	MW (g/mol)	Molecular Formula	Classification	Structure
Sulfamethoxazole	253.3	$C_{10}H_{11}N_3O_3S$	Antibiotics	
Ketoprofen	254.28	$C_{16}H_{14}O_3$	Anti-inflammatory, Analgesic	
Propranolol	259.34	$C_{16}H_{21}NO_2$	Beta-blocker	
Atenolol	266.336	$C_{14}H_{22}N_2O_3$	antihypertensive agent	
Pentoxifylline	278.3	$C_{13}H_{18}N_4O_3$	Blood pressure regulator	
Diazepam	284.7	$C_{16}H_{13}ClN_2O$	Tranquillizers	
Triclosan	289.5	$C_{12}H_7Cl_3O_2$	Antimicrobial Agent	
Trimethoprim	290.32	$C_{14}H_{18}N_4O_3$	Antimibactrial	
Diclofenac	296.15	$C_{14}H_{11}C_{l2}NO_2$	Anti-inflammatory, Analgesic	
Fluoxetine	309.3	$C_{17}H_{18}F_3NO$	Anti-depressants	
Ranitidine	314.4	$C_{13}H_{22}N_4O_3S$	histamine-2 blockers	

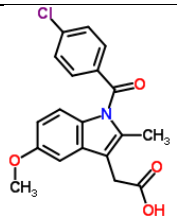
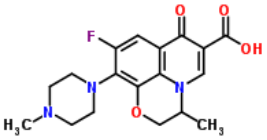
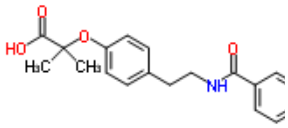
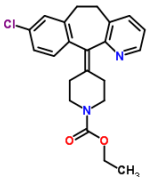
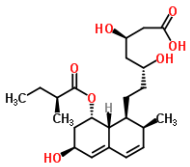
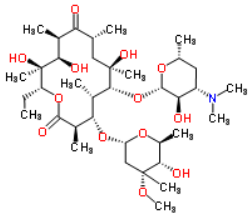
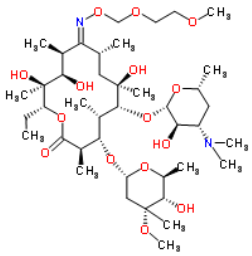
Compound Name	MW (g/mol)	Molecular Formula	Classification	Structure
Indometacin	357.78	$C_{19}H_{16}ClNO_4$	anti-inflammatory	
Ofloxacin	361.36	$C_{18}H_{20}FN_3O_4$	antibiotic	
Bezafibrate	361.82	$C_{19}H_{20}ClNO_4$	Lipid Regulator	
Loratidine	382.88	$C_{22}H_{23}ClN_2O_2$	antihistamine	
	High molecular weight compounds			
Pravastatin	424.53	$C_{23}H_{36}O_7$	Lipid Regulator	
Erythromycin	733.93	$C_{37}H_{67}NO_{13}$	antibiotic	
Roxithromycin	837.05	$C_{41}H_{76}N_2O_{15}$	Antibiotics	

Table 2.2.3 Physico-chemical properties of the selected pharmaceutically active compounds

Compound	logKow	pKa	Charge at (pH 7)	logD[b] (pH 7)	DM (D)	ML (nm)	EW (nm)
Ofloxacin	-2	5.8	-	-0.25	9.18	1.2	0.75
Metronidazole	-0.02	2.5	-	-0.27	6.3	0.93	0.66
Atenolol	0.16	9.6	Neutral	-1.85	4.13	1.37	0.49
Ranitidine	0.27	8	Neutral	-0.63	5.61	1.43	0.49
Pentoxifylline	0.29	0.97	Neutral	0.54	3.07	1.1	0.68
Acetaminophen[a]	0.46	9.5	Neutral	0.23	1.38	1.14	0.53
Sulfamethoxazole	0.89	1.7, 5.6	-	-0.45	6.31	1.031	0.55
Primidone	0.91	-1, 12.2	-	0.83	2.69	0.72	0.62
Trimethoprim	0.91	7.2	Neutral	1	2.21	1.1	0.59
Propyphenazone	1.94	0.8	-	2.13	4.10	1.01	0.50
Carbamazepine	2.45	13	Neutral	2.58	1.67	1.20	0.73
Clofibric acid	2.57	3.35	-	-1.08	1.86	0.99	0.40
Roxithromycin	2.75	9	Neutral	??	9.5	1.38	1.09
Diazepam	2.82	3.4	-	2.92	2.65	1.03	0.55
Erythromycin	3.06	8.9	Neutral	1.55	9.47	1.42	1.05
Pravastatin	3.1	4.2	-	-1.21	2.61	1.32	0.81
Ketoprofen	3.12	4.29	-	0.41	3.42	1.16	0.83
Naproxen	3.18	4.2	-	0.34	0.34	1.37	0.76
Propranolol	3.48	9.6	Neutral	1.15	0.6	1.29	0.55
Fenoprofen	3.9	4.21	-	0.38	1.99	1.16	0.83
Ibuprofen	3.97	4.47	-	1.44	1.8	1.39	0.64
Fluoxetine	4.05	8.7	Neutral	1.75	1.3	1.24	0.56
Indometacin	4.23	3.8	-	0.75	1.45	1.31	0.67
Bezafibrate	4.25	3.44	-	0.69	1.57	1.67	0.46
Diclofenac	4.51	4.08	-	1.59	0.96	0.83	0.49
Gemfibrozil	4.77	4.45	-	2.22	3.57	1.58	0.78
Mefenamic acid	5.12	3.8	-	2.04	4.75	0.95	0.58
Triclosan	5.17	7.8	Neutral	5.28	2.33	1.42	0.69
Loratidine	5.2	4.4	-	5.32	4.14	1.21	0.71

a: References: [24-29]

b: A compound is hydrophobic when logD>2.6 or logKow>2

DM: Dipole moment

ML: Molecular length

EW: Equivalent width

Table 2.2.4 Removal rates of osmosis systems for different pharmaceutically active compounds

Compound	Conc. (µg/l)	Brand Name	Rejection rate (%)	Ref
Acetaminophen	1.120	RO (X20)	(82.1)[a] (94.5)[b] (92.2)[c] (99.7)[d]	[29]
	0.05	RO (BW30LE-440)	(85.6)	[34]
	n.a.	RO (SW30-2540)	(99.9)	[35]
	10	FO (HTI)	(48.3)[e] (44.3)[f]	[24]
	n.a.	FO (HTI)	(70)	[35]
Metronidazole	10	FO (HTI)	(69.7)[e] (62.5)[f]	[24]
Carbamazepine	0.33	RO (SW 30-4040)	(99.7)	[8]
	1.130	RO (X20)	(91)[a] (97.9)[b] (91.5)[c] (97.0)[d]	[29]
	100	RO (XLE)	(91)	[32]
	100	RO (SC-3100)	(85)	[32]
	0.05	RO (BW30LE-440)	(98.5)	[34]
	250	FO (HTI)	(91.5)[g] (93.6)[h] (93.1)[i]	[36]
	250	FO (HTI)	(95.7)	[37]
	250	FO (made in lab)	(95.9)	[37]
	10	FO (HTI)	(66.4)[e] (76.3)[f]	[24]
Ibuprofen	10	FO (HTI)	(92.6)[e] (98.2)[f]	[24]
	250	FO (HTI)	(82.2)	[37]
	250	FO (made in lab)	(95.6)	[37]
	n.a.	FO (HTI)	(99.9)	[35]
Naproxen	0.038	RO (BW30LE-440)	(95)	[8]
	10	FO (HTI)	(96.3)[e] (96.9)[f]	[24]
	250	FO (HTI)	(73.4)	[37]
	250	FO (made in lab)	(93.9)	[37]
	n.a.	FO (HTI)	(99.8)	[35]
Fenoprofen	10	FO (HTI)	(93.5)[e] (96.8)[f]	[24]
Gemfibrozil	1.370	RO (X20)	(97.7)[a] (99.6)[b] (96.9)[c] (98.5)[d]	[29]
	0.05	RO (BW30LE-440)	(49.9)	[34]
	10	FO (HTI)	(95.4)[e] (97.0)[f]	[24]
Ketoprofen	0.05	RO (BW30LE-440)	(98.1)	[34]
	10	FO (HTI)	(96.2)[e] (96.7)[f]	[24]
Primidone	100	RO (XLE)	(87)	[32]
	100	RO (SC-3100)	(85)	[32]
	100	RO (XLE)	(84)	[38]
	0.1	RO (XLE)	(78)	[38]
Sulfamethoxazole	1.190	RO (X20)	(94.1)[a] (99.4)[b] (95.6)[c] (98.8)[d]	[29]
	100	RO (XLE)	(70)	[32]

Compound	Conc. (µg/l)	Brand Name	Rejection rate (%)	Ref
	100	RO (SC-3100)	(82)	[32]
	n.a.	RO (XLE)	(94.6)[c] (89.2)[j] (95)[k] (94.8)[l]	[39]
	0.05	RO (BW30LE-440)	(99.9)	[34]
	n.a.	RO (SW30-2540)	(99.8)	[35]
	n.a.	FO (HTI)	(99.9)	[35]
	250	FO (HTI)	(54.3)[g] (65.2)[h] (99.2)[i]	[36]
Diclofenac	0.329	RO (BW30LE-440)	(99.7)	[8]
	500	RO (HTI)	(90)	[26]
	100	RO (XLE)	(95)	[38]
	0.05	RO (BW30LE-440)	(99.9)	[34]
	250	FO (HTI)	(94.5)	[37]
	500	FO (HTI)	(99)	[26]
	250	FO (made in lab)	(95.8)	[37]
	n.a.	FO (HTI)	(99.9)	[35]
Triclosan	500	FO (HTI)	(99)	[26]
	500	RO (HTI)	(99)	[26]
Clofibric acid	0.155	RO (BW30LE-440)	(99.4)	[8]

a: Raw Lake Ontario water b: 5-µm filtered Lake Ontario water c: laboratory-grade water (Milli-Q®) d: MBR effluent e: Clean FO f: Fouled FO g: pH= 3.5 h: pH=5.5 i: pH=7.5 j: model water k: tap water l: pharmaceutical wastewater

Table 2.2.5 Removal rates of nanofiltration systems for different pharmaceutically active compounds

Compound	Conc. (µg/l)	Brand Name	Rejection rate (%)	Ref.
Acetaminophen	1.120	TS80	(47.9)[a] (67.1)[b] (0)[c] (76.7)[d]	[29]
	1	TS80	(28.9)[c] (27.7)[n] (39.4)[o] (34.6)[p]	[45]
	1.120	NF270	(31.2)[a] (0)[b] (1.3)[c] (8.2)[d]	[29]
	0.05	NF90	(44.6)	[34]
	0.027	ESNA	(44.4)	[46]
Carbamazepine	1.130	TS80	(66.9)[a] (97.3)[b] (8.9)[c] (92.6)[d]	[29]
	1	TS80	(93.4)[c] (95.8)[n] (99.3)[o] (99.4)[p]	[45]
	2	TS-80	(84.3)[h] (85.5)[i] (82.3)[j] (87.6)[k]	[47]
	750 (500)	TFC-SR2	(9.4)[e] (9.4)[f] (5.2)[g]	[48, 49]
	750	NF90	(98.6)[e] (97.9)[f] (97.1)[g]	[49]
	0.05	NF90	(98.1)	[34]
	1.130	NF270	(6.6)[a] (51.7)[b] (5.8)[c] (71.2)[d]	[29]
	750	NF270	(81.9)[e] (83.8)[f] (83.3)[g]	[49]
	1000 (1300)	NF270	(92)[q] (53)[r]	[50]
	2	Desal HL	(82.1)[h] (75.9)[i] (71)[j] (76)[k]	[47]
	0.061	ESNA	(63.4)	[46]
Gemfibrozil	1.370	TS80	(86.5)[a] (98.4)[b] (27.1)[c] (97.4)[d]	[29]
	100	TS-80	(99)[l] (40.7)[m]	[25]
	1	TS80	(95.2)[c] (67.5)[n] (99.8)[o] (99.8)[p]	[45]
	2	TS-80	(91.4)[h] 90.3)[i] (96.2)[j] (93.4)[k]	[47]
	0.05	NF90	(53.1)	[34]
	1.370	NF270	(16.7)[a] (85.7)[b] (10.7)[c] (92.9)[d]	[29]
	2	Desal HL	(82.9)[h] (90.4)[i] (94.2)[j] (90.3)[k]	[47]
	0.061	ESNA	(61)	[46]
Sulfamethoxazole	1.190	TS80	(76.4)[a] (98.8)[b] (21.5)[c] (96.7)[d]	[29]
	750 (500)	TFC-SR2	(2.7)[e] (19.1)[f] (64.5)[g]	[48, 49]
	0.05	NF90	(96.3)	[34]
	n.a.	NF 90	(98)[c] (99.6)[s] (99.6)[t] (98.3)[u]	[39]
	750	NF90	(97.8)[e] (98.4)[f] (99.7)[g]	[49]
	1.190	NF270	(13.5)[a] (88.3)[b] (9.7)[c] (90.1)[d]	[29]
	750	NF270	(31.4)[e] (82.8)[f] (98.7)[g]	[49]
	n.a.	NF 270	(77.3)[c] (80.1)[s] (87.7)[t] (62.2)[u]	[39]
	0.029	ESNA	(53.7)	[46]
	n.a.	Desal HL	(51.4)[c] (88)[s] (93.2)[t] (64.9)[u]	[39]
Ibuprofen	30	TS-80	(99)[l] (53)[m]	[25]
	2	TS-80	(88.9)[h] (92.1)[i] (97.1)[j] (93.5)[k]	[47]
	750	NF90	(99.9)[e] (99.9)[f] (99.9)[g]	[49]
	1500	NF 90	(84.1)[e] (83.7)[f] (87.8)[g]	[51]

Compound	Conc. (µg/l)	Brand Name	Rejection rate (%)	Ref.
	1500	NF 200	(69.4)[e] (74.8)[f] (76.7)[g]	[51]
	10	NF 270	(83.9)[e] (94.2)[f] (95.6)[g]	[10]
	750	NF270	(89.6)[e] (98.5)[f] (99.1)[g]	[49]
	750 (500)	TFC-SR2	(32.9)[e] (62.6)[f] (82.3)[g]	[48, 49]
	750	TFC-SR2	(36.2)[e] (64.4)[f] (82.3)[g]	[49]
	2	Desal HL	(83.9)[h] (90.2)[i] (95.1)[j] (90.7)[k]	[47]
	0.037	ESNA	(52.9)	[46]
	100	TS-80	(99)[l] (84.2)[m]	[25]
Clofibric acid	2	TS-80	(88.1)[h] (87.1)[i] (94.5)[j] (91.6)[k]	[47]
	2	Desal HL	(86.5)[h] (88.5)[i] (92.1)[j] (90.7)[k]	[47]
	50	TS-80	(99)[l] (76.8)[m]	[25]
Ketoprofen	2	TS-80	(88.8)[h] (89.1)[i] (95.9)[j] (93.7)[k]	[47]
	2	Desal HL	(84.1)[h] (85.5)[i] (95.6)[j] (95)[k]	[47]
	0.05	NF90	(97.2)	[34]
	5	TS-80	(99)[l] (99)[m]	[25]
	2	TS-80	(89.2)[h] (89.9)[i] (96.3)[j] (93.2)[k]	[47]
	100	ESNA	(93)	[38]
Diclofenac	0.029	ESNA	(48.9)	[46]
	2	Desal HL	(86.8)[h] (91.5)[i] (94.7)[j] (91.8)[k]	[47]
	0.05	NF90	(99.9)	[34]
	10	NF 270	(92.5)[e] (94.2)[f] (95.6)[g]	[10]
	50	TS-80	(99)[l] (88.6)[m]	[25]
Bezafibrate	2	TS-80	(89.3)[h] (91.7)[i] (97.4)[j] (93.6)[k]	[47]
	2	Desal HL	(88.9)[h] (93)[i] (97.2)[j] (91.8)[k]	[47]
Fenoprofen	2	TS-80	(91.2)[h] (89.9)[i] (96.2)[j] (92.9)[k]	[47]
	2	Desal HL	(73.8)[h] (87.4)[i] (89.8)[j] (86.5)[k]	[47]
	2	TS-80	(88.7)[h] (88.7)[i] (95.1)[j] (92.9)[k]	[47]
Naproxen	2	Desal HL	(77.6)[h] (87.8)[i] (92.5)[j] (98.6)[k]	[47]
	0.016	ESNA	(50)	[46]
Primidone	100	ESNA	(87)	[38]
	0.1	ESNA	(72)	[38]
Triclosan	43	ESNA	(93)	[46]

a: Raw Lake Ontario water b: 5-µm filtered Lake Ontario water, c: Milli-Q® water d: MBR effluent e: pH=5 f: pH=7 g: pH=9 h: Clean membrane i: fouled with river water j: fouled with Ion-Exchange effluent k: fouled with UF effluent l: Recovery rate= 10% m: Recovery rate= 80% n: Milli-Q® water with cations o: Milli-Q® water with NOM p: Milli-Q® water with cations and NOM q: in summer r: in winter s: model water t: tap water u: pharmaceutical wastewater

Table 2.2.6 Properties of typical membranes used for water treatment

Type	Brand Name[a]	Manufacturer	PS[b] (nm)	PWP[c]	MWCO (Da)	$MgSO_4$ Rej. (%)	NaCl Rej.	ZP[d] (mV)	CA[e] (°)
UF	UE 10	TriSep	10	32	100000	0	0	-92	49.3
	GM	Osmonics	2.3	14.5	8000	0	0	-28.8	46
NF	NF 270	Filmtec	0.84	15.5	400	97	58	-87	29.8
	NF 200	Filmtec	0.93	5	300	97	65	-15.3	30.3
	TS 80	TriSep	0.80	4.5	<200	96.3	30	-15	56.6
	NF 90	Filmtec	0.68	6	200	97	85	-48	58
	ESNA	Hydronautics	0.90	7.4	600	89	80	-10.6	57
	Desal-HL	Osmonics	0.96	15.4	150-300	98	60	-30	41
	TFC-SR2	Koch	1.28	15.4	400	99.8	9.8	-10.4	55.3
FO	HTI	Hydration Technology Innovations	-	0.36	200	98	94	-25	58.8
RO	X 20	TriSep	-	2.5	<200	99.5	98	-87	55
	BW30LE-440	Filmtec	-	3.75	100	100	99	-23	42
	XLE	Filmtec	-	3.8	150	>99.2	99	-19	46.9
	SW 30 4040	Filmtec	-	0.75	<100	99.9	99.7	-15	62
	SC-3100	Toray	-	n.d.	200-300	n.d.	94	n.d.	n. d.

a: References: [24, 32, 39, 45, 46, 49, 51, 56, 58, 65, 69-72]
b: Pore size
c:Pure water permeation (L/m^2 h bar)
d: Zeta potential
e: Contact angle
n.d.: no data

Table 2.2.7 Removal efficiencies of pharmaceutically active compounds by membrane bioreactor systems

Compound	Concentration (µg/l)	SRT (day)	HRT (h)	Removal efficiency (%)	Ref
Acetaminophen	5	n.d.	24	87	[90]
	1000000	n.d.	48	99.9	[88]
	50	(15) (30)	13	(99.9) (99.9)	[91]
	1	3	14	99.6	[85]
	9.25	n.d.	(15) (7.2)	(99.8)[a] (99.9)[b]	[89]
Diclofenac	5	n.d.	24	15	[90]
	2.8	37	24	23	[92]
	0.251	(15) (65)	6.7	(50.6) (81.6)	[93]
	10	72	12	0	[94]
	25000	n.d.	48	50	[95]
	690	n.d.	48	55	[96]
	1	3	14	87.4	[85]
	(3.2) (4.1)(3.2)	(10) (27) (55)	(12) (29) (96)	(0) (50.5) (32.9)	[86]
	5	88	26	26	[97]
	2	70	24	17.4	[80]
	1.3	n.d.	(15) (7.2)	(65.8)[a] (62.6)[b]	[89]
	2	70	24	(43)[c] (0.8)[d] (1.1)[e]	[98]
Ketoprofen	5	n.d.	24	66	[90]
	50	(15) (30)	13	(98) (99.9)	[91]
	0.47	37	24	62	[92]
	0.979	(15) (65)	6.7	(82.5) (97.9)	[93]
	25000	n.d.	48	94	[95]
	1	3	14	91.9	[85]
	5	88	26	94	[97]
	2	70	24	70.5	[80]
	0.95	n.d.	(15) (7.2)	(43.9)[a] (44)[b]	[89]
	2	70	24	(89.1)[c] (3.9)[d] (62.5)[e]	[98]
Bezafibrate	2.6	37	24	91	[92]
	1	3	14	95.8	[85]
	(1.9) (2) (6.8)	(10) (27) (55)	(12) (29) (96)	(94.7) (96.4) (77.3)	[86]
	15.85	n.d.	(15) (7.2)	(90.3)[a] (88.2)[b]	[89]
Naproxen	5	n.d.	24	45	[90]
	50	(15) (30)	13	(86) (89)	[91]
	0.95	37	24	71	[92]
	0.276	(15) (65)	6.7	(96) (96.3)	[93]
	10	72	12	84	[94]

Compound	Concentration (µg/l)	SRT (day)	HRT (h)	Removal efficiency (%)	Ref
Ibuprofen	25000	n.d.	48	99	[95]
	1	3	14	99.3	[85]
	5	88	26	82	[97]
	2	70	24	40.2	[80]
	0.4	n.d.	(15) (7.2)	(90.7)[a] (91.6)[b]	[89]
	5	n.d.	24	96	[90]
	5.7	37	24	97	[92]
	1.966	(15) (65)	6.7	(94.6) (98.2)	[93]
	10	72	12	98	[94]
	25000	n.d.	48	97.7	[95]
	1	3	14	99	[85]
	(1.5) (2.7) (2.5)	(10) (27) (55)	(12) (29) (96)	(98.5) (99.1) (97.2)	[86]
	5	88	26	99	[97]
	2	70	24	96.8	[80]
	22.95	n.d.	(15) (7.2)	(99.2)[a] (99.5)[b]	[89]
	2	70	24	(98.8)[c] (72.7)[d] (5.5)[e]	[98]
Carbamazepine	5	n.d.	24	32	[90]
	20	72	12	10	[94]
	25000	n.d.	48	21.6	[95]
	1	3	14	0	[85]
	(1.8) (1.2) (0.7)	(10) (27) (55)	(12) (29) (96)	(12.5) (4.4) (0)	[86]
	5	88	26	58	[97]
	2	70	24	13.2	[80]
	2	70	24	(28.1)[c] (21.5)[d] (25)[e]	[98]
Clofibric acid	0.028	(15) (65)	6.7	(76.9) (82.1)	[93]
	25000	n.d.	48	65	[95]
	1	3	14	71.8	[85]
	5	88	26	81	[97]
Primidone	25000	n.d.	48	94	[95]
	2	70	24	13.2	[80]
Indomethacin	1	3	14	46.6	[85]
	0.83	n.d.	(15) (7.2)	(41.4)[a] (29.7)[b]	[89]
Mefenamic acid	0.221	(15) (65)	6.7	(50) (93.2)	[93]
	1	3	14	74.8	[85]
	1	n.d.	(15) (7.2)	(40.5)[a] (35.5)[b]	[89]
Ranitidine	1	3	14	95.0	[85]
	0.31	n.d.	(15) (7.2)	(44.2)[a] (29.5)[b]	[89]
Propyphenazone	1	3	14	64.6	[85]
	0.071	n.d.	(15) (7.2)	(64.5)[a] (60.7)[b]	[89]
Erythromycin	1	72	12	91.5	[94]
	1	3	14	67.3	[85]

Compound	Concentration (µg/l)	SRT (day)	HRT (h)	Removal efficiency (%)	Ref
	1.51	n.d.	(15) (7.2)	(43)[a] (25.2)[b]	[89]
roxithromycin	50	(15) (30)	13	(57) (81)	[91]
	10	72	12	77.4	[94]
	(0.02) (0.06) (0.12)	(10) (27) (55)	(12) (29) (96)	(99.9) (34.4) (73.5)	[86]
Ofloxacin	1	3	14	94	[85]
	16.29	n.d.	(15) (7.2)	(95.2)[a] (91.3)[b]	[89]
Sulfamethoxazole	50	(15) (30)	13	(55) (64)	[91]
	10	72	12	52	[94]
	1	3	14	60.5	[85]
	0.14	10	12	61.3	[86]
	2	70	24	91.4	[80]
	0.77	n.d.	(15) (7.2)	(80.8)[a] (78.3)[b]	[89]
	2	70	24	(93.7)[c] (92.6)[d] (68.4)[e]	[98]
Metronidazole	5	n.d.	24	40	[90]
	25000	n.d.	48	40.7	[95]
	5	88	26	80	[97]
Gemfibrozil	5	n.d.	24	98	[90]
	25000	n.d.	48	97.7	[95]
	5	88	26	96.5	[97]
	3.95	n.d.	(15) (7.2)	(42.2)[a] (32.5)[b]	[89]
Triclosan	25000	n.d.	48	97.5	[95]
Diazepam	20	72	12	26.5	[94]
Atnolol	2	70	24	96.9	[80]
	1.82	n.d.	(15) (7.2)	(76.6)[a] (65.9)[b]	[89]
Trimethoprim	10	72	12	36.4	[94]
	2	70	24	17.4	[80]
	0.29	n.d.	(15) (7.2)	(66.7)[a] (47.5)[b]	[89]
Loratidine	0.029	n.d.	7.2	33.5	[89]
Floxetine	1.21	n.d.	(15) (7.2)	(98)[a] (98)[b]	[89]
Propranolol	0.62	n.d.	(15) (7.2)	(77.6)[a] (65.5)[b]	[89]
Pravastatin	0.98	n.d.	(15) (7.2)	(86.1)[a] (83.1)[b]	[89]

a: Flat sheet membrane b:Hollow fiber membrane c: pH=5 d: pH=7 e: pH=9

Table 2.2.8 Summary of influencing parameters on rejection of PhACs by different membrane processes

Membrane process	FO	RO	NF	MBR
Hydrophobicity	+	+	+	+
Membrane surface charge	+	+	+	+
Solute charge	+	+	+	+
Polarity	+	+	+	-
Molecule geometry	+	+	+	-
Membrane salt rejection	+	+	+	-
Membrane MWCO	+	+	+	-
Diffusion of solute	+	+	+	-
Fouling	+	+	+	-
Structural complexity	-	-	-	+
Microorganisms	n.a.	n.a.	n.a.	+

n.a: not associated

Table 2.2.9 Qualitative prediction of rejection of pharmaceutically active compounds

Hydrophobicity	MW	Charge	MWd	Mechanism	Predicted rejection
Log Kow<2	MW>MWCO	pH>pKa	MWd > Pore Size	SE, CR	Very high
			MWd < Pore Size	SE, CR	High-Very high
		pH<pKa	MWd > Pore Size	SE	Moderate-high
			MWd < Pore Size	SE	Low-Moderate
	MW<MWCO	pH>pKa	MWd > Pore Size	CR	Moderate-high*
			MWd < Pore Size	CR	Low- Moderate*
		pH<pKa	MWd > Pore Size	--	Low
			MWd < Pore Size	--	Low
Log Kow>2	MW<MWCO	pH<pKa	MWd < Pore Size	--	Very low
			MWd > Pore Size	--	Low
		pH>pKa	MWd < Pore Size	CR	Low-Moderate*
			MWd > Pore Size	CR	Moderate-high*
	MW>MWCO	pH<pKa	MWd < Pore Size	SE	Low-Moderate
			MWd > Pore Size	SE	Moderate-high
		pH>pKa	MWd < Pore Size	SE, CR	High-very high
			MWd > Pore Size	SE, CR	Very high

SE: size exclusion CR: charge repulsion MWd: molecular width

* Depend on surface charge of membrane and dissociation fraction of compound

Table 2.2.10 Comparison of capital and operational costs for different technologies

Technology	Energy consumption (KWh/ m^3)	Capital cost ($/$m^3$/d)	Operational cost ($/$m^3$)	Reference
RO[a]	4.7	334.3	0.72	[123]
NF[b]	3.4	338.2	0.57	[123]
FO[c]	0.84	> 500	0.66	[124]
MBR[d]	1.07	408.7	0.13	[125]

a & b) capacity; 3000m^3/d, energy cost: 0.09 $/kWh
c) capacity: 3785 m^3/d, energy cost: n.d.
d) capacity: 20850 m^3/d, price of energy: 0.1 $/kWh

Table 2.2.11 Removal efficiencies of PhACs by combinational treatments

Components	Type	compound	Removal rate (%)	Ref.
Reverse osmosis + Ultra-violet photolysis	Combined	Naproxen	>99	[28]
Nanofiltration/ Reverse osmosis + photo-fenton process	Integrated	Diclofenac Ranitidine sulfamethoxazole	>80 >99 >99	[131]
Nanofiltration + Ultra-violet /Ozone		Roxithromycin	>98	[132]
Membrane bioreactor + Ultra-violet photolysis	Combined	Carbamazepine	>96	[90]
Membrane bioreactor + Ozonation	Integrated	Acyclovir	>99	[133]
Nanofiltration + Granular activated carbon	Combined	Atnolol	>99	[25]
Membrane bioreactor + Granular activated carbon	Combined	Diclofenac Carbamazepine	>98 >98	[134]
Membrane bioreactor + Granular activated carbon	Combined	Sulfamethoxazole Carbamazepine	>82 >92	[100]

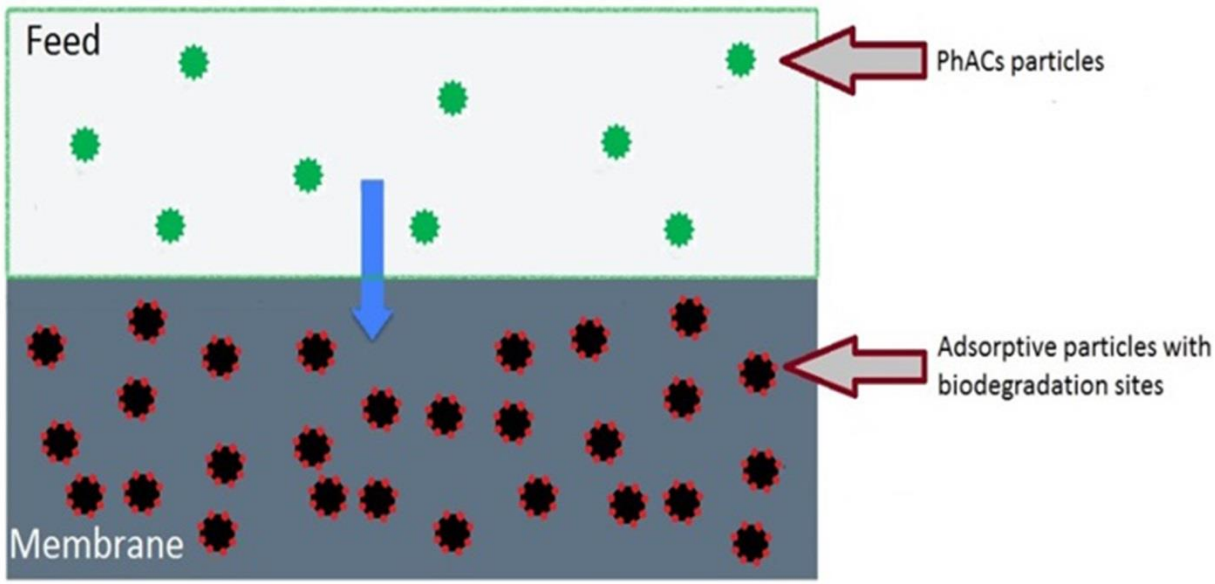


Figure 2.2.1 A schematic for proposed separation-adsorption-oxidation system for removal of pharmaceutically active compounds

Part 3

Adsorption Study of Environmentally relevant concentrations of Chlortetracycline on Pinewood Biochar

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Résumé

La présence de composés actifs pharmaceutiques (PhACs) dans l'eau de surface et les eaux usées a suscité l'intérêt des chercheurs en raison de leurs impacts environnementaux potentiels, par conséquent, leur élimination est devenue très imporant. Le comportement d'adsorption de la chlorotétracycline (CTC) dans une solution aqueuse sur le biocharbon de pin brut et activé a été étudié à 298 K. L'effet du pH initial de la solution a été étudié en réalisant des expériences à trois pH différents (1, 5 et 9). A chaque pH, la CTC a montré une charge électrostatique variée (+ 1, 0 et - 1, respectivement) qui a affecté directement son adsorption. Les résultats indiquent que la CTC a suivi l'isotherme de Langmuir, et les paramètres connexes ont été calculés. De plus, nous avons observé que l'adsorption maximale se produisait à un pH égal à 1. La capacité d'adsorption de la CTC pour le biocharbon brut et activé était respectivement 2,1 et 208,3 mg/g d'adsorbant. Les biocharbons ont été caractérisés selon différentes méthodes analytiques, telles que, par un analyseur de potentiel zêta, par la granulométrie à laser et à l'aide d'un microscope électronique à balayage (SEM). Les résultats ont montré que les biocharbon bruts et activés sont des candidats potentiels et prometteurs pour l'élimination de la CTC de l'eau en raison du caractère acide de la pinède qui pourrait entraîner une meilleure interaction avec les composés ionisables à des pH plus faibles.

Mots clés

Pinewood, Biochar, activation, chlortétracycline, adsorption, isotherme

Abstract

The presence of pharmaceutically active compounds (PhACs) in water and wastewater has raised concerns because of potential environmental impacts and thus their removal is of high importance. The adsorption behavior of chlortetracycline (CTC) from aqueous solution on raw and activated pinewood biochar was studied at 298 K. The effect of initial pH of the solution was studied by performing the experiment at three different pHs (1, 5 and 9). At each pH, CTC showed varied electrostatic charge (+1, 0 and -1, respectively) which affected its adsorption. The results indicated that CTC followed Langmuir isotherm and the related parameters were calculated. Also, it was observed that the maximum adsorption occurred at pH 1. The adsorption capacity of CTC for raw and activated biochar was at least 2.1 and 208.3 mg/g adsorbent, respectively. The characteristics of biochars were studied using zeta potential analyzer, laser size analyzer and scanning electron microscopy (SEM). The results showed that raw and activated biochars are promising candidates for removal of CTC from water due to the acidic character of pinewood that can result in better interaction with ionizable compounds at lower pHs.

Keywords

Pinewood, Biochar, activation, chlortetracycline, adsorption, isotherm

Introduction

Chlortetracycline (CTC) is a broad-spectrum antibiotic from the family of tetracyclines that is commonly used as veterinary medicine for poultry, swine, and livestock for preventing, controlling, and treating animal health issues as well as increasing their growth rates [1]. This pharmaceutically active compound (PhAC) can enter the environment through the application of animal manure to agricultural fields and thereafter it can go to rivers, ground waters and lakes by surface runoff [2]. The concentration of CTC is reported from several ppb in the effluent of municipal wastewater treatment plant to several mg/L in pharmaceutical production wastewater [3, 4]. Generally, PhACs can enter the human body through the drinking of contaminated water which has raised concerns over potential human health risks [5]. Antibiotic resistance can also develop to chlortetracycline in human and animals which in turn necessitates the prescription of higher dosages of antibiotics and finally invention of new compounds [6]. Therefore, removal of this compound from water and wastewaters should be considered to avoid potential problems.

There are different methods including physical (membrane separation, adsorption), chemical (ozonation, chlorination, UV irradiation) and biological (microbial or enzyme bioreactor) for removal of PhACs from aqueous media [7-15]. Among them, adsorption onto carbonaceous materials is of interest because of its feasibility, scale-up capability and reasonable capital and operational costs [16]. Ji *et al.* studied the removal of tetracycline with carbon nanotube and compared it with graphite and activated carbon. They observed that single-walled carbon nanotube has higher adsorbent to solution distribution coefficient compared to multi-walled carbon nanotube, activated carbon and graphite [17]. Choi *et al.* used granular activated carbons (GAC) made from coal or coconut for removal of CTC and achieved more than 80 % removal efficiency for both carbon sources [18]. Gao *et al.* investigated the adsorption capacity of graphene oxide for tetracycline and achieved 313 mg/g maximum adsorption capacity [19]. Recently, biochars produced by pyrolysis of different biomass has attracted the attention of researchers for removal of PhACs because they are cost effective, porous and environmentally friendly and have functional groups. For example, Zhang *et al.* used corn straw biochar for sorption of tetracycline and observed that, the corn straw biochar is not an ideal sorbent for immobilization of tetracycline though its performance for simazine is acceptable [20,

21]. In another study, Liu *et al.* enhanced the adsorption capacity of rice-husk biochar with acid and alkali treatment and achieved more than 58.8 mg/g adsorption on alkali treated biochar [16]. Among different sources of biochar, pinewood biochar is of high interest. Pine trees account for the majority of forest trees in Canada and other regions and each year millions of them are cut and transported to all around the world for industrial uses which leaves lots of biomass in the form of wooden chips. Therefore, availability and low cost make pinewood biomass as a promising source for production of biochar which is also a value adding process for wooden residues. To the best of our knowledge, there is no report on adsorption of chlortetracycline on pinewood biochar and in this study the adsorption behavior of this compound is investigated at different pHs on raw and activated biochar.

Materials and methods

Materials

Chlortetracycline (CTC, purity > 97%) was purchased from Toronto Research Chemicals (TRC-Canada). HPLC grade water was prepared in the laboratory using milli-Q/Millli-Ro Milli pore system (Massachusetts, USA). Sodium hydroxide and hydrogen chloride (purity > 97%) were purchased from Fisher Scientific. Biochar was donated by Pyrovac Inc. (Quebec, Canada) derived from pine white wood (80%) purchased from Belle-Ripe in Princeville and the rest was spruce and fir (20%). This biochar was produced at 525°C under atmospheric pressure for 2 minutes and used as obtained from the reactor outlet.

Biochar Characterization

Supplied biochar was grinded by a Retsch RS 200 vibratory disc mill at 750 rpm for 60 seconds. For activated biochar, this process was performed again for 30 seconds after activation due to agglomeration. Particle size distribution of biochar was measured by a LA-960 laser particle size analyzer (Horiba, Japan). For this analysis, about 0.5 g of samples was dispersed into 100 mL of water and the mixture was introduced into sample space. The zeta potential of biochar and activated biochar were measured by zetasizer nano ZS (Malvern instruments Inc., UK) at different pH. Prior to analysis, a 200 mg/L mixture of biochar or activated biochar in water was prepared and injected into sample holder. For each sample, measurement was done

30 times and the average value was reported. Fourier-transform infrared-attenuated total reflectance (FTIR-ATR) spectra were recorded on a Nicolet iS50 spectrometer (Thermo scientific, USA) at 0.04 cm^{-1} resolution and in the range of 400-4000 cm^{-1}. Morphological characteristics of biochar and activated biochar were investigated by an EVO® 50 scanning electron microscope (Zeiss, Germany). For this analysis, small amounts of the samples were transferred to the stub for gold coating by a SPI Module sputter coater. Energy-dispersive X-ray analysis (EDS) was performed by Inca 250 x-ray analyzer (Oxford instrument, UK). The BET specific surface areas were obtained from the N_2 adsorption isotherms recorded at 77 K ((Autsorb-1, Quantachrome Instruments) at the relative pressure range from 0.05 to 0.3. Powder X-ray diffraction (XRD) patterns of raw and activated biochar were collected using D5000 diffractometer (Siemens, Germany). The diffractometer was operated at 40 kV and 40 mA using Cu Kα radiation. Diffractograms were obtained from 4º to 84º (2θ scale) at a step size of 0.02º and a counting time of 1.2 s steps.

Adsorption measurements

The biochar was grinded and sieved to powder with diameter less than 70 µm. The adsorption measurements were carried out by batch equilibrium experiments in 50 mL glass flasks. About 20 mL of CTC solutions with specified concentrations (0.2, 0.5, 1, 2, 4, 8, 12, 16 and 20 mg/L for raw biochar and 0.2, 0.5, 1, 5, 20, 50, 75, 100, 125 and 150 mg/L for activated biochar) were mixed with fixed amounts of adsorbent (0.0400 g raw biochar and 0.0010 g activated biochar). Before addition of adsorbent, the pH of mixtures was adjusted to required value (1, 5, or 9) either by 1 N NaOH or HCl. The ionic strength of mixtures was kept constant with calcium chloride at 0.01 M. The flasks were sealed and incubated at 150 rpm for 72 h to ensure equilibrium. Later, the mixtures were filtered with 0.45 µm cellulose acetate syringe filters prior to concentration measurement. CTC concentrations were estimated by using Laser Diode Thermal Desorption (LDTD) (Phytronix technologies, Canada) coupled with a LCQ Duo ion trap tandem mass spectrometer (Thermo Finnigan, USA). The daughter ions identified for CTC in LDTD were 464 and 444 Da. The detailed method was explained elsewhere [22]. A calibration curve of CTC concentration versus absorbance was developed with seven standard solutions and with R^2 no less than 0.98. All experiments were carried out in duplicates and the average values were

used for analysis. The equilibrium concentration of CTC in biochar was calculated based on following equation 1:

$$q_e = \frac{(C_0 - C_e) * V}{M} \quad (1)$$

where q_e *(mg/g)* is the equilibrium CTC concentration in biochar, *V (L)* is the volume of solution, *M(g)* is the weight of biochar and C_0 and *Ce (mg/L)* are initial and equilibrium concentrations of CTC, respectively.

Activation of biochar

About 20 g NaOH was dissolved in 100 mL of water and 10 g of biochar was added to this solution. The mixture was stirred with a magnetic stirrer (150 rpm) at room temperature for 2 h and it was then dried at 80 °C for 24 h. The prepared sample was placed in quartz tube to be heated in a horizontal furnace under nitrogen flow of 200 mL/min. The temperature of the quartz tube was increased to 800 °C at 10 °C/min, and held at this temperature for 2 h before cooling down. Later, the product was washed with water, and the sodium hydroxide was neutralized with 0.1 M HCl. Finally, for removal of sodium salt, the product was washed with water and dried at 60 °C for 24 h.

Results and Discussion

Biochar Characteristics

Figure 2.3.1 shows the particle size distribution of biochar. The mean particle size of the raw biochar was 25.7 µm and 90 % of the particles were smaller than 47.8 µm. For activated biochar, the mean particle size was 12.25 µm and 90 % of the particles were smaller than 19.1 µm. The smaller sizes of activated biochar can be due to second grinding process and also heat treatment.

Figure 2.3.2 illustrated the FTIR spectra of raw and activated biochar samples. According to these spectra, there were four significant bands at 3324 cm^{-1} (alcohol, O-H stretching), 1582 (alkene/aromatic, C=C stretching), 1185 (phenolic, C-O stretching), and 872 (aromatic, C-H out of plane bending) cm^{-1} in raw biochar. For activated biochar, no characteristic band was observed which confirmed the gasification and removal of functional groups.

Figure 2.3.3 presents the SEM images of raw and activated biochar. SEM images demonstrated that raw biochar had particles with polygonal shape and smooth surface but after activation they were converted into porous structure. It is in agreement with the results of Angin et al. and Azargohar and Dalai that reported formation of macropores in biochar after activation [23, 24]. The elemental composition of raw and activated biochar that was measured by EDS are summarized in Table 2.3.1. The results showed that during activation process, the content of oxygen decreased by 2.66 %. The presence of silicon in activated biochar was due to its migration from quartz tube during activation process. Also the results of BET analysis showed that the specific surface area of activated biochar was 57 times more than that of raw biochar.

According to Yu *et al.* and Pei *et al.,* decreasing oxygen content, can have positive effects on adsorption capacity [16, 19] though in some cases, oxygen-bearing functional groups, such as OH, C-O and C=O can be beneficial for adsorption of organic compounds [25].

The structure of biochars was believed to be consisting of short stacks of small graphite-like sheets in a highly disordered form and with O-containing groups on the edge to form connected microporous networks [16, 26]. As observed in Figure 2.3.4, there is no remarkable peak in the XRD pattern of raw biochar except a small peak at around 2θ=26.54º indicating the d-spacing of 0.335. In contrast, the appearance of two broad peaks at 2θ=26.16º and 2θ=41.12º in the XRD pattern of activated biochar implied turbostratic crystallites .i.e. intermediate structure between graphite and amorphous states [27, 28]. The d-spacing of activated biochar was estimated to be 0.340 nm which is higher than that of raw biochar and close to the conventional graphene [29].

Adsorption model

Figure 2.3.5 and Figure 2.3.6 demonstrate the adsorption isotherms of CTC on raw and activated pinewood biochar, respectively. There are several adsorption models, such as Langmuir and Freundlich that can describe the interactions between adsorbates and adsorbents. In Langmuir model, adsorbent surface is uniform and adsorbate molecules form a monolayer around adsorbent surface without interaction between them. In Freundlich model, surface energies are heterogeneous and depend on surface coverage.

The Langmuir and Freundlich models are defined by following equations 2 and 3, respectively:

$$\frac{C_e}{q_e} = \frac{C_e}{q_m} + \frac{1}{bq_m} \quad (2)$$

$$q_e = kC_e^{\frac{1}{n}} \quad (3)$$

In these two equations, *q*m (mg/g) and *b* (L/mg) are constants for Langmuir model, and *k* ((mg/g).(mg/L)$^{-1/n}$) and n are constants the Freundlich model. These two models were used to correlate experimental data and the results are summarized in Table 2.3.2.

The results indicated that at three studied pHs, adsorption process on raw and activated biochars was dominated by Langmuir model rather than Freundlich. It meant that both raw and activated biochar followed the single layer theory which necessitates the existence of a saturation point or maximum adsorption capacity.

There was a large difference between the adsorption capacity of raw and activated biochar which was due to the modification of hydrophobic property and also specific surface area. Biochar has a highly hydrophobic surface and showed strong adsorption affinity for hydrophobic organic compounds [30, 31]. Based on FTIR spectra (Figure 2.3.2), confirmed the presence of hydrophobic groups e.g. aromatics on the surface of raw biochar. Therefore, CTC with K_{ow} of around 0.24 is not expected to be adsorbed well on raw biochar. The lower specific surface of raw biochar is another reason for lower adsorption capacity of raw biochar for CTC. On the other hand, according to Table 2.3.3, activated biochar showed a remarkable adsorption capacity for CTC (181.8-434.8 mg/g) that was considerably higher than those obtained in previous investigations for different carbonaceous materials including carbon nanotubes (57.13-303.1 mg/g), graphene oxide (212.3 mg/g) and biochar (58.8 mg/g). The significant improvement in the adsorption capacity of activated biochar compared to raw biochar can be attributed to the intercalation process which led to the formation of nano-porous structure and consequently increased the specific surface area to a large extent (from 14.86 m^2/g to 852.95 m^2/g). Also, the outgassing of hydrophobic groups from raw biochar could modify the affinity of activated biochar for CTC [32]. The disappearance of peaks at 1582 cm^{-1}

and 872 cm^{-1} in FTIR spectrum of activated biochar confirmed the complete outgassing of hydrophobic groups. The intercalation and formation of nanostructured carbon were confirmed by XRD patterns as discussed earlier in biochar characterization.

Effect of pH

According to Figure 2.3.5 and Figure 2.3.6, adsorption of CTC on raw and activated biochar decreased as the pH increased. The π –π interactions between graphite-like structure, as a π -acceptor, and aromatic ring structures, as an electron donor, was theoretically demonstrated [16]. Therefore, the graphite-like structure of biochar was supposed to have good interaction with aromatic rings of CTC. On the other hand, CTC is an amphoteric molecule and three groups in its structure can undergo protonation–deprotonation reactions depending on the pH of the solution which consequently leads to formation of four different species as given in Figure 2.3.7.

At lower pH, CTC was fully protonated and had one positive charge (H^+). Therefore, apart from π–π interaction, H-π bonding can also occur and increase the tendency of biochar to adsorb ionized compound [34]. At higher pH, following the first deprotonation (pKa=3.3), a species was formed that had a negatively charged group and a positively charged group (zero net charge). By increasing pH, the second deprotonation occurred (pKa=7.44) and a species with two negatively charged groups and a positively charged group (net charge = -1) was formed. The third deprotonation with pKa=9.5, resulted in species with net charge=-2 [35]. Increasing the negative charges of CTC molecule weakened its electron accepting capabilities which affected the interaction with adsorbate [36]. Biochars are generally known for having negatively charged surface [37]. Their negative charge is increased by increasing solution pH [38]. Figure 2.3.8 presents zeta potential of raw and activated biochar at different pH. According to Figure 2.3.8, increasing pH resulted in increasing zeta potential of raw and activated biochar which can repel the molecules with negative charge. Therefore, it was expected that increasing the pH of the solution reduced the adsorption rate by inhibition of H-π bonding and increasing electrostatic repulsion. A similar decreasing trend was observed in the study of Essandoh *et al.* for adsorption of ibuprofen and salicylic acid on pinewood biochar by increasing solution pH [39]. They calculated the point of zero charge (pHpzc), at which the net charge on the surface was zero, to be around 2. This value was in

agreement with the x-intercept in the zeta potential diagram (Figure 2.3.8). They concluded that at pH <2, the electrostatic repulsion was minor and increasing the solution pH resulted in increased magnitude of biochar negative charge which consequently enhanced the electrostatic repulsion between biochar and anions. They attributed this behavior to the acidic character of pinewood biochar, which was in favor of adsorbing ionizable compounds, and also to the presence of carboxylic acids and phenols.

Biochar is gradually finding its application as one of the best options for carbon sequestration and soil amendment [40]. Meanwhile, other applications are emerging for this new class of environmentally-friendly material. Removing persistent organic compounds from water and wastewater are among these applications which are attracting the attention of researchers due to the interesting features of biochar. However, due to versatile structures of biomass, different roles of functional groups and effects of activation processes, more studies are needed to optimize the application of biochars in purification industries. Furthermore, integrating other contaminant removal methods, such as biodegradation processes with the adsorption potential and chemical interaction capability of biochar can be of high interest to researchers in the future.

Conclusion

The adsorption behavior of CTC from water on raw and activated pinewood biochar was studied at 298 K and different pH (1, 5 and 9). The results showed that CTC followed Langmuir isotherm for adsorption on raw and activated biochar and that the maximum adsorption occurred at pH 1. Also, it was found that the maximum adsorption capacity (q_m) of the synthesized activated biochar in this study was considerably higher than previous investigations. Thus, pinewood biochar is a good candidate for production of a new generation of high performance adsorbents from renewable resources and using pollution-free methods, especially for the emerging contaminants, such as CTC at their environmentally relevant concentrations and hence consequent applications in wastewater/water treatment plants.

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Table 2.3.1 Elemental analysis and specific surface areas of raw and activated biochar

Property	Raw biochar	Activated biochar
Carbon content (Wt%)	78.28	80.73
Oxygen content (Wt%)	21.72	18.06
Silicon content (Wt%)	ND	1.21
Specific surface area (m^2/g)	14.86	852.95
ND: Not detected		

Table 2.3.2 Fitting parameters of Langmuir and Freundlich models for CTC adsorption on biochar

Adsorbent	pH	Langmuir model			Freundlich model		
		q_m	B	R^2	K	n	R^2
Biochar	1	2.383	0.851	0.9895	0.743	2.023	0.9887
	5	2.014	0.228	0.9844	0.297	0.6915	09754
	9	1.998	0.118	0.9928	0.1864	0.765	0.9761
Activated Biochar	1	434.783	0.299	0.9929	51.003	1.808	0.9548
	5	222.261	0.188	0.9916	21.810	1.783	0.9163
	9	181.818	0.080	0.9912	10.728	1.573	0.9649

Table 2.3.3 Comparison of removal of tetracycline family with carbonaceous material

Adsorbent	Adsorbate	Adsorption Conditions	Max. adsorption (mg/g)	Ref.
MWCNT*	Tetracycline	Ambient temperature, 72 h, pH 5	57.13	[9]
Alkali-treated biochar	Tetracycline	303 K,24 h, neutral pH	58.8	[16]
Graphene oxide	Oxytetracycline	298 K, 24 h, pH 3.6	212.3	[19]
MWCNT	Tetracycline	298 K, 24 h, pH 4	269.2	[33]
SWCNT**	Tetracycline	Ambient temperature, 72 h, pH 5	303.1	[9]
Alkali-treated biochar	Chlortetracycline	298 K, 24 h, pH 1	434.8	This study
MWCNT: Multi-walled carbon nanotube SWCNT: Single-walled carbon nanotube				

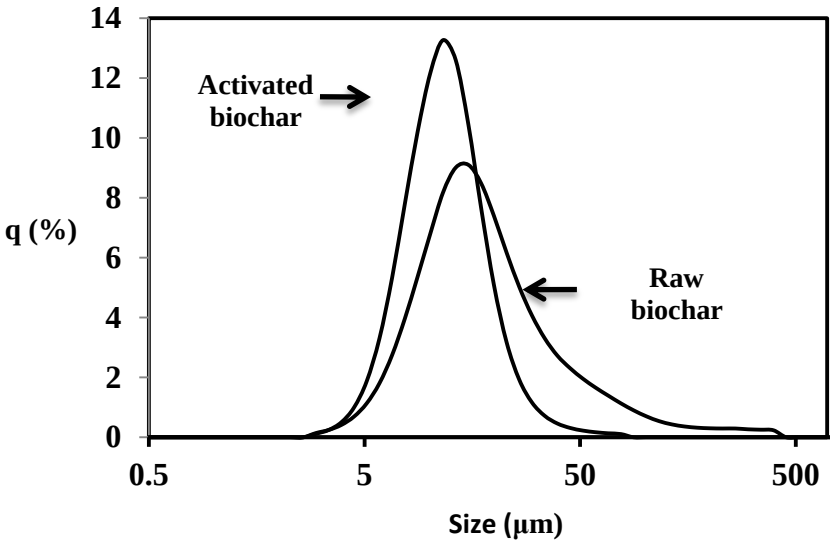

Figure 2.3.1 Particle size distribution of biochars (q: amount of each size by volume)

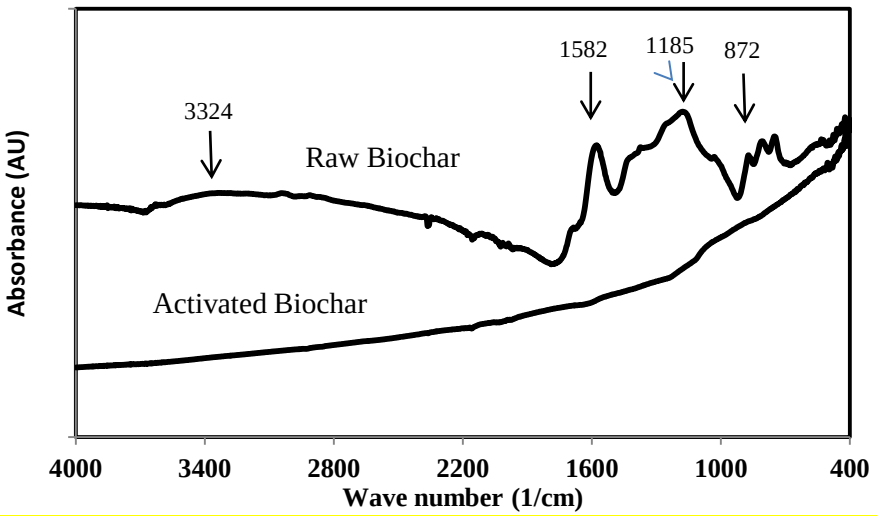

Figure 2.3.2 FTIR spectra of raw and activated biochars

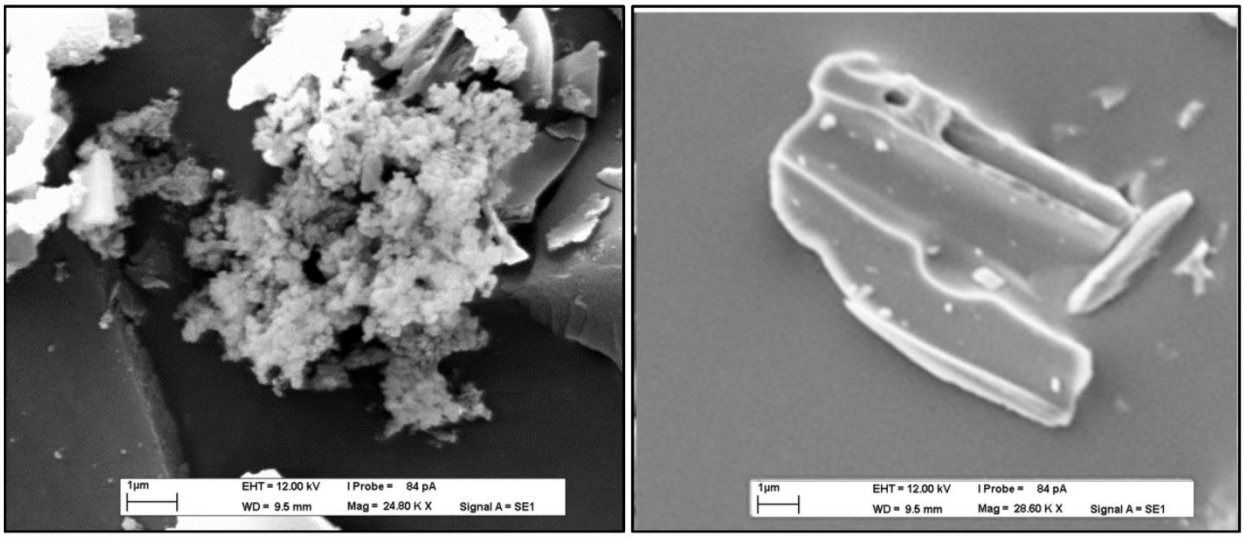

Figure 2.3.3 SEM images of raw biochar (right) and activated biochar (left)

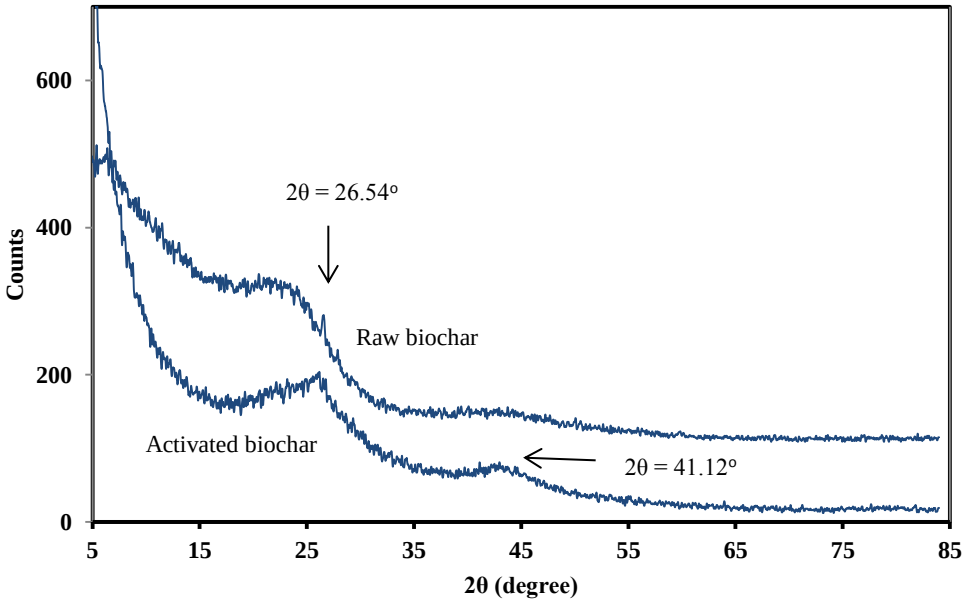

Figure 2.3.4 XRD patterns of raw and activated biochars (pattern for raw biochar was shifted by +100 counts for better discrimination)

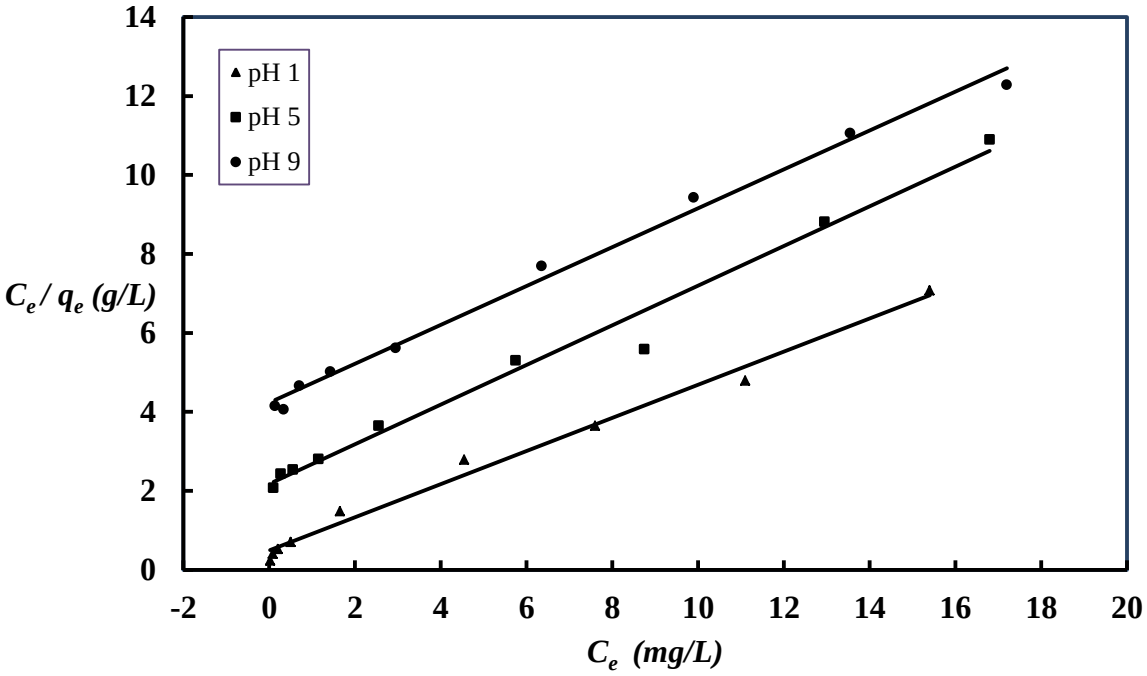

Figure 2.3.5 Adsorption isotherms of CTC on raw biochar at different pH (T = 298 K)

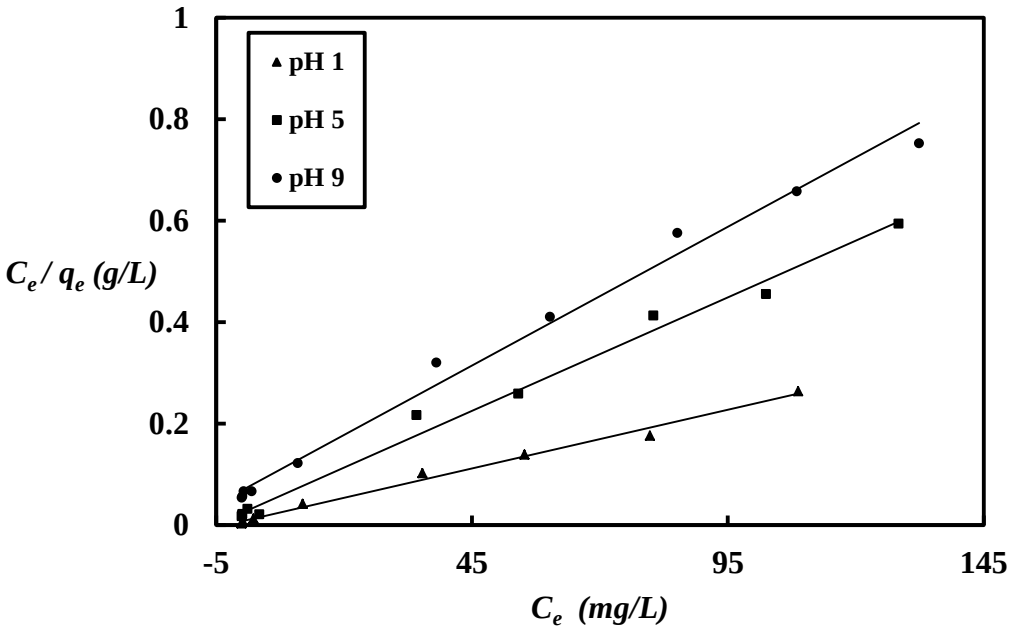

Figure 2.3.6 Adsorption isotherms of CTC on activated biochar at different pH (T = 298 K)

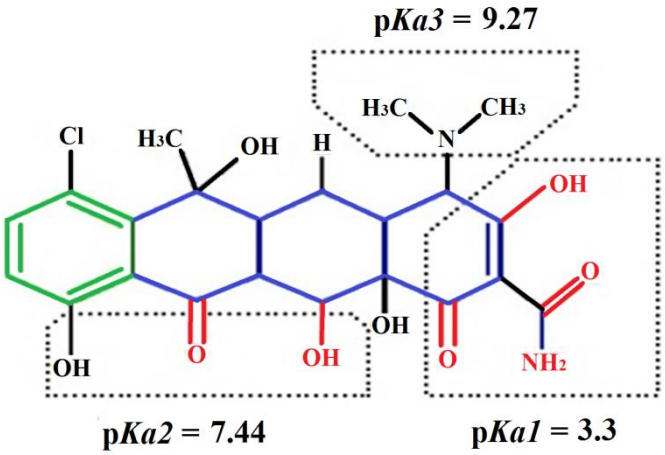

Figure 2.3.7 Three different protonation–deprotonation reactions of CTC

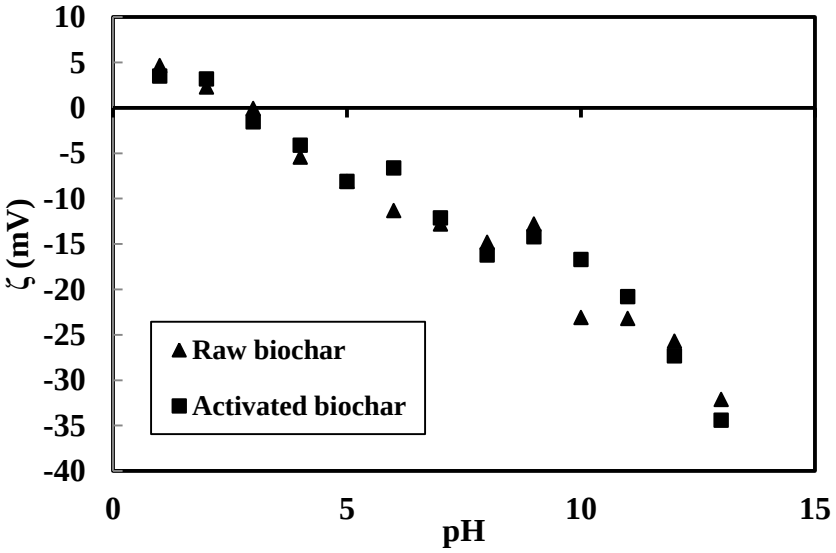

Figure 2.3.8 Zeta potential of raw and activated biochar at different pH

Part 4

Development of adsorptive membrane by confinement of activated biochar into electrospun nanofibers

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Résumé

Les membranes absorbantes ont de nombreuses applications dans l'élimination des contaminants, tels que les métaux lourds et les contaminants organiques de l'eau. Récemment, des concentrations croissantes de composés pharmaceutiques actifs, en particulier les antibiotiques, tels que la chlorotétracycline (CTC) dans l'eau potable et les eaux usées, ont soulevé des préoccupations quant à leurs effets potentiellement néfastes sur l'environnement et la santé humaine. Dans cette étude, une série de membranes à base de nanofibres (NFMs) formées de polyacrylonitrile (PAN)/biocharbon activé avec différentes charges de biocharbon (0-2%, w/w) ont été fabriquées avec la technique d'électrofilature. La morphologie et la structure des membranes fabriquées ont été analysées par microscopie électronique à balayage (SEM), par spectroscopie infrarouge à transformée de Fourier (FTIR) et par l'analyse infrarouge et thermogravimétrique. Les résultats ont montré qu'à 1,5% (w/w) de biocharbon la surface atteint la valeur maximale de 12,4 m^2/g, au-delà de cette quantité utilisée de biocharbon il y a agglomération des particules ce qui empêche leur interaction avec la matrice de nanofibres. De plus, les essais d'adsorption avec la chlorotétracycline ont montré que, à des concentrations pertinentes pour l'environnement, les MNF fabriquées par adsorption avaient le potentiel d'éliminer ces types de contaminants émergents de l'eau et des eaux usées.

Mots clés

Membrane d'adsorption, Nanofibres, Chlorotétracycline, Biocharbon

Abstract

Adsorptive membranes have many applications in removal of contaminants, such as heavy metals and organic contaminants from water. Recently, increasing concentrations of pharmaceutically active compounds, especially antibiotics, such as chlortetracycline in water and wastewater sources has raised concerns about their potentially adverse impacts on environment and human health. In this study, a series of polyacrylonitrile (PAN)/activated biochar nanofibrous membranes (NFMs) with different loadings of biochar (0-2 %, w/w) were fabricated using electrospinning method. The morphology and structure of fabricated membranes was investigated by scanning electron microscopy, Fourier transform infrared and thermogravimetric analysis. The results showed that at 1.5% of biochar loading, the surface area reached the maximum value of 12.4 m^2/g and beyond this loading value, agglomeration of particles inhibited fine interaction with nanofibrous matrix. Also, the adsorption tests using chlortetracycline showed that, at environmentally relevant concentrations, the fabricated adsorptive NFMs had a potential for removal of these types of emerging contaminants from water and wastewaters.

Keywords

Adsorptive membrane, Nanofibers, Chlortetracycline, Biochar

Introduction

Adsorptive membranes have many applications in clarification, concentration, fractionation and purification processes and offer several advantages over conventional packed bed systems including low backpressure, short residence times and high volumetric throughputs [1]. Adsorptive membranes can be fabricated using membrane precursors with affinity to target compounds, modification of membrane surface with functional groups or embedding adsorbents into membrane matrices [2]. There are many reports on functionalization or embedding adsorbents into conventional ultrafiltration membranes for immobilization of different compounds and researchers continuously have tried to improve the performance of adsorptive membranes [3-7]. After demonstration of submicron fibers produced by spinning techniques in 1990s, new horizons emerged for different fields, especially membrane processes [8]. Nanofibrous membranes (NFMs) that are produced by electrospinning can impact the performance of separation technologies due to their high surface to volume ratio, being pore size tunable and the ease of functionalization [9]. Adsorptive NFMs can be used for removal of heavy metals, organic compounds, microorganisms and biomolecules that makes them ideal candidates for environmental applications. There are many recent reports on functionalization of NFMs for removal of compounds of environmental concern. For example, Vanraes *et al.* used polyamide NFM in combination with electrical discharge to adsorb and degrade atrazine from water [10]. Kampalanonwat and Supaphol and also Neghlani *et al.* used aminated polyacrylonitrile (PAN) nanofibers to remove heavy metals from water and achieved up to 150 mg/g adsorption capacity for copper [11, 12]. Haider and Park fabricated chitosan nanofibers to take advantage of its affinity towards metallic ions, such as copper and lead [13]. In a similar study, Aliabadi et al. used PEO/Chitosan for NFM fabrication to remove nickel, cadmium, lead and copper from aqueous solutions and reported no considerable change in the adsorption capacity after five cycle [14]. Also there are reports on embedding adsorbent materials into NFMs to enhance adsorption capability of composite membranes. For example, Wu *et al.* [15], Xu *et al.* [16] and Wang *et al.* [17] used SiO_2 particles for fabrication of composite poly(vinyl alcohol), poly (acrylic acid) and PAN NFMs in order to adsorb metallic ions, malachite green and methylene blue respectively from water. Embedding carbonaceous materials, such as carbon nanotubes and graphene in

NFMs have been investigated for different applications, such as glucose sensors, hydrogen storage and enzyme immobilization [18, 19]. However, to the best of our knowledge, there is no report on fabrication of NFM containing carbonaceous adsorbents for removal of pollutants from aqueous media.

In recent years, emerging contaminants such as pharmaceutically active compounds (PhACs) and endocrine disrupters have been the focus of attention due to their long term effects on human health and environment. Chlortetracycline (CTC), a broad-spectrum antimicrobial agent, is commonly used as veterinary medicine for poultry, swine, and livestock [20]. This compound can enter the environment through the application of animal manure for agriculture, moving to rivers, ground waters and lakes by surface runoff [21]. Presence of CTC and similar compounds in water cycle has raised concerns over potential human health risks. Therefore, removal of PhACs from water sources through possible methods is necessary. Adsorption of these compounds onto different media, such as carbonaceous materials is among efficient removal methods due to feasibility, high efficiency and scalability. Using biochar derived from pinewood for adsorption is of high interest as pine trees account for the majority of forests around the world and more so in Canada. Each year, millions of them are cut and are used for industrial purpose which produces lots of biomass. Therefore, low cost and availability make pinewood biomass as a promising source for production of biochar which is also a value addition strategy for wooden residues [22]. In this study, activated pinewood biochar, with its interesting properties, was incorporated into PAN NFM for the first time to take advantages of both systems. For this purpose, different concentrations of activated biochar (0-2 %, w/w) were added into polymeric solution and the morphological, chemical and thermal properties were characterized. Also, the performance of fabricated membrane for removal of CTC from water was investigated.

Materials and Methods

Materials

PAN, with an average weight molecular weight of 1.5×10^5 (g/mol), was obtained from Scientific Polymer Product Company (USA) and used without further purification. Biochar was donated by Pyrovac Inc. (Canada) and it was derived from pine white wood (80%) purchased from Belle-Ripe in Princeville and the rest was spruce and fir

(20%). This biochar was produced at 525±1 °C under atmospheric pressure for 2 minutes and used as obtained from the reactor outlet. Sodium hydroxide and hydrogen chloride with 98% purity and N,N'-Dimethyl-Formamide (DMF) and Dimethyl-Sulfoxide (DMSO) with 99.5% purity were supplied by Fisher Scientific (USA). Chlortetracycline (CTC, purity > 97%) was purchased from Toronto Research Chemicals (TRC-Canada). HPLC grade water was prepared in the laboratory using milli-Q/Milli-Ro system (Millipore, USA).

Activation of biochar

About 20 g NaOH was dissolved in 100 mL of water and 10 g of biochar was added to this solution. The mixture was stirred with a magnetic stirrer (150 rpm) at room temperature for 2 h and it was then dried at 80±1 °C for 24 h. The prepared sample was placed in quartz tube to be heated in a horizontal furnace under nitrogen flow of 200 mL/min. The temperature of the quartz tube was increased to 800±1 °C at 10 °C/min, and held at this temperature for 2 h before cooling down. Later, the product was washed with water, and sodium hydroxide was neutralized with 0.1 M HCl. Finally, for removal of sodium salt, the product was washed with water and dried at 60±1 °C for 24 h.

Preparation of PAN-biochar membrane

A schematic of electrospinning process for preparation of NFMs is illustrated in Figure 2.4.1. In brief, PAN was dissolved in DMF/DMSO solvent mixture (9:1 v/v) at the concentration of 10 Wt% and stirred until a clear solution was obtained. Activated biochar at the ratios of 0, 0.5, 1, 1.5 and 2 % (w/w) of the polymer was added to the solution and the mixture was stirred for 48 h. Nanofibrous membranes fabricated via electrospinning process under ambient conditions (T=25 °C, RH=32%) and with a rotary drum collector (length= 25 cm, diameter= 10 cm). The flow rate, electric field strength and collector rotational speed were 1.2 mL/h, 1.1 KV/cm and 400 rpm respectively. Also the needle gauge was 22 and the distance of needle tip to the center of collecting drum was 18 cm. The electrospinning continued for 12 h and the deposited mats were soaked in methanol for 60 min to remove residual solvents. The soaked mats were washed with distilled water several times and dried for 10 h at 50±1 °C. To determine the amount of residual solvent, samples for thermogravimetric analysis were not subjected to methanol and heat treatment.

Characterization of fabricated NFMs

The surface morphology of the fabricated membranes was examined using a JSM-840A (JEOL, Japan) scanning electron microscope (SEM) at acceleration voltage of 10 kV. For this analysis, small amounts of the samples were coated with a thin layer of gold-palladium alloy using a SPI Module sputter coater. Fourier transform infrared-attenuated total reflectance (FTIR-ATR) spectra were recorded on a Nicolet iS50 spectrometer (Thermo scientific, USA) at 0.04 cm^{-1} resolution and in the range of 400-4000 cm^{-1}. Brunauer–Emmett–Teller (BET) specific surface areas were obtained from the N_2 adsorption isotherms recorded at 77 K using Autsorb-1 gas analyzer (Quantachrome Instruments, USA) at the relative pressure range from 0.05 to 1. Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) were performed using a STA 449C (Netzsch, Germany) thermogravimetric analyzer. Samples of 10 mg were heated from ambient temperature to 400±1 $^{\circ}C$ at a constant rate of 10 $^{\circ}C$/min under nitrogen with a flow rate of 20 mL/min.

Adsorption properties of fabricated NFMs

The capability of fabricated membrane for adsorbing micropollutants was studied on the sample with highest surface area (NFM-1.5%). Adsorption test was performed on a 15*15 cm^2 stainless steel (SS-316) membrane test module connected to a peristaltic pump. A solution with CTC concentration of 200 ppb in milli-Q water was pumped at a flux of 3 $mL/cm^2.h$ into the test setup in dead-end configuration. Samples for measuring CTC concentration were taken at 1 liter interval for 40 liters of total passed volume. CTC concentrations were estimated by using Laser Diode Thermal Desorption (LDTD) (Phytronix technologies, Canada) coupled with a LCQ Duo ion trap tandem mass spectrometer (Thermo Finnigan, USA). The daughter ions identified for CTC in LDTD were 464 and 444 Da. The detailed method was explained elsewhere by Pulicharla *et al.* [23].

Results and Discussion

Nanofiber morphology

The SEM micrographs of fabricated nanofibrous membrane with different contents of activated biochar are illustrated in Figure 2.4.2 Generally, the nanofibers were uniform in shape and size and the moderate speed of rotational drum led to

formation of randomly-oriented fibers which is in favor of membrane fabrications due to required mechanical strength in all directions. Also the entrapment of biochar particles among fibers was perfect since after washing several times with methanol, no leaching was observed visually.

The distribution of fibers' diameter was analyzed using Image-J software with Diameter-J module and the average diameters are listed in Table 2.4.1. According to Table 2.4.1, the average diameter of fibers was increased from 242 nm for NFM-0% to 316 nm for NFM-2% as the concentration of biochar increased which can be attributed to the increasing of solution viscosity as a result of adding biochar. Increasing viscosity of the solution enhances the resistance of the solution against being stretched by the charges on the jet and therefore increases fiber diameter [24]. Generally, increasing solution viscosity through addition of more polymer to the solution causes the jet to be more stable and reduce bead formation [25]. However, in this case, viscosity was elevated due to increasing the concentration of biochar particles which simultaneously disturbed the jet and blocked the needle several times. The particle size of activated biochar was in the range of 5-20 micrometers, however there were also few particles with more than tens of micrometer in size. On the other hand, the inner diameter of employed needle was around 400 micrometer. Consequently, increasing the content of biochar in polymeric solution will increase the chance of agglomerating big particles in the needle which may lead to disturbing the jet, clogging the needle and formation of biochar aggregates in NFMs. Observing large beads in NFM-2% and also the trend in the BET surface areas of fabricated samples indicated that increasing biochar more than 1.5% disturbed the uniformity of fibers and decreased the surface area. A similar behavior was reported by Ji *et al.*, as they tried to add silica to PAN nanofibers up to 5 % and observed beads and particle aggregates for more than 2% silica loading [26].

BET surface area

According to Table 2.4.1, the enhancement of specific surface area of electrospun NFMs with increasing biochar content from 0% to 1.5% (w/w) confirmed the entrapment of biochar particles without adverse effect on fibers structure. However, increasing biochar content beyond 1.5% caused formation of large beads which possess much lower surface to volume ratio compared to cylindrical geometry and therefore reduced specific surface area. The statistical analysis confirmed that

addition of activated biochar was a significant contributor (p-value=0.040, F factor=11.56) to enhancement of specific surface area.

The nitrogen adsorption isotherms at 77 K against relative pressure and differential and cumulative pore surface area against pore width for NFM-0% and NFM-1.5% are plotted in Figure 2.4.3 and Figure 2.4.4 The adsorption isotherms indicated that raw NFM-0% had significantly lower N_2 adsorption capacity than NFM-1.5% so that they had a total pore volume of 0.024 and 0.128 mL/g at 0.99 P/P_o, respectively.

According to the differential surface area curves in Figure 2.4.4, NFM-0% had pores in two size ranges of 1.5-10 nm and 38-46 nm and the most probable pore size was 2 nm. Also, NFM-1.5% had pores in two size ranges of 2.5-10 nm and 20-50 nm and the most probable pore size was 3.2 nm. The occurrence of pore diameter in the range of 1-10 nm suggested that both samples had pores inside the single fibers. Both samples showed pores higher than 10 nm which corresponded to the interspace between the fibers [27]. Furthermore, cumulative surface area curves showed that in both samples, around 50 % of the surface area corresponded to the pores smaller than 10 nm and 50 % corresponded to pores larger than 10 nm.

FTIR spectroscopy

In Figure 2.4.5(a) and Figure 2.4.5(b), the FTIR spectra of pure PAN powder, activated biochar, NFM-0% and NFM-1.5% are illustrated. Raw biochar (Data are not shown) had two peaks at around 1185 cm^{-1} and 1580 cm^{-1} which correspond to C-H and C=C in aromatic rings [28]. However, activated biochar showed no characteristic peak which indicates that all of the functional groups left the surface during activation.

The PAN molecule consists of nitrile (CN) and methylene (CH2) in a linear arrangement. Therefore, the strong peak at 2243 cm^{-1} must be assigned to nitrile and the peaks at 1072 cm^{-1}, 1453 and 2940 must be representative of methylene groups [26, 29]. Other researchers reported peaks at around 1700 cm^{-1} which corresponded to the carbonyl groups of residual DMF solvent [26]. In this study, due to methanol washing step, no additional peaks were observed. The characteristic peaks of methylene and nitrile groups were observed in pure PAN powder, NFM-0% and NFM-1.5% at similar wave numbers. However, the pattern for NFM-1.5% was affected by activated biochar so that it looks like a combination of patterns for pure PAN powder and activated biochar.

Thermal behavior of electrospun PAN nanofibers

In DSC process, if PAN is heated in presence of oxygen, it begins to degrade near its melting point through an exothermic reaction which can obscure its melting endotherm. Therefore, the melting point cannot be observed for PAN. However, if DSC is conducted in N_2 atmosphere, the degradation exotherm is observed [30, 31]. In Figure 2.4.6 the DSC thermogram of pure PAN powder, NFM-0% and NFM-1.5% are illustrated. The sharp peaks located at 291.48 °C, 289.15 °C and 303.67 °C are attributed to the nucleophilic attack at a nitrile and cyclization to an extended conjugated structure [26, 30]. The shift of exothermic peak to lower temperature from pure PAN powder to PAN nanofiber (NFM-0%) suggests that cyclization is more easily initiated due to molecular rearrangement during electrospinning which resulted in improved orientation in molecular chains. On the other hand, the shift to higher temperature from NFM-0% to NFM-1.5% confirmed the inhibitory effect of confined particles [26].

Similarly, Figure 2.4.7 illustrated the TGA thermograms of pure PAN powder, NFM-0% and NFM-1.5%. The onset temperature of these samples were 288.9 °C, 286.2 °C and 301.2, respectively °C that are in the same order as their exothermic peaks in DSC thermograms. The shifting of onset temperature of electrospun membrane to higher values indicated the strong interfacial interactions between activated biochar and PAN nanofibers.. Also, there was around 8% weight reduction after temperature exceeded 80 °C for NFM-0% and NFM-1.5% due to evaporation of residual solvents in nanofibers.

Adsorption properties

Figure 2.4.8 illustrated the performance curve of fabricated adsorptive membrane with highest surface area (NFM-1.5%) for removal of CTC from aqueous media. CTC is one of the most hydrophilic veterinary pharmaceutical compounds with Log Kow=-0.52 [20]. The physicochemical properties of CTC favor its mobility in the environment [32]. In fact, if the adsorptive membrane succeed in removal of CTC, it would be possible to apply for removal of other micropollutants. The CTC concentration in feed stream was set to 200 ppb since the reported values for the influent and effluents of wastewater treatment plants ranged from 1.2 ppb in municipal wastewater to 32 mg/L in pharmaceutical wastewater effluent [32-34].

Therefore, the studied concentrations were reasonably in the relevant environmental concentration range. According to the performance curve in Figure 2.4.8, more than 95% of the spiked CTC was removed for the first 22 liters that passed through the membrane. At first, the entrapped biochar particles are fresh with all their adsorption sites and essentially a small part of the target compound can escape. As time passes, some of the adsorption sites are occupied and the concentration in the effluent starts to rise until reaching the same concentration as inlet. From the rising point in Figure 2.4.8 it is implied that after passing around 25 liters, the membrane should be regenerated. In our previous research, we observed that the adsorption capacity of activated pinewood biochar towards CTC was up to 434 mg/g which was comparable with graphene oxide and carbon nanotubes [22]. However, in this research, due to the low loading of activated biochar onto membrane, the adsorption capacity was around 6.3 mg/g of membrane. Therefore, further research is still needed to increase the adsorption capacity through increased adsorbent loading or adsorbent specific surface area. Also, working on other applications of these adsorptive membranes, such as immobilization of enzyme would be of interest due to their capability to enhance enzyme loading.

Conclusion

Adsorptive nanofibrous membranes were fabricated through electrospinning of PAN solution containing 0-2% activated biochar. SEM micrographs showed that biochar particles entrapped well among nanofibers with the diameters in the range of 100-400 nm. The shift in endothermic peak and onset temperature of NFM-1.5% compared to NFM-0% and pure PAN indicated interactions between polymer and activated biochar. The results of BET sorption test on fabricated membranes showed that at 1.5% biochar loading, maximum surface area occurred which was due to aggregation of particles at higher concentrations and also formation of large beads which reduce surface to volume ratio. Adsorption test in continuous mode indicated that the fabricated membrane can efficiently remove micropollutants, such as CTC from aqueous media. This shows the promising nature of these kinds of systems in removal of emerging contaminants from aqueous environmental streams. However, further research is needed to increase the adsorption capacity of fabricated membranes to compete with commercial adsorbents.

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Table 2.4.1 Average diameter and BET surface area of fabricated nanofibrous membranes

Sample ID	Activated biochar concentration (%,w/w)	Average diameter (nm)	BET surface area (m^2/g)
NFM-0%	0	242	5.45
NFM-0.5%	0.5	257	5.84
NFM-1%	1	278	9.55
NFM-1.5%	1.5	293	12.52
NFM-2%	2	316	10.87
Raw biochar			14.86
Activated biochar			853.95
Raw PAN			1.14

Legends:
NFM-0%: Nanofibrous membrane with 0% biochar
PAN: Polyacrylonitrile
BET: Brunauer–Emmett–Teller

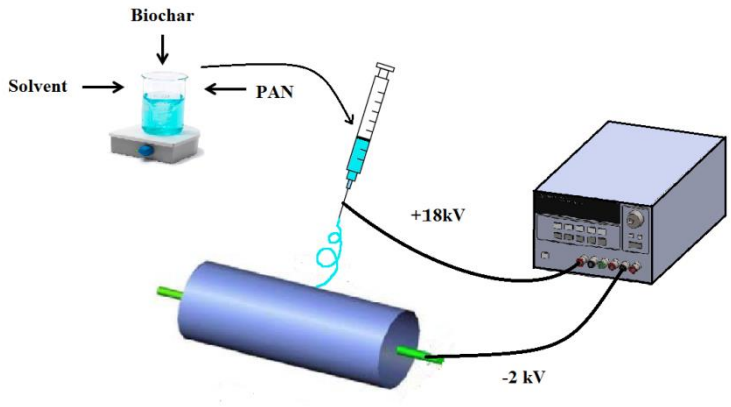

Figure 2.4.1 Schematic of Electrospinning system

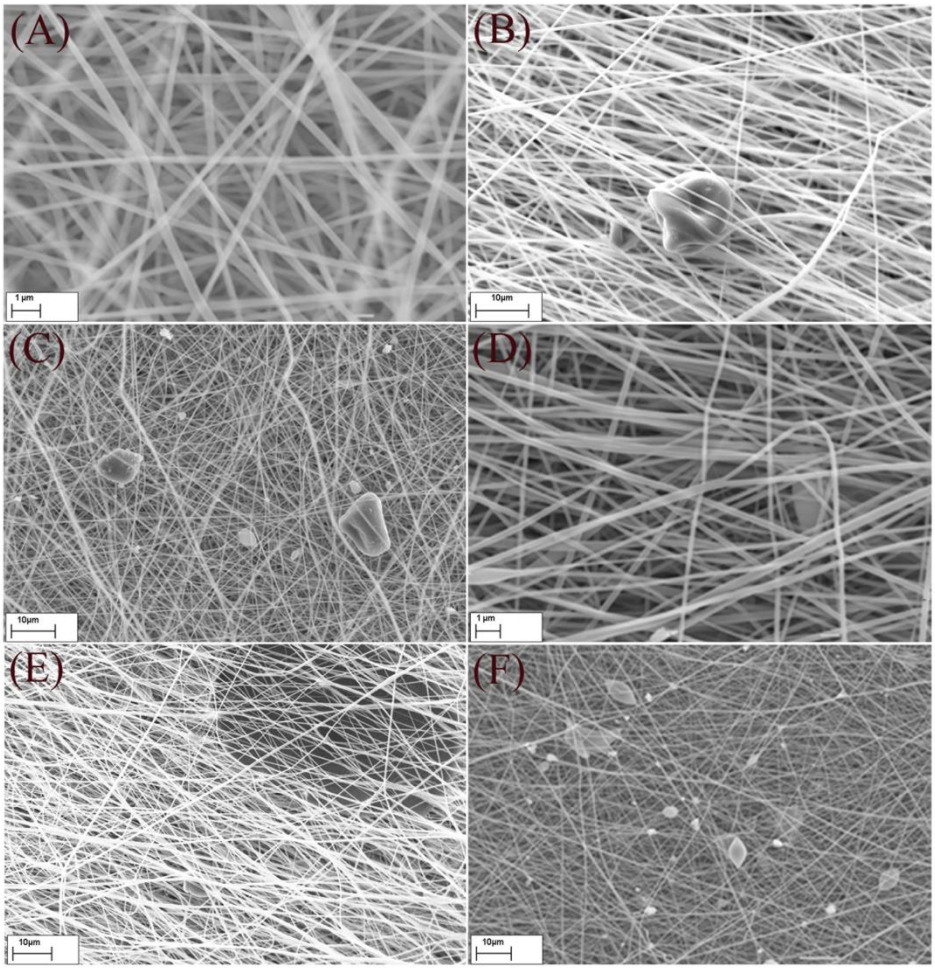

Figure 2.4.2 SEM micrographs of NFMs, a) smooth and randomly-oriented fibers in NFM-0%, b & c) entrapment of biochar among fibers in NFM-0.5% and NFM-1%, d & e) NFM-1.5% at different magnifications and F) formation of beads in NFM-2%

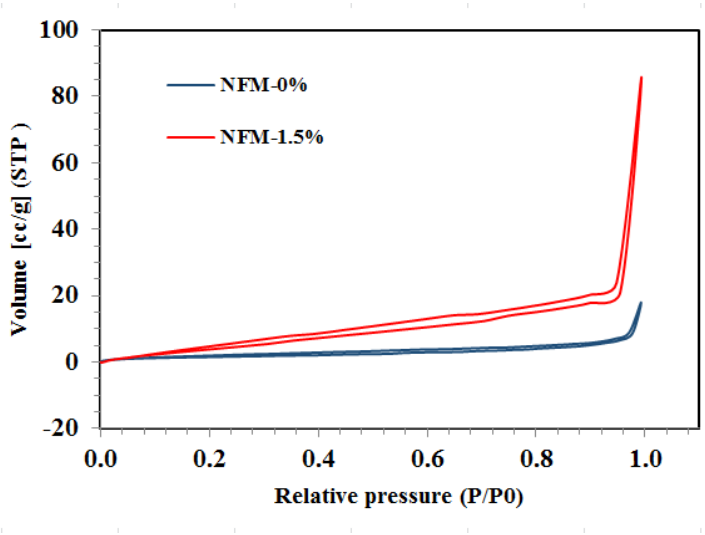

Figure 2.4.3 Nitrogen adsorption isotherms at 77 K for NFM-0% and NFM-1.5% (NFM: Nanofibrous membrane)

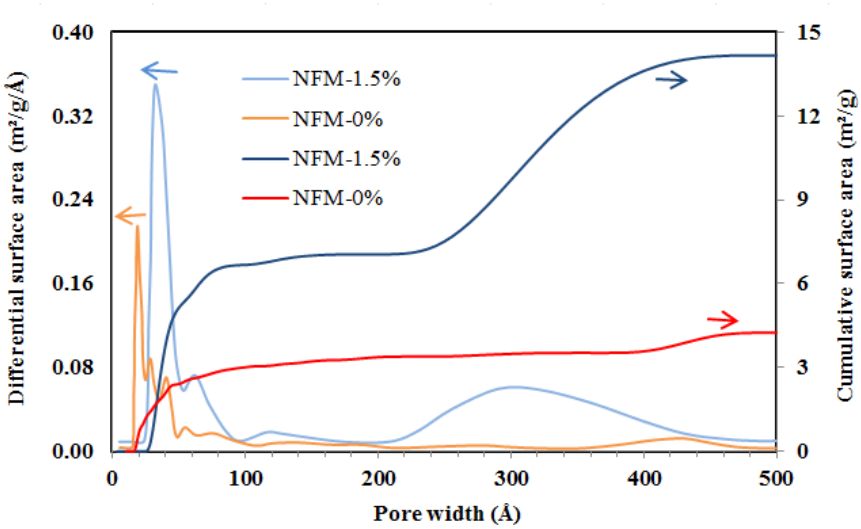

Figure 2.4.4 Cumulative surface area versus pore width for NFM-0% and NFM-1.5% (NFM: Nanofibrous membrane)

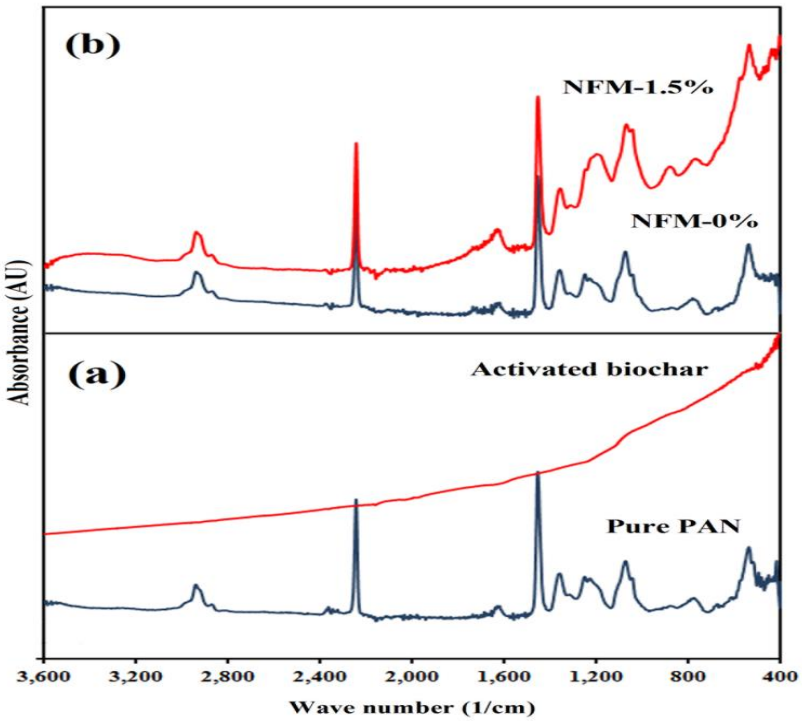

Figure 2.4.5 FTIR spectra of (a) pure PAN powder and activated biochar & (b) NFM-0% and NFM-1.5% (NFM: Nanofibrous membrane)

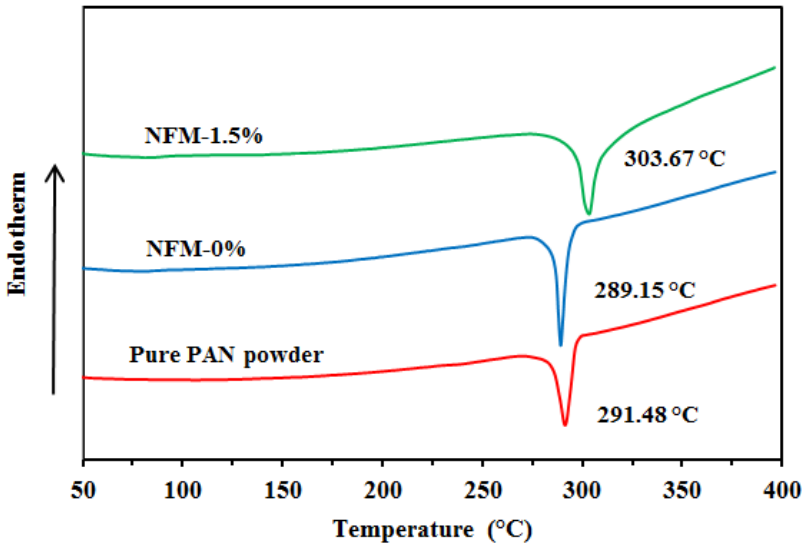

Figure 2.4.6 DSC thermograms for pure PAN powder, NFM-0% and NFM-1.5% (NFM: Nanofibrous membrane)

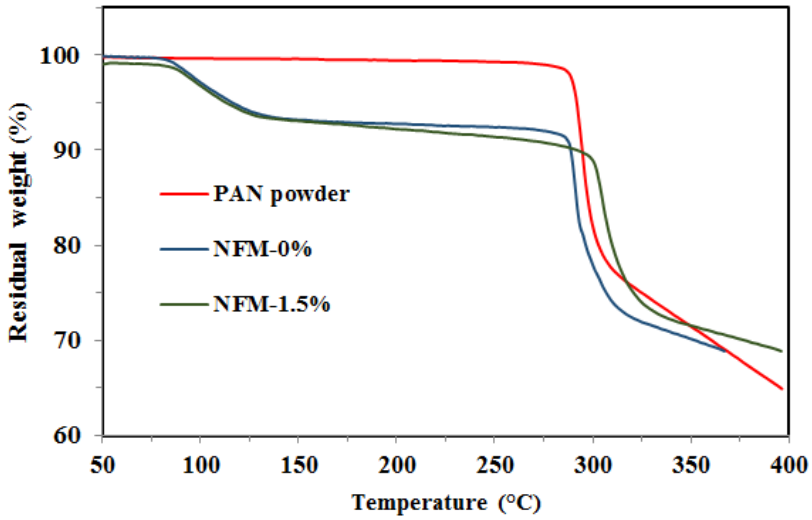

Figure 2.4.7 TGA thermograms for pure PAN powder, NFM-0% and NFM-1.5% (NFM: Nanofibrous membrane)

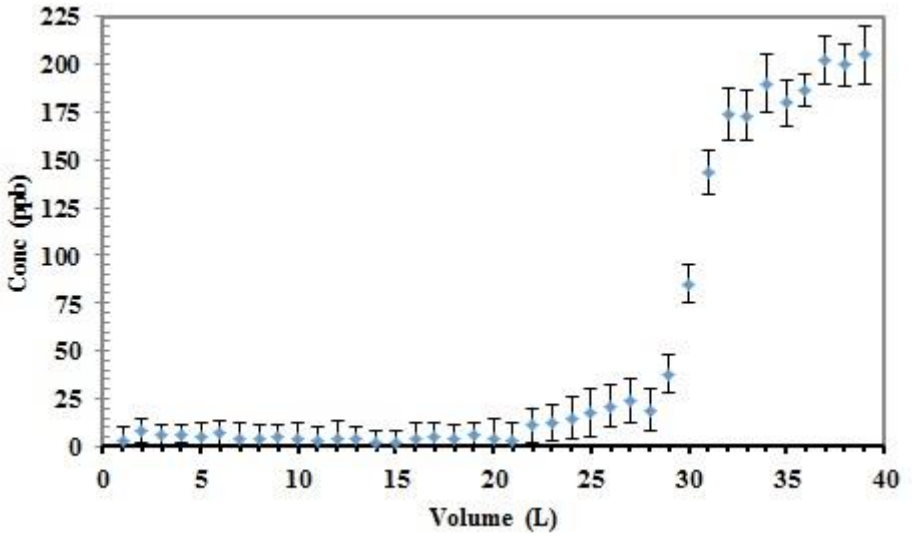

Figure 2.4.8 Performance of adsorptive membrane for removal of chlortetracycline from aqueous media

CHAPTER 3

Application of BIMeMS for CTC removal

Part 1

Biodegradation of Chlortetracycline by *Trametes versicolor* produced laccase: By-products Identification

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Résumé

La chlorotétracycline (CTC) est l'une des antibiotiques les plus utilisés dans les fermes d'élevage, par conséquent il est couramment présent dans les eaux usées et les eaux de surface. Par conséquent, le développement de nouvelles technologies de traitement est nécessaire pour éviter les effets néfastes sur l'homme et l'environnement. L'utilisation de la laccase produite à partir de champignons pourriture blanche est une solution intéressante pour le traitement de l'eau et des eaux usées contaminées par des micropolluants. Dans ce travail, la laccase a été produite par *Trametes versicolor*. La planification des expériences a été réalisée en utilisant un plan central composite (CCD), la méthodologie de la réponse de surface (RSM) a été par la suite utilisée pour étudier l'effet de différents paramètres tels que pH, température, concentration du médiateur et concentration d'enzyme sur la biodégradation de la CTC dans la phase aqueuse.

Un modèle quadratique a été ajusté pour exprimer les effets de chaque paramètre, y compris les termes quadratique linéaire et d'interaction. Les valeurs pour R^2 et R^2 ajusté étaient respectivement de 0,85 et 0,70, ce qui indique que le modèle est raisonnablement bon pour les applications pratiques. Parmi les paramètres examinés, les termes linéaires de température et de pH ont eu les effets les plus importants. Nous avons observé que l'efficacité de dégradation maximale d'environ 95 % peut être atteinte à un pH de 5,2, une température de 35,5 °C, une concentration enzymatique 62,3 unités/L et une concentration de médiateur de 10,9 µM. De plus, trois structures ont été proposées pour la biodégradation des sous-produits de la CTC à l'aide de données de spectroscopie de masse, le test de dépistage des œstrogènes de levure a montré que ces sous-produits ne sont pas toxiques.

Mots clés

Chlorotétracycline, biodégradation, laccase, sous-produits

Abstract

Chlortetracycline (CTC) is among widely used antibiotics in animal farms commonly found in wastewater and surface water and therefore development of new treatment technologies is necessary to avoid adverse effects on human and environment. White-rot fungi laccase is among attractive candidates for safe treatment of water and wastewater contaminated with micropollutants. In this work, *Trametes versicolor* laccase was produced and response surface methodology with a central composite design was employed to investigate the effects of different parameters including pH, temperature, mediator concentration and enzyme concentration on biodegradation of CTC in aqueous phase. A quadratic model was fitted to express the effects of each parameters including quadratic, linear and interaction terms. The values for R^2 and adjusted R^2 were 0.85 and 0.70, respectively indicating a reasonably good model for practical applications. Among examined parameters, linear terms of temperature and pH had the largest effects. It was observed that the maximum degradation efficiency of around 95% can be achieved at pH, temperature, enzyme concentration and mediator concentrations of 5.2, 35.5 °C, 62.3 U/L and 10.9 µM, respectively. Also, three structures were proposed for the by-products of CTC biodegradation using mass spectroscopy data and the yeast estrogen screen test showed that these by-products are not toxic.

Keywords:

Chlortetracycline, Biodegradation, Laccase, By-products

Introduction

Antibiotics have been widely used to control and prevent the diseases in animal farms. However, high percentage of prescribed antibiotics are excreted since they are not completely metabolized in animal [1]. Chlortetracycline (CTC) is one of these veterinary antibiotics which is utilized for prevention of diseases and promotion of growth rate in animal farms [2]. The excess amount of this pharmaceutically active compound end up in rivers, ground waters and lakes through feces and urine [3]. The continuous discharge of pharmaceutically active compounds (PhACs) into water sources cause their presence for long period of time which has raised concerns over potential ecological and human health effects. Therefore, the removal of these compounds from water and wastewaters is crucial to avoid consequent problems such as antibiotic resistance [4, 5].

Recently, different removal methods, such as filtration, adsorption, ozonation, chlorination, UV irradiation and biological approaches have been investigated for removal of PhACs from aqueous media [6-14]. Among them, using oxidoreductase enzymes that are capable of breaking organic molecules is of high interest for wastewater treatment due to their low environmental footprint and the fact that they are non-specific towards target compounds [5]. Laccase is a member of ligninolytic enzymes known for their catalysis activity in oxidation of various compounds and can be easily produced through fermentation of a broad spectrum of microorganisms, such as white-rot fungi on lignocellulose-rich biomass, such as wood chips. Numerous studies reported use of laccase for degradation of different PhACs, such as ibuprofen, diclofenac, naproxen and sulfamethoxazole [15-21]. Also, there is some research on the performance of laccase for degradation of CTC or other members of tetracycline family. For example, Suda *et al.* studied the effect of using 1-hydroxybenzotriazole as mediator for degradation of CTC and reported 48% and 100% degradation in presence and absence of mediator after 4 hours of reaction [22]. Similarly, Ding *et al.* reported >90% degradation efficiency in degradation of CTC with laccase in presence of syringaldehyde and 1-hydroxybenzotriazole as mediator after 3 hours of reaction [23]. In another study, Cazes *et al.* reported that free laccase can degrade up to 30% of tetracycline after 24 hours [24].

Although the capability of laccase for degradation of tetracyclines have been verified by other researchers, high concentrations of target compounds (>10 mg/L) were

used that do not match with their real concentrations in surface water and municipal wastewater. Another deficiency in the literature is using pure laccase which is very costly while it is possible to use the crude laccase obtained from fermentation of cost-effective of substrate by white-rot fungi. Moreover, the toxicity of CTC degradation by-products has not been evaluated as yet. Also, the influences of operational parameters have not been statistically investigated in order to develop a reliable and efficient treatment method. In this work, the effects of four parameters including enzyme concentration, mediator concentration, pH and temperature on the biodegradation rate of laccase for CTC were studied using response surface methodology (RSM). This statistical approach is widely used for modelling and optimization of multivariable processes and in this research, we employed a central composite experimental design to provide raw rata for analysis of variance (ANOVA).

Materials and methods

Chemicals

CTC (purity > 97%) was purchased from Toronto Research Chemicals (TRC-Canada). 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was purchased from Sigma-Aldrich (Oakville, Canada). Tween 80 and methanol were purchased from Fisher scientific (Ottawa, Canada). HPLC grade water was prepared in the laboratory using milli-Q/Milli-Ro system (Millipore, USA).

Preparation of inoculum

Fungus, *Trametes versicolor* (ATCC 20869) was grown aerobically in liquid medium by inoculating three 50 mL flasks containing potato dextrose broth (PDB) using lyophilized powder and then incubating the flasks in orbital shaker at 30±1 °C and 150 rpm for 7 days. Later, 100 µL of PDB medium was inoculated in potato dextrose agar (PDA) plates at 30±1 °C for 9 days and the plates were stored at 4±1 °C, prior to use.

Solid-state fermentation

Apple pomace (Vergers Paul Jodoin Inc., Quebec, Canada) was used as solid substrate for the production of laccase by *T. Versicolor.* About 40 grams of solid substrate (70% (w/w) of moisture and pH=4.5), along with 0.5% v/w Tween 80 in a

500 mL flasks were thoroughly mixed and autoclaved at 121±1 °C for 30 min. Later, each flask was inoculated with the biomass content of one petri plate and incubated in a static incubator at 30±1 °C for 15 days. The biomass from petri plates was removed using a spatula, mixed with three mL of sterilized water and transferred using a pipette.

Enzyme extraction and assay

One gram of fermented sample was mixed with 20 mL of 50 mM sodium phosphate buffer (pH=6.5), agitated at 35±1 °C for 1 h and then centrifuged at 7,000 × g for 30 min. The relative laccase activity of collected supernatant was analyzed spectrophotometrically at pH=4.5 and 45±1 °C monitoring the oxidation of ABTS at 420 nm (ε_{420} = 36,000 M^{-1} cm^{-1}) [25]. Each unit of laccase was defined as the amount of laccase required to oxidize one micromole of ABTS per minute under assay condition. Enzyme assay was replicated twice and the average value was used for further calculations.

Experimental design

Central composite design was used to investigate the degradation efficiency of CTC as a function of temperature (°C), ABTS concentration (µM), enzyme concentration (U/L) and pH using response surface methodology (RSM). Independent parameters and corresponding levels are listed in Table 3.1.1. Design-Expert®-7 software (Stat-Ease Inc., Minneapolis, USA) was employed to construct the experimental design which resulted in 30 experiments with 6 replicates in the center (Table 3.1.2). All the samples were prepared as 10 mL, collected after 8 h and analyzed as described below in Table 3.1.2.

Degradation experiments

For each experiment listed in Table 3.1.2, the calculated amounts of ABTS, enzyme and CTC were mixed at desired pH and the volumes were adjusted to 10 mL. The CTC concentration in all experiments was fixed to 1 mg/L. All the flasks were then incubated at specified temperature and 150 rpm. The degradation efficiency (%) calculated for each run was used as the response variable. By taking the response surface quadratic model for interactions into consideration, the relationship between the response variable and the studied parameters was determined. Considering the

significance level of 0.05, model terms with p-values higher than 0.05 were not included. The best fit of the quadratic models was evaluated from R^2 values and final equation was obtained from the analysis of variance (ANOVA).

Quantification of CTC

After 8 h of reaction, 500 µL of each sample was diluted with methanol to 1 mL. Later, 100 µL of the obtained solution was mixed with 20 µL of 200 ppb sulfamethazine (as internal standard) and 80 µL of 1 mM ethylene diamine tetra-acetic acid. CTC residual concentrations were estimated using Laser Diode Thermal Desorption (LDTD) (Phytronix technologies, Canada) coupled with a LCQ Duo ion trap tandem mass spectrometer (Thermo Finnigan, USA). The daughter ions identified for CTC in mass spectrum were 462 and 444 Da. The detailed method was explained elsewhere [26]. The degradation efficiency for each experiment was calculated using the following equation 1:

$$Degradation\ Efficiency\ (\%) = 100 * \frac{C_i - C_r}{C_i} \quad (1)$$

Where C_i and C_r are initial and residual concentration of CTC. For each sample, two measurements were performed.

By-product identification

For identification of by-products, the biodegradation reaction was performed under optimum conditions and then the liquid sample was analyzed to find the produced compounds using the Laser Diode Thermal Desorption coupled with tandem mass spectroscopy (LDTD-MS-MS) in the m/z range of 10-1000. Also, the optimum biodegradation reaction was replicated in a 100 mL serum bottle which was already purged with CO_2–free air. After 8 h of reaction, 3 mL of headspace gas was injected directly into a Thermo-Finnigan MAT 4500 gas chromatography- mass spectroscopy (GC–MS) to analyze the gaseous products.

Toxicity assessment of the CTC by-products

The Yeast Estrogen Screen (YES) assay described by Routledge and Sumpter was employed to measure estrogenic activity of CTC and its degradation by-products. All samples were sterilized at 121±1 °C for 30 min before the assay. The procedure for determination of the total estrogenic activity was carried out by serial dilution of 17β-

estradiol in ethanol as standard across 12 wells in a 96-well plate (Costar Brand, NY, USA). In the first row of the plate, 10 µL of ethanol was placed in each well as blank. In the second and third rows (in duplicate), 10 µL 17β-estradiol with different concentrations (0, 0.5 ng/L, 5 ng/L,…, 5 mg/L, 50 mg/L) was placed in wells. In the fourth-row, CTC sample at 1 mg/L and its degradation by-products (with and without ABTS) were placed in quadruplicate. Later, the plate was conditioned under laminar flow for complete drying of the samples. Aliquots (200 µL) of the seeded assay medium containing chlorophenol red-β-D-galactopyranoside (CPRG) and the yeast (hER-transfected recombinant yeast) were dispensed into samples. The sealed plate with parafilm was incubated for 3 days at 32±1 °C. The color development of the samples was checked periodically for qualitative assessment of toxicity [27].

Result and Discussion

Effects of parameters

The effect of different parameters and their interactions on the degradation of CTC was studied. The central composite design considered very low (-2), low (-1), center (0), high (+1) and very high (+2) levels for the selected parameters. The levels for all experiments, the corresponding results and the predicted results for each experiment are listed in Table 3.1.2. The results were analyzed to develop a 2^{nd}-order equation including quadratic, linear and interaction terms between the variables. The ANOVA results are listed in Table 3.1.3 and the equation obtained based on this statistical analysis considering significant terms (p value < 0.05) is given below in Equation 2 in terms of actual factors:

Degradation (%) = -525.17+24.74*Temp.+11.18*ABTS Conc.+18.98*pH+1.44*Enz. Conc.-0.07*Temp.*Enz. Conc. -0.36*Temp.^{2}-0.40*ABTS Conc.^{2}-6.83* pH^2 (2)

According to ANOVA table, the critical F values at 0.05 confidence level are 2.42 for the whole model, 3.05 for linear and square effects and 2.79 for interaction effect. Therefore, by comparing the F values with corresponding critical values, one can conclude that the model, linear effects and square effects are significant and interaction effects are insignificant ($p<0.05$). However the detailed ANOVA results showed that among six possible interaction terms, the interactions of temperature and enzyme concentration were significant (p value=0.049) in biodegradation of

CTC. In terms of model accuracy, the values for R^2 and adjusted R^2 were 0.85 and 0.70, respectively indicating a reasonably good model for practical applications.

The response surface for the effects of temperature and the enzyme concentration is depicted in Figure 3.1.1 (keeping ABTS concentration at 14 µM and pH at 6) and as expected, the lowest degradation rate (<10 %) occurred at low temperature and enzyme concentrations. At any enzyme concentration, increasing temperature from 25° C to 45° C exponentially improved the degradation efficiency before reaching a maximum at around 36° C. Increasing the temperature facilitates the supply of activation energy of reactions and simultaneously increases collisions between reactants. This behavior is similar to the trend observed by Liao *et al.* for the degradation of CTC using microbiota [28]. As Sigoillot *et al.* indicated, the activation energy (Ea) of laccase reaction with ABTS is up to 42 kJ/mol and therefore according to Arrhenius equation (k=exp[-Ea/RT]), the rate constant of reaction at 40 °C is expected to be 3 and 4 times higher than those at 20 °C and 10 °C, respectively [29]. Further increasing of temperature may also result in denaturation of enzyme and reduce the degradation efficiency. Tavares et al. found that the optimum temperature for degradation of textile dyes using laccase-ABTS system is 35-40 °C while Weng et al. reported 40-60 °C as optimum temperature for degradation of sulfonamide antibiotics using laccase-ABTS system [1, 30]. Therefore, the optimum temperature for degradation depends not only on enzyme and mediator, but also on the nature of substrate or the target compound. On the other hand, at low temperatures (25-35 °C), increasing enzyme concentration from 20 to 100 U/L resulted in linear increase of degradation efficiency which is due to the higher collision frequency of CTC and ABTS molecules with enzyme. But at high temperature, increasing the enzyme concentration slightly changed the degradation rate. It can be due to the partial denaturation of enzyme molecules at higher temperatures. In this research, cost-effective crude laccase was employed for degradation of CTC instead of purified laccase which is costly and compromises the feasibility of the process since recovering and keeping the free enzyme stable is difficult. Furthermore, there are natural mediators (mostly, phenolic compounds) in the crude laccase obtained from apple pomace which can enhance the effectiveness of the treatment [31, 32].

Figure 3.1.2 illustrates the effects of temperature and pH on degradation of CTC, keeping ABTS and enzyme concentration at 10 µM and 60 U/L, respectively. The

projection of red circle on gray plane showed that in the pH range of 5-6 and temperature range of 35-40 °C, the CTC degradation was more than 90%. The enzymes are proteins and their stability is dependent on several interactions, such as hydrogen bonds which ensure that the active sites are held in the right shape. The pH of solution affects these interactions and consequently affects enzyme activity. Each enzyme can have its optimum pH depending on its own molecular stability as well as the nature of substrate and mediator. In reported research on biodegradation of micropollutants using laccases, the optimum pH was mentioned to be in the range of 3-7 [1, 30, 33-35]. In addition, the pH can affect the substrate charge and consequently alter the interaction of enzyme and substrate. The net charge of CTC molecule in the pH range of 4-6 is near zero and at higher pH, the net charge is negative and for laccase, it is negatively charged at pH higher than 3 [36, 37]. Therefore, at pH higher than 6, electrostatic repulsion is expected to take place between laccase and CTC which reduces collision frequency and finally degradation efficiency in a certain time period. Furthermore, at higher pH, more hydroxyl ions can bind to active copper atoms of laccase and inhibit its activation [38] which is another reason for the sharp decrease in degradation efficiency at higher pH.

The response surface of pH and ABTS concentration, keeping temperature and enzyme concentration at 35 °C and 60 U/L, respectively are depicted in Figure 3.1.3. According to this surface plot, more than 95% of degradation is expected at ABTS concentration of around 10 µM. The confirmation experiment, explained in the following section, showed more than 92% degradation at the mentioned optimum levels. Laccase alone can degrade pollutants with high redox potential and using mediators, such as ABTS as electron carrier between enzyme and compounds extending laccase application to other contaminants [1]. In laccase-ABTS system, laccase transforms ABTS to cationic radical, $ABTS^{\bullet+}$ which oxidises the contaminant molecules by transferring the electron and returning to its original form [30].

Kinetics of degradation of chlortetracycline

Analysis of data using Design-Expert can estimate the degradation efficiency at different points within the considered range of parameters and also can suggest several optimum points which in this case are the conditions with maximum degradation efficiency. For the obtained data in this research, a set of parameters

i.e. pH, temperature, enzyme concentration and mediator concentrations of 5.2, 35.5 °C, 62.3 U/L and 10.9 µM were among the offered optimum points with the highest degradation rates (>92%) and these parameters along with CTC concentration of 1 mg/L were further selected for kinetic study. The concentration of CTC ranged from several ppb in the effluent of municipal wastewater treatment plant to several mg/L in pharmaceutical wastewater and therefore the studied concentration was reasonably in the relevant environmental concentration range [39-41].

In Figure 3.1.4, the time evolution trends of residual concentration of CTC in the optimized conditions along with another sample with same conditions except using ABTS are illustrated. According to this figure, laccase-ABTS system degraded more than 95% of CTC in less than 8 hours while laccase without ABTS could not reach more than 47 % even after 24 hours. Generally, enzymatic degradation processes follow Michaelis-Menten kinetic model in which the degradation rate is of zero order at higher substrate concentrations and of first order at lower substrate concentrations. Due to very low concentrations (µM level), degradation of micropollutants can be reasonably fitted by a first order reaction rate [42]. In the case of reaction conditions of Figure 3.1.4, the concentration of substrate, CTC was 1 mg/L and the first order rate constants (K) were 0.230 h^{-1} and 0.030 h^{-1} for reactions with and without ABTS, respectively. CTC can be degraded autonomously under environmental conditions and according to Soeborg *et al.,* the natural degradation half-life time ($t_{1/2}$) of CTC is in the range of 10-50 days which causes the persistence of this compound in the nature [43]. Therefore, using laccase-ABTS system under optimized conditions can reduce the degradation half-life time to 3 h and efficiently prevent its accumulation in the environment with consequent reduction in concentration. In the previous studies, higher concentration of tetracyclines (100-150 mg/L) was applied for enzymatic biodegradation [22] which may shift the reaction to zero order and in this case, the half-life will be under evaluated to 2-3 min. Therefore, the results of this study are much closer to what is going to happen at environmentally-related concentrations of CTC.

Degradation by-products

Laccase is well known for oxidation of phenolic compounds, such as hydroquinone to benzoquinone [44] and especially white-rot fungi laccase is known to oxidize polyaromatic hydrocarbons (PAHs) to their corresponding quinones and subsequently degrade the material to CO_2 [38]. In laccase-ABTS reaction with CTC

(Figure 3.1.5), laccase oxidizes ABTS to $ABTS^+$ to be able to attack substrate. In this step, mediator subtracts hydrogen from alcohol groups of substrate or substitutes oxygen with possible groups in the structure.

According to the mass spectrum obtained for the optimized sample after biodegradation (Data in Supplementary Material), three compounds were identified as major by-products of CTC degradation. The detected molecular weights of by-products in mass spectroscopy are described in Table 3.1.4 and the proposed pathway and structures of by-products is illustrated in Figure 3.1.6. The first left ring in CTC (Figure 3.1.5) resembles p-chlorophenol which was already reported to transform to benzoquinone in the presence of laccase [45]. Also, the second ring can be transformed to quinone structure by subtracting a methanol molecule from CTC [46]. The dechlorination and quinone formation in the first left ring is similar to the pathway of ozonation process reported for CTC [47].

These two reactions resulted in compound A (m/z: 427) which could undergo dehydrogenation process and transformed into compound B (m/z: 423). The third by-product, compound C (m/z: 431), is the analogous of enzymatic degradation of tetracycline proposed by Llorca et al. in which a C=C bond is formed on the second ring (from left) and a dimethleneamine molecule along with 1.5 hydrogen molecule are subtracted from parent compound [48]. The values of chromatographic peak area ratio (A/A_0) corresponding to each by-products showed that compound B was the most probable by-product followed by compound A and compound C. However, the sum of chromatographic area of by-products and remaining CTC was not equal to that of initial CTC concentration. For further investigation, the gas phase above the solution was analyzed with GC-MS and CO_2 was detected as the major component in the gas mixture compared to the control sample. Detection of CO_2 confirmed the mineralization of CTC by-products, however, further research is needed to investigate the complete pathway of CTC mineralization.

Toxicity of by-products

Recent studies indicated the presence of compounds in the wastewater effluent that can affect the human endocrine system and bring adverse outcome, such as malformations, infertility and cancers [49]. The presence of these compounds is due to the release of chemical compounds and their subsequent transformation to by-products in wastewater treatment plants. For example, in a research by Isidori *et al*.,

nine out of fourteen pharmaceutical compounds from different therapeutic categories showed estrogenic activity [50]. Therefore, efficient technologies for treatment of wastewater and control of effluent quality are important for prevention of the pollution of the receiving bodies. The conventional methods for quality control relies on measuring the concentration of target compounds, such as 17beta-Estradiol, however, these methods are not able to address the complex matrices with unknown estrogenic compounds. In this case, YES test is able to confirm the estrogenic activity of complex environmental samples, such as wastewater effluent [51]. In YES assay, the human estrogen receptor (hER) is expressed in yeast to enable activating transcription of a promoter carrying estrogen-responsive element, in an estrogen-dependent manner [27]. The recombinant yeast hosts expression plasmids that carry the beta-galactosidase-encoding reporter gene lac-Z. After activation of lac-Z gene in the presence of estrogenic compounds, beta-galactosidase degrades CPRG substrate. Degradation of CPRG causes a color change from yellow to red which confirms the estrogenicity of the compound [49]. The repeatability of this assay was assessed by comparing the response of the yeast to 17β-estradiol with ones for CTC and its by-products. The change in color as a result of responding yeast to 17β-estradiol, CTC and its by-products is presented in Figure 3.1.7. As it is observed, the color of medium containing yeast and high concentrations of 17β-estradiol turned red after 3 day of incubation. However, none of the samples with CTC or its by-products showed any change in color. It can be concluded that CTC at 1 mg/L and its by-products formed after degradation with laccase and laccase-ABTS system has no estrogenic activity towards yeast. Therefore, the by-products of CTC degradation will have no effects on the endocrine system.

Conclusion

Laccase obtained from *Trametes Versicolor* was employed for biodegradation of veterinary antibiotic CTC and the effects of pH, temperature, enzyme concentration and mediator concentration on degradation efficiency were investigated. A quadratic model was obtained using Design-Expert software in which R^2 and adjusted R^2 were 0.85 and 0.70, respectively indicating a reasonably good model for practical application such as pharmaceutical wastewater treatment systems. According to the obtained equation, temperature and pH had the largest effects on biodegradation of

chlortetracycline. Optimization data predicted that setting the values of pH, temperature, enzyme concentration and mediator concentration to 5.2, 35.5 °C, 62.3 Unit/L and 10.9 µM resulted in maximum biodegradation of approximately 95% for chlortetracycline after 8 hours. Also, the YES assay showed that the by-products of enzymatic degradation with and without ABTS have no estrogenic activity which is important for safe disposal of wastewater.

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Table 3.1.1 Independent variables used for degradation study

	Values of independent variables in experimental design				
Levels	-2	-1	0	+1	+2
A: Temperature (°C)	25	30	35	40	45
B: ABTS concentration (μM)	2	6	10	14	18
C: pH	4	5	6	7	8
D: Enzyme concentration (U/L)	20	40	60	80	100

Table 3.1.2 Variable parameters and their level in designed experiments

No.	Temperature (°C)	ABTS Conc. (μM)	pH	Enzyme Conc. (U/L)	Actual Degradation (%)	Predicted Degradation (%)
1	30	6	5	40	58.52	55.09
2	40	6	5	40	77.42	78.21
3	30	14	5	40	80.59	72.08
4	40	14	5	40	78.02	98.53
5	30	6	7	40	17.14	13.80
6	40	6	7	40	52.30	58.07
7	30	14	7	40	11.45	28.09
8	40	14	7	40	78.47	75.69
9	30	6	5	80	77.73	75.12
10	40	6	5	80	82.89	71.53
11	30	14	5	80	80.42	79.94
12	40	14	5	80	81.73	79.68
13	30	6	7	80	72.21	56.97
14	40	6	7	80	71.42	74.53
15	30	14	7	20	65.29	59.10
16	40	14	7	80	71.28	79.99
17	25	10	6	60	19.10	30.63
18	45	10	6	60	86.06	74.65
19	35	2	6	60	39.01	52.11
20	35	18	6	60	87.54	74.56
21	35	10	4	60	78.44	81.95
22	35	10	8	60	44.37	40.97
23	35	10	6	20	87.39	74.51
24	35	10	6	100	85.84	98.83
25	35	10	6	60	88.29	88.77
26	35	10	6	60	87.95	88.77
27	35	10	6	60	89.32	88.77
28	35	10	6	60	89.57	88.77
29	35	10	6	60	88.54	88.77
30	35	10	6	60	88.92	88.77

Table 3.1.3 ANOVA results for the quadratic equation of degradation of chlortetracycline

Source	Degree of Freedom	Sum of squares (SS)	F value	Critical F value	P value
Model	14	12680.98	5.92	2.42	0.0009
Linear	4	7068.27	11.58	3.05	0.0002
Square	4	4632.36	7.57	3.05	0.0019
Interaction	6	1863.23	2.024	2.79	0.1087
Residual error	15	2295.29			
Lack-of-fit	10	2293.37			
Pure error	5	1.92			
Total	29	14976.26			

Table 3.1.4 Molecular mass of chlortetracycline by-products and proposed elemental composition

Compound	Ion	Area ratio* (%)	Mass (m/z)	Elemental Composition
CTC	$[M+H]^+$	7.34	479.05	$C_{22}H_{23}ClN_2O_8$
	$[M-NH_3+H]^+$		462.02	
Compound A	$[M+H]^+$	6.58	427.08	$C_{21}H_{18}N_2O_8$
	$[M-NH_3+H]^+$		410.04	
Compound B	$[M+H]^+$	11.17	423.04	$C_{21}H_{14}N_2O_8$
	$[M-NH_3+H]^+$		406.01	
Compound C	$[M+H]^+$	4.79	431.12	$C_{20}H_{13}ClNO_8$
	$[M-NH_3+H]^+$		414.09	

Ratios of by-products expressed as [A/Ao CTC * 100] where A is chromatographic peak area of the corresponding compounds and Ao is chromatographic peak area of CTC at t=0.

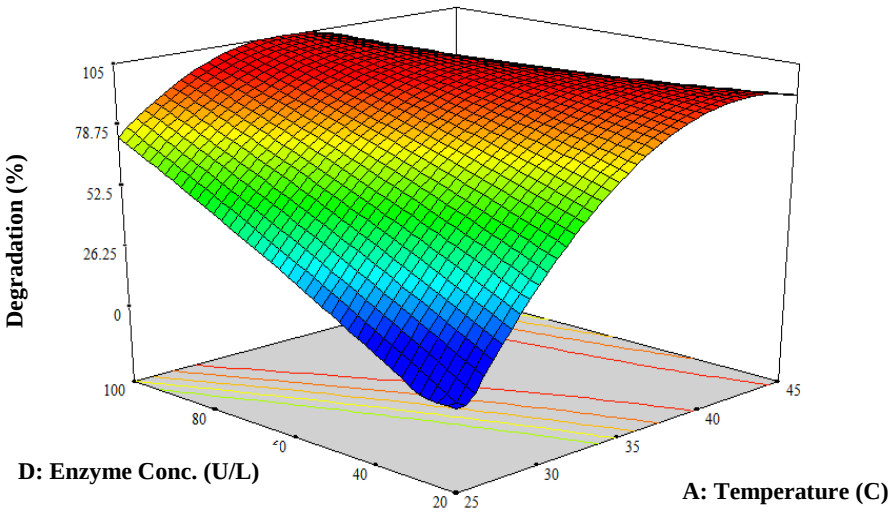

Figure 3.1.1 Response surface plot for the effects of temperature and enzyme concentration on degradation efficiency of chlortetracycline

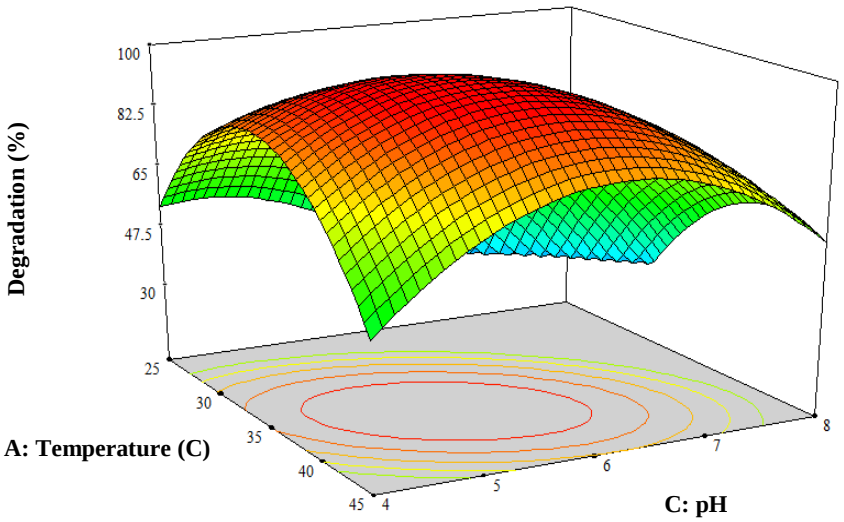

Figure 3.1.2 Response surface plot for the effects of temperature and pH on chlortetracycline degradation

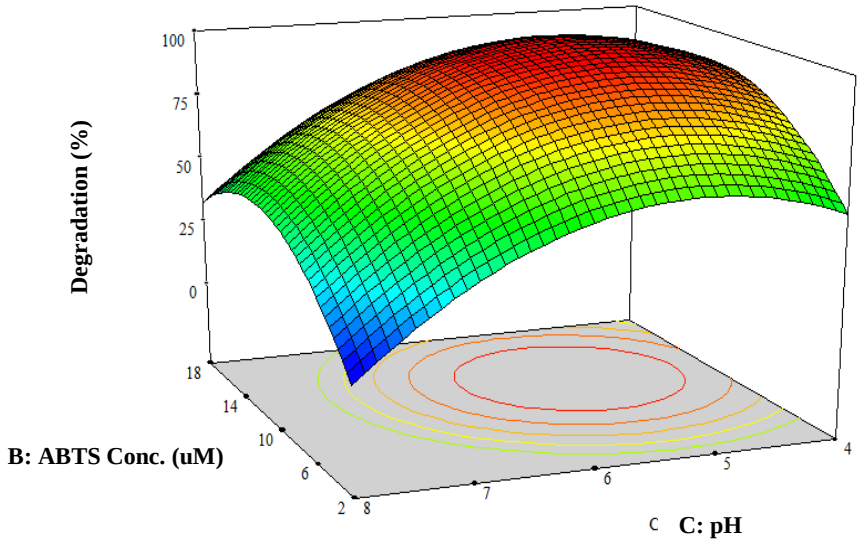

Figure 3.1.3 Response surface plot for the effects of ABTS concentration and pH on degradation efficiency of chlortetracycline

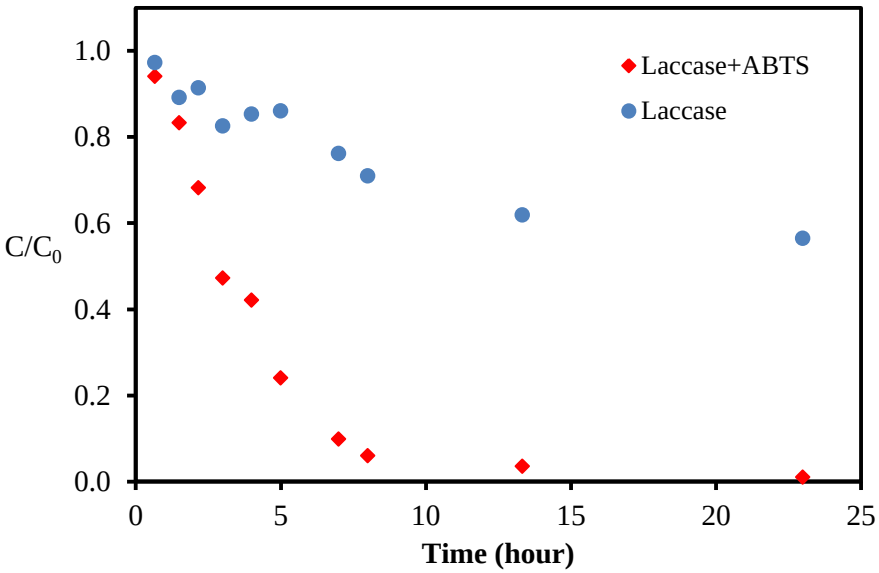

Figure 3.1.4 Effect of time on biodegradation of chlortetracycline using laccase in presence and absence of ABTS (T=35.5 °C, Enzyme Conc.=62.3 U/L, ABTS Conc.=11.9 µM, pH=5.2, CTC Conc.=1 mg/L)

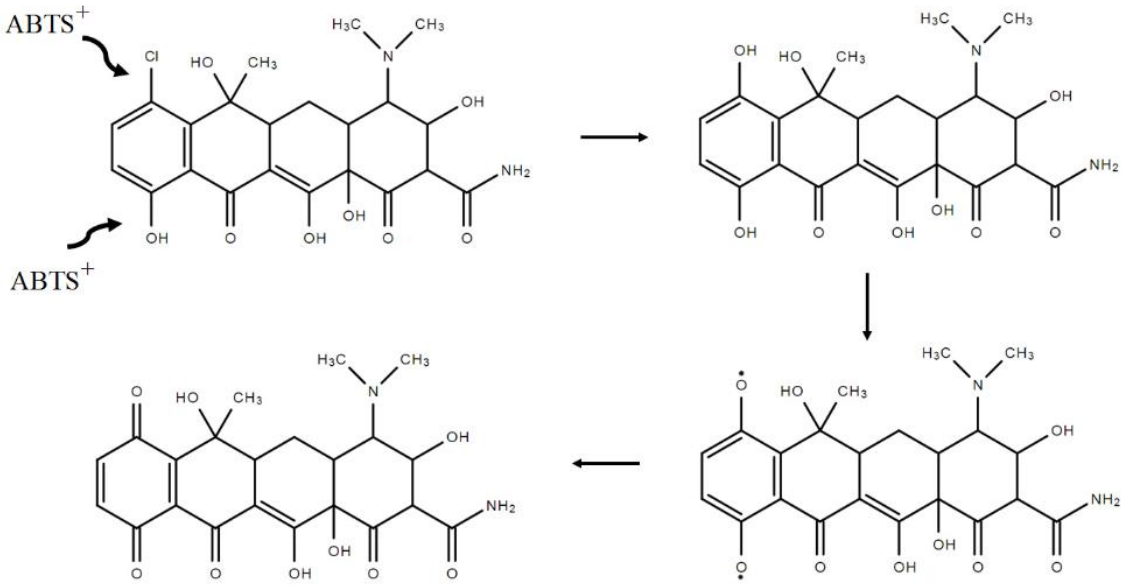

Figure 3.1.5 Mechanism of chlortetracycline biodegradation with laccase-mediator system

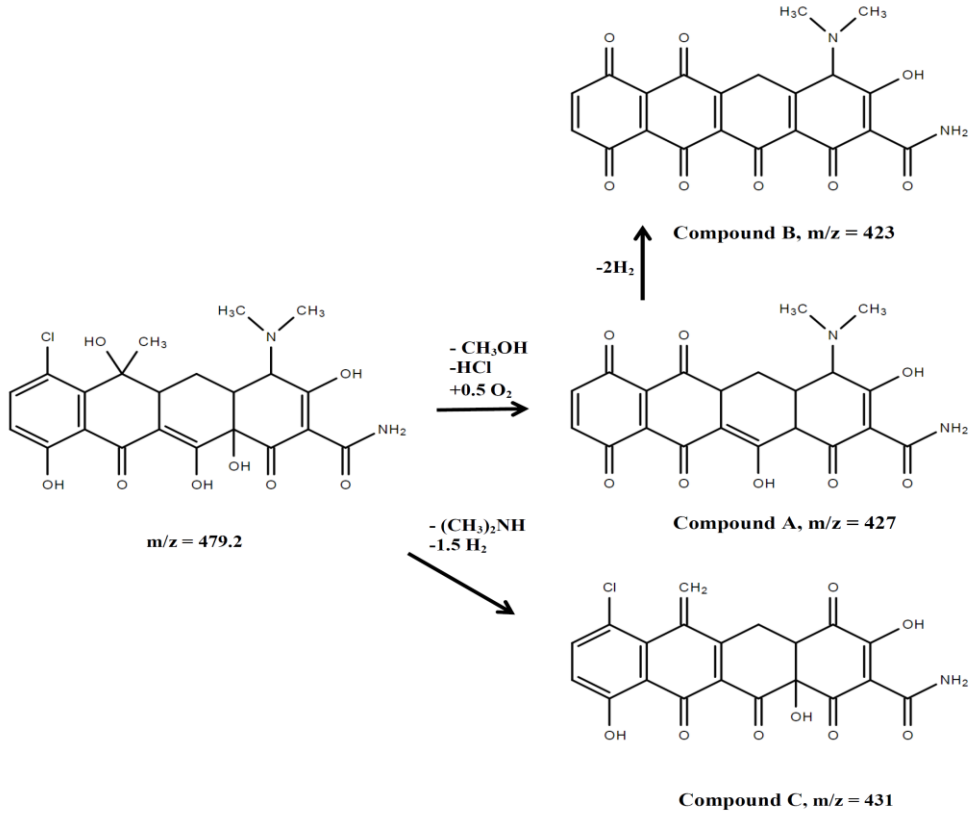

Figure 3.1.6 Proposed pathway for biodegradation of chlortetracycline using laccase-ABTS system

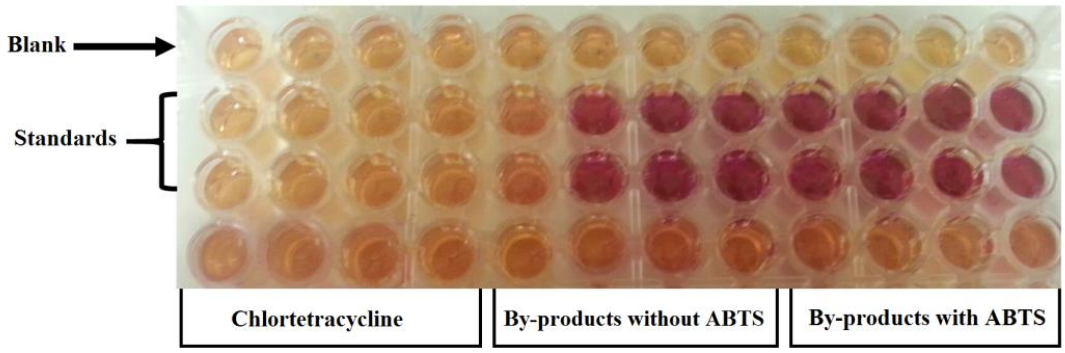

Figure 3.1.7 Yeast estrogen screen assay for blank, 17 β-Estradiol, Chlortetracycline and its degradation by-products

Part 2

Degradation of Chlortetracycline using immobilized laccase on Polyacrylonitrile-biochar composite nanofibrous membrane

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Résumé

Le rejet continuel des antibiotiques dans l'environnement à travers l'effluent usé a soulevé des inquiétudes vu leurs effets potentiels sur les différents organismes vivants. La dégradation enzymatique en utilisant la laccase est une alternative verte pour l'élimination des composés pharmaceutiques des milieux aqueux. Dans cette étude, la laccase a été immobilisée sur une membrane à base de nanofibres fabriquée à partir d'un mélange polyacrylonitrile-biocharbon. Le biocatalyseur obtenu a été utilisé pour l'élimination d'un antibiotique largement utilisé qui est la chlorotétracycline, à partir de milieux aqueux en mode continu. Les résultats ont montré que la laccase immobilisée a montré des meilleurs résultats en termes de stockage, température et stabilité du pH par rapport à la laccase libre. En outre, il a conservé plus de 50% de son activité initiale après 7 cycles d'oxydation ABTS, qui améliore significativement la réutilisation de l'enzyme. Enfin, l'utilisation de laccase immobilisée pour la dégradation de la chlorotétracycline en mode continu a conduit à une efficacité d'élimination importante de chlorotétracycline de 58,3%, 40,7% et 22,6% à des vitesses de flux de 1, 2 et 3 mL/h·cm^2.

Mots clés

Nanofibrous, Immobilisation, Antibiotiques, Enzyme, Dégradation.

Abstract

The continuous release of antibiotic compounds through wastewater effluent into environment has raised concerns about their potential problems for different organisms. Enzymatic degradation with laccase is a green option for removal of pharmaceutical compounds from aqueous media. In this study, laccase was immobilized onto homemade Polyacrylonitrile-biochar composite nanofibrous membrane and the obtained biocatalyst was employed for removal of chlortetracycline, a widely used antibiotic, from aqueous media in continuous mode. The results showed that the immobilized laccase has improved storage, temperature and pH stability compared to free laccase. Also, it retained more than 50% of its initial activity after 7 cycles of ABTS oxidation which indicated improved enzyme reusability. Finally, while using immobilized laccase for degradation of chlortetracycline in continuous mode exhibited 58.3 %, 40.7 % and 22.6 % chlortetracycline removal efficiency at flux rates of 1, 2 and 3 mL/h.cm^2.

Keywords

Nanofibrous, Immobilization, Antibiotics, Enzyme, Degradation.

Introduction

In the past 70 years, antibiotics have been widely used in animal husbandry for controlling and preventing the infectious diseases. However, a considerable portion of the prescribed antibiotics is not metabolized in animal body and therefore it is released into soil, rivers, lakes and groundwater through animals' urine and feces [1, 2]. Chlortetracycline (CTC) is among the known veterinary antibiotics which is used for controlling the diseases as well as for promoting the growth rate of cattle and swine on a regular basis [3]. The presence of CTC along with other pharmaceutically active compounds (PhACs) in water bodies raised concerns among environmentalists over their potential effects on ecosystem and human health [4]. For example, it is reported that tetracycline compounds caused histological alteration in gills of fish as well as exerting a pro-oxidative activity [5]. Also, there are numerous reports on development of antibiotic resistance among bacteria against members of tetracycline family [6, 7]. Therefore, removing these compounds from water and wastewater is crucial to prevent their release into the environment and to avoid consequent ecological and health problems [8].

As conventional water treatment systems cannot efficiently remove these micropollutants from aqueous media, different physical, biological and chemical removal methods have been proposed and studied for removal of PhACs from aqueous media. However, technical, economical and environmental issues related to each approach halted the development of an acceptable strategy for removal of these compounds [9-11]. For example, production of toxic by-products in advanced oxidation processes, production of a concentrated stream in membrane separation and instability and sensitivity of biodegradation system are among the challenges that need to be addressed.

Using ligninolytic enzymes, especially laccases, that are capable of non-specifically oxidizing a broad range of organic molecules is a promising option for future strategies of wastewater treatment due to the low environmental impact of enzymatic treatment [12]. Numerous researchers reported the capability of laccase for degradation of different PhACs, such as ibuprofen, diclofenac, naproxen and sulfamethoxazole and reported degradation efficiencies up to 95% [13-15]. For example, Suda *et al.* degraded CTC with laccase and obtained 48% degradation after 4 hours of reaction [16]. In another study, Cazes *et al.* reported that laccase can

degrade up to 30% of tetracycline after 24 hours [4]. In a related work, Ding *et al.* reported >90% efficiency for degradation of CTC with laccase in presence of mediator after 3 hours of reaction [17]. However, using enzymes in their free forms has drawbacks, such as high cost of production, low stability and problems with reusability which should be addressed before scale-up [18]. Immobilization of enzyme onto a variety of solid supports and especially porous membranes, which are called enzymatic membrane reactors (EMR), is a promising approach to overcome these problems since membranes can provide high surface area for catalytic reaction [4]. The benefits of laccase immobilization on membranes, such as longer shelf life, reusability and stability against temperature and pH variations have been extensively studied in numerous research works [19, 20]. In most of the reported investigations, pure and costly laccase was employed while it is possible to use the white-rot fungi crude laccase. Furthermore, high concentrations of target compounds (>20 mg/L) reported in the literature is far beyond the real concentrations in surface water and municipal wastewater. Moreover, there is scant literature on removal of micropollutants using EMRs in continuous mode. In this work, the enzymatic degradation of chlortetracycline (CTC) in continuous mode and at environmentally relevant concentration was studied. The laccase was produced by growing *Trametes versicolor* fungi and was immobilized onto an electrospun Polyacrylonitrile-biochar composite membrane. Using biochar for treatment of wastewater is of high interest due to its low cost, availability, interesting physico-chemical properties and role in value-addition to wooden residues [21]. In this work, the activated biochar was incorporated into nanofibers to create the adsorption capability in the membrane and increase the contact time between pollutants and immobilized enzyme. The properties of free and immobilized enzymes were compared and the performance of the EMR in continuous mode was investigated.

Materials and methods

Chemicals

CTC (purity > 97%) was purchased from Toronto Research Chemicals (TRC-Canada). 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was purchased from Sigma-Aldrich (Oakville, Canada). Tween 80 and methanol were purchased from Fisher scientific (Ottawa, Canada). HPLC grade water was prepared

in the laboratory using milli-Q/Milli-Ro system (Millipore, USA). Polyacrylonitrile (PAN), with an average weight molecular weight of 1.5×10^5 (g/mol), was obtained from Scientific Polymer Product Company (USA) and used without further purification. Biochar was donated by Pyrovac Inc. (Canada) and it was derived from pine white wood (80%) purchased from Belle-Ripe in Princeville and the rest was spruce and fir (20%). This biochar was produced at 525±1 °C under atmospheric pressure for 2 minutes and used as obtained from the reactor outlet. Sodium hydroxide and hydrogen chloride with 98% purity and N,N'-Dimethyl-Formamide (DMF) and Dimethyl-Sulfoxide (DMSO) with 99.5% purity were supplied by Fisher Scientific (USA). HPLC grade water was prepared in the laboratory using milli-Q/Milli-RO system (Millipore, USA).

Preparation of inoculum

Fungus, *Trametes versicolor* (ATCC 20869) was grown aerobically in liquid medium by inoculating the flasks containing potato dextrose broth (PDB) using lyophilized powder and then incubating the flasks in orbital shaker at 30±1 °C and 150 rpm for 7 days. Later, 100 µL of PDB medium was inoculated in potato dextrose agar (PDA) plates at 30±1 °C for 9 days and the plates were stored at 4±1 °C, prior to use.

Solid-state fermentation

Apple pomace (Vergers Paul Jodoin Inc., Quebec, Canada) was used as solid substrate for the production of laccase by *T. versicolor.* About 40 grams of solid substrate (70% (w/w) of moisture and pH 4.5), along with 0.5% v/w Tween 80 in 500 mL flask was thoroughly mixed and autoclaved at 121±1 °C for 30 min. Later, each flask was inoculated with the biomass content of one petri plate and incubated in a static incubator at 30±1 °C for 15 days.

Enzyme extraction and assay

One gram of fermented sample was mixed with 20 mL of 50 mM sodium phosphate buffer (pH 4.5), mixed at 35±1 °C for 1 h and then centrifuged at 7,000 × g for 30 min. The relative laccase activity of collected supernatant was analyzed spectrophotometrically at pH=4.5 and 45±1 °C by monitoring the oxidation of ABTS at 420 nm (ε_{420} = 36,000 M^{-1} cm^{-1}) . Each unit of laccase was defined as the amount of required laccase to oxidize one micromole of ABTS in one minute under assay

condition. For evaluation of laccase activity of immobilized enzyme, 20 mg of the sample was thrown in a 4 mL sodium phosphate buffer (pH 4.5) containing 0.5 mM ABTS and after 10 min of agitation at 45 °C, the absorbance at 420 nm was recorded. The measuring procedure was performed in triplicate and the averages were reported.

pH, temperature and storage stability

For evaluation of pH stability, 50 µL of free laccase and 20 mg of immobilized laccase onto PAN-biochar nanofibrous membrane were added to separate tubes containing 3 mL of buffers with different pH values (1.5 to 9.5) and kept for 24 h at 25 °C and 200 rpm. Then, the laccase activities of immobilized and free samples were measured (Section 2.4). For thermal stability, samples were incubated at different temperatures (20–70 °C) for 1 h at constant pH of 4.5 and then activities were measured at same temperature. The storage stability of the immobilized laccase was evaluated by keeping the samples at 4 °C and 25 °C for one month and measuring their laccase activity during one month period. Same procedure was performed for free laccase for comparison. The measuring procedure was performed in triplicates and the averages along with their standard errors are presented in figures. The ANOVA, obtained from Excel software, showed p-value<0.01 for each graph which confirmed the changes in the laccase activity are directly related to changes in studied parameters i.e. temperature, time, number of cycles and pH.

Activation of biochar

About 10 g of biochar was added to 100 mL of water containing 20 g of NaOH. The mixture was stirred with a magnetic stirrer at room temperature and 150 rpm and for 2 h and dried at 80±1 °C for 24 h. The sample was heated in a horizontal furnace under nitrogen flow of 200 mL/min. The temperature of the furnace was increased to 800±1 °C at the rate of 10 °C/min, and held for 2 h before cooling. The sample was later washed with water and neutralized with 0.1 M HCl. Finally, the sample was again washed with distilled water to remove sodium salt and dried at 60±1 °C for 24 h. The properties of activated biochar were discussed elsewhere [22].

Preparation of PAN-biochar membrane

About 2 g of PAN was dissolved in DMF/DMSO solvent mixture (9:1 v/v) at the concentration of 10 Wt% and stirred on a magnetic stirrer until a clear solution was obtained. Activated biochar at the ratios of 1.5 % (w/w) of the polymer was added to the solution and the mixture was stirred for another 48 h. PAN-biochar nanofibrous membrane was fabricated through electrospinning process under ambient conditions (T=25 °C, RH=35%) and with a rotary drum collector (length= 25 cm, diameter= 10 cm). The electric field strength, flow rate and collector rotational speed was adjusted to 1.1 KV/cm, 1.4 mL/h and 400 rpm, respectively. Also, the distance of center of collecting drum to the needle tip was 18 cm and the needle gauge was 22. The electrospinning continued for 10 h and the deposited membrane was washed with methanol for 120 min for removal of residual solvents. Later, nanofibrous membrane sample was washed with distilled water and dried for 10 h at 50±1 °C.

Characterization of fabricated NFMs

The surface morphology of the fabricated membranes was examined using an EVO-50 (Zeiss, Germany) scanning electron microscope (SEM) at acceleration voltage of 10 kV. For this analysis, a small piece of the nanofibrous membrane was coated with a thin layer of gold using an SPI Module sputter coater.

Immobilization of laccase onto PAN-biochar membrane

Immobilization of laccase onto PAN membranes was described elsewhere in details. In brief, samples were treated with 0.5 mol/L hydroxylamine hydrochloride aqueous solution at pH 6 and 70±1 $^{\circ}$C for 2 h. After completion of this reaction, the treated samples were rinsed with distilled water and dried at 40±1 $^{\circ}$C. In this case, amidoxime linkage is formed on the surface of nanofibers (Figure 3.2.1). Later, the samples were immersed in 3 g/L laccase solution (acetate buffer with pH 4.5) at 4±1 $^{\circ}$C for 12 h. Finally, the samples were washed several times with same buffer, until no laccase was detected in the drained buffer.

Biodegradation in continuous mode

The capability of immobilized laccase on PAN membrane for degradation of micropollutants in continuous mode was studied using a 15*15 cm^2 stainless steel (SS-316) membrane test module connected to a precise syringe pump. A solution with CTC concentration of 200 ppb in milli-Q water was pumped at three different

fluxes (1, 2 and 3 mL/cm^2.h) into the test setup in dead-end configuration. Samples for measuring CTC concentration were taken at 1 liter interval for 30 liters of total passed volume. CTC concentrations were estimated by using Laser Diode Thermal Desorption (LDTD) (Phytronix technologies, Canada) coupled with a LCQ Duo ion trap tandem mass spectrometer (Thermo Finnigan, USA). The daughter ions identified for CTC in LDTD were 464 and 444 Da.

Results and discussion

Immobilization

Determination of laccase activity revealed that the immobilization of laccase onto PAN-biochar electrospun membrane using amidoxime linkage resulted in the immobilization loading of around 10.1 unit/g dry membrane under experimental conditions. Also, it was observed that for unmodified membranes, almost no laccase was immobilized. Binding onto the activated surfaces of membrane is driven by the hydrogen bonding of amine structures in the proteins toward amine and hydroxyl structures in amidoxime-modified PAN-biochar membrane. Figure 3.2.2 demonstrates the SEM pictures of PAN nanofibers with and without laccase immobilization. The surface of nanofibers before laccase immobilization is quite smooth but after immobilization, they showed a rough texture which may be due to formation of amidoxime bonds as well as attachment of enzyme macromolecules. Figure 3.2.2 also showed that activated biochar particles are entrapped into the nanofibers and increase the specific surface area of membrane to more than 12 m^2/g. These adsorbent particles can adsorb the target compounds from influent and consequently prepare enough time for degradation with laccase [21].

Storage stability of immobilized laccase

Stability of the enzyme as reaction biocatalyst is very important for various biotechnological processes. However, the denaturation of enzyme and depletion of its activity is a natural phenomenon that happens in a period of time. Fortunately, immobilization of enzyme onto supports could considerably mitigate the degree of enzyme activity reduction. Generally, the immobilization restricts the freedom of macromolecules and leads to increased stability towards deactivation [23]. Figure 3.2.3 shows the residual activity of free and immobilized enzymes stored at 4±1 °C

and 25±1 °C The immobilized enzymes retained more than 71% and 31% of their initial activities after one month, but the free laccase samples preserved only 37% and 2% of their initial activities. It indicated that the immobilization of laccase on PAN-biochar nanofibrous membrane enhanced the biocatalyst storage stability compared to free laccase. This enhancement is due to the fact that enzyme immobilization restricts conformational changes, which in turn prevent denaturation and increase the stability [20]. Similar results for residual activities of immobilized enzymes through amidoxime linkage after storage for 20 days at 4±1 °C were reported by Wang *et al.* (50%), Feng *et al.* (52%) and Zhang *et al.* (60%) [19, 20, 24]. In a related study, Xu *et al.* reported high storage stability of the immobilized laccase onto PAN nanofibrous membrane as it retained 92% of the initial activity after 18 days of storage at 4±1 °C, whereas the free laccase showed only 20% of initial activity [25]. Further, they investigated the storage stability of immobilized laccase on chitosan nanofibers and observed 60% residual activity after 10 days of storage at room temperature while free laccase lost most of its activity under same conditions [26]. Also, Jiang *et al.* immobilized laccase onto chitosan microspheres and observed 70% of activity retention after one month storage at 4±1 °C, while free enzyme resulted in 30% of its initial activity [27].

Operational stability of immobilized laccase

Reusing laccase in free form is a big issue since it is soluble in aqueous reaction media and therefore its discharge along with effluent or product flow increases the operational cost. Unlike free laccase, immobilized laccase can be easily separated from the reaction media and reused, which considerably decreases the cost for practical application. However, still decreasing enzyme activity as a result of repeated usage is expected due to possibility of denaturation during the process. Therefore, the knowledge of operational stability of immobilized laccase is essential to evaluate its industrial exploitation. This stability parameter is estimated by reusing the same immobilized laccase sample for 7 successive cycles of ABTS oxidation and the retained activity in each run was determined. Each run had duration of 30 min and the activity of the immobilized laccase was measured in each cycle and the results are shown in Figure 3.2.4. There is a sharp reduction of activity (32.7%) from first to third run and then the activity was reduced slightly until run #7 (16.2%). Decrease in the laccase activity is expected due to denaturation or desorption of

enzymes during repeated usage. Denaturation may happen due to formation of radicals during the reaction of laccase with ABTS which can block the active sites on the enzyme [28]. Zhang *et al*. used the same procedure for immobilization of laccase onto PAN nanofibrous membrane and observed around 60% activity loss after 7 cycle of ABTS oxidation [24]. Feng *et al.* modified this method by using glutaraldehyde as a crosslinking agent between amidoxime and laccase and therefore lost only 30% of laccase activity after 7 cycles of ABTS oxidation [29]. Likewise, Xu *et al.* immobilized laccase onto PAN nanofibers through amidination process and reported that the immobilized laccase lost 30 % of the initial activity after 7 cycles [25].

pH stability of immobilized laccase

The solution pH determines the ionization state of amino acids in enzymes which affect their 3-D structure, activity and denaturation [30, 31]. At a specific pH, the best combination between enzyme and the substrate can occur which results in a highly efficient catalytic reaction. In this work, the stability of immobilized and free laccases was evaluated in the solution pH range of 1.5 to 9.5 and the results are shown in Figure 3.2.5. Accordingly, the optimum pH value to obtain maximum activity of laccase was shifted from around 4.5 for free laccase to around 4 for immobilized laccase. The partitioning concentrations of H^+ and OH^- in microenvironment of the bulk solution and the immobilized enzyme is usually unequal due to electrostatic interactions with the matrix [27]. These interactions led to the displacement in the pH activity profile and optimum pH [32]. In this work, it seemed that biochar and amidoxime groups affected the partitioning of H^+ and OH^- and shifted the optimum pH. In a study by Wang *et al.,* it is reported that the optimum pH for maximal activity of laccase shifted from 3.5 for free laccase to 4 for immobilized laccase onto PAN membrane with metal chelation. They attributed this shift to the influence of electrostatic interactions exerted by the carrier on the microenvironment [19]. In contrast, Xu *et al.* immobilized laccase onto PAN nanofibers through amidination process and observed no changes in the optimum pH for laccase activity. They attributed this behavior to neutral amidine bonds [25]. Similar results were reported by Catapane *et al.* who employed diazotization process for linking enzyme to PAN beads as they observed no changes in the optimum pH for maximum activity of laccase and related it to the zero surface electric charge of PAN beads [33].

Furthermore, the immobilized laccase onto PAN-biochar membrane in this work showed lower sensitivity to pH variation. This enhancement is due to the multi-point attachments between proteins and nanofibers which in turn rigidify the enzyme and protect it from deactivation [31]. Also, the effect of electrostatic charge of biochar in resisting against pH variation in enzyme microenvironment can be considered. Other researchers observed same behavior for immobilized laccase [24, 27]. For example Xu *et al.* found that immobilized laccase onto chitosan/poly(vinyl alcohol) nanofibrous membrane was less sensitive to the pH than free laccase, especially in a basic environment. It was related to the effect of the charge of the carrier since the surface of nanofiber had a large amount of free hydroxyl groups that resulted into an acidic microenvironment for immobilized laccase [26]. Also Nicolucci *et al.* found that the pH range where the enzyme activity is more than 90% of the maximum activity pH 4.0-6.2 while for the free form it is 4.6-5.4 which showed less sensitivity of immobilized laccase to the pH variations [34].

Thermal stability of immobilized laccase

Increasing the temperature enhanced the mobility of enzyme macromolecules which significantly affect its activity. In one hand, increasing the temperature leads to better supply of activation energy of reactions but in the other hand enhancing he mobility of macromolecule branches can increase the possibility of denaturation. Therefore an optimum temperature is expected for obtaining maximum catalytic activity. According to Figure 3.2.6, free laccase showed its maximum activity at 30 °C-40 °C while for immobilized laccase it is shifted to 40 °C-50 °C. Also the immobilized enzyme showed a slight improvement in thermal stability which is due to the bonds between enzyme and membrane [18]. The multipoint interactions between membrane and laccase can reduce the extent of conformational change at higher temperature and protected it from denaturation and shifted the optimum temperature to higher values [20]. However, the reported effects of temperature on stability and shifting optimum temperature are not in accordance with each other. For example, Jiang *et al.* reported higher sensitivity of immobilized laccase onto magnetic chitosan microspheres compared to free laccase while other researchers reported at least a slight improvement in thermal stability of immobilized laccase [19, 27, 29].

Also, Wang *et al.* and Xu *et al.* immobilized laccase on PAN and chitosan nanofibers and observed no changes in the optimum temperature [19, 25, 26] while other

researchers, such as Nicolucci *et al.* and Zhang *et al.* reported a 10±1 °C shift in optimum temperature of free laccase and immobilized laccase on PAN support [24, 33, 34]. Therefore, the behavior of immobilized laccase at different temperatures should be carefully investigated for practical applications.

CTC Biodegradation in continuous mode

The hydrophilicity of CTC favors its mobility in the environment and the multi-ring structure of CTC complicates its degradation [3, 21]. Laccase is a single-electron oxidoreductase known for non-specific oxidation of organic molecules. The four copper ions in laccase play important role in generation of free radicals [15]. In this research work, the immobilized laccase onto PAN-biochar nanofibrous membrane was used for degradation of CTC in continuous mode and the results are depicted in Figure 3.2.7. The concentration of CTC in feed stream was set to 200 ppb since the reported concentrations in literature ranged from 1.2 ppb in municipal wastewater to several mg/L in wastewater effluent from pharmaceutical industries [35, 36]. According to Figure 3.2.7, at the flux rate of 1 mL/h.cm^2, the concentration of CTC decreased to the average of 83.25 ppb which is equivalent to 58.3 % removal. By increasing the flux rate to 2 mL/h.cm^2 and 3 mL/h.cm^2, the removal efficiency was reduced to 40.7% and 22.6%, respectively. Changing the flux rate directly affected the contact time among CTC molecules, biochar particles and enzyme biomolecules. Therefore, it is expected that by increasing the flux rate, the collision frequency among them is decreased and as a result, the removal efficiency is decreased. Xu *et al.* took advantage of immobilized laccase onto PAN nanofibers for removal of 2,4,6-trichlorophenol from aqueous media in a batch reactor and obtained more than 40% and 90% removal efficiency in 1 and 4 h contact time, respectively [25]. Also, they used immobilized laccase onto chitosan/poly(vinyl alcohol) nanofibers for degradation of 2,4-dichlorophenol and observed 87.6% removal after 6 h of contact time [26]. In a related work, Nicolucci *et al.* used immobilized laccase on PAN beads for removal of bisphenol A, bisphenol B, bisphenol F and tetrachlorobisphenol A in a batch reactor and obtained more than 90% of removal efficiency for these compounds after 90 min of treatment [34]. They also used immobilized laccase on PAN beads for removal of octylphenol and nonylphenol and obtained more than 60% and 80% of removal efficiency for octylphenol and nonylphenol, respectively after 90 min [33]. In this work, considering the membrane thickness to be around 200 µm, the

contact time (or residence time) corresponding to flux rates of 1, 2 and 3 mL/h.cm^2 were 1.2, 2.4 and 3.6 min, respectively are far lower than those reported in literature. The better performance of this system can be attributed to the presence of adsorptive particles i.e. activated biochar which adsorbed CTC and provided enough time for biodegradation with laccase. In real field applications, the reduction in residence time can result in a downsized reactor and less energy consumption for mixing. Therefore, a significant reduction in capital and operational costs may be expected [37, 38].

Conclusion

An adsorptive membrane was fabricated by entrapment of activated biochar into PAN nanofiber through electrospinning process and laccase was immobilized onto this membrane through formation of amidoxime linkage. The obtained biocatalyst showed the enzyme loading of 10.1 Unit/g and it was used in a continuous mode for degradation of a widely used veterinary antibiotic, chlortetracycline. Compared to free laccase, pH, temperature and storage stability of immobilized laccase was improved. Also, it retained more than 50% of its initial activity after 7 cycles of ABTS oxidation which indicated improved reusability. Finally, using immobilized laccase for degradation of chlortetracycline in continuous mode exhibited 58.3%, 40.7% and 22.6% removal efficiency at 1, 2 and 3 mL/h.cm^2 demonstrating its potential application in wastewater treatment.

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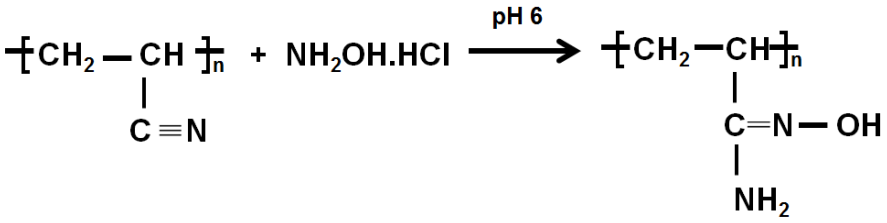

Figure 3.2.1 Formation of amidoxime linkage

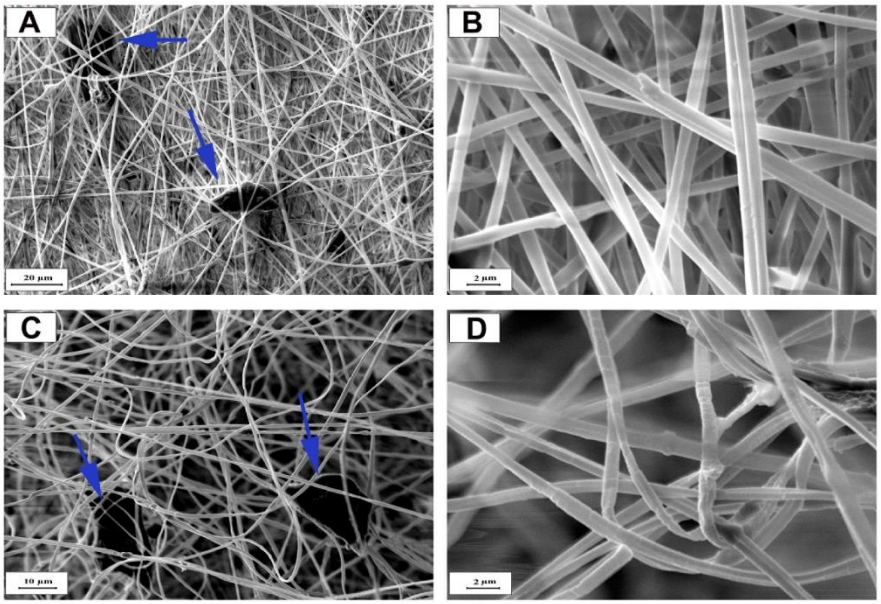

Figure 3.2.2 SEM micrographs of PAN nanofibers at different magnifications A & B: before laccase immobilization and C & D after laccase immobilization

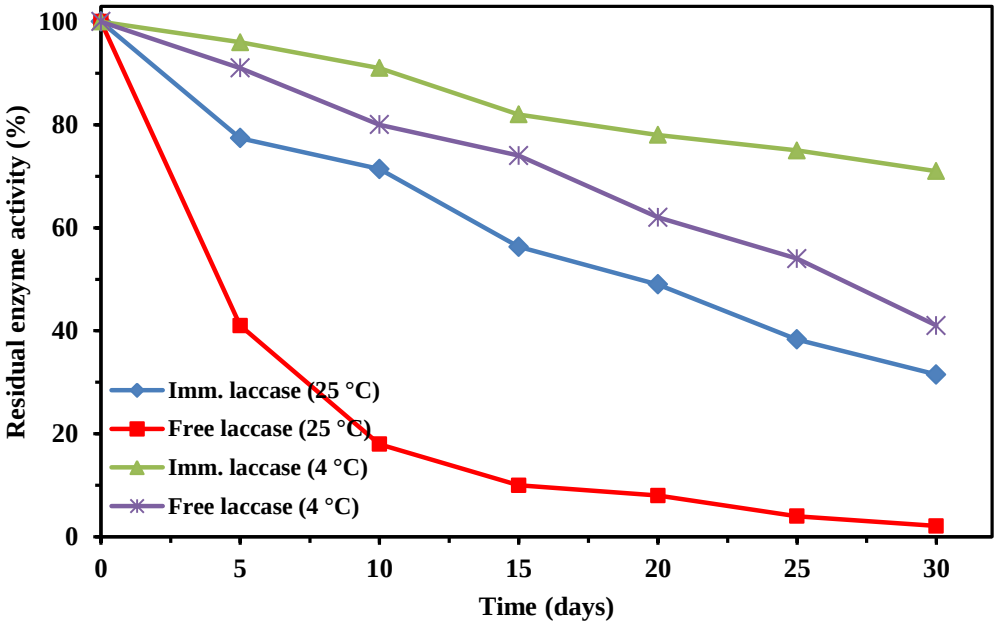

Figure 3.2.3 Storage stability of the free and immobilized laccase stored at 4±1 °C and 25±1 °C

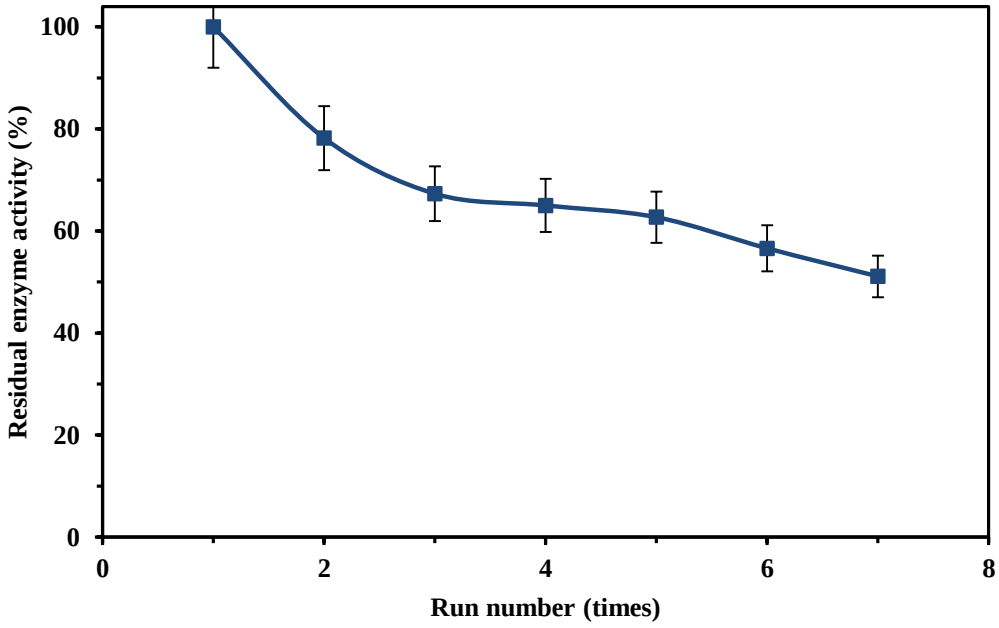

Figure 3.2.4 Operational stability of immobilized laccase

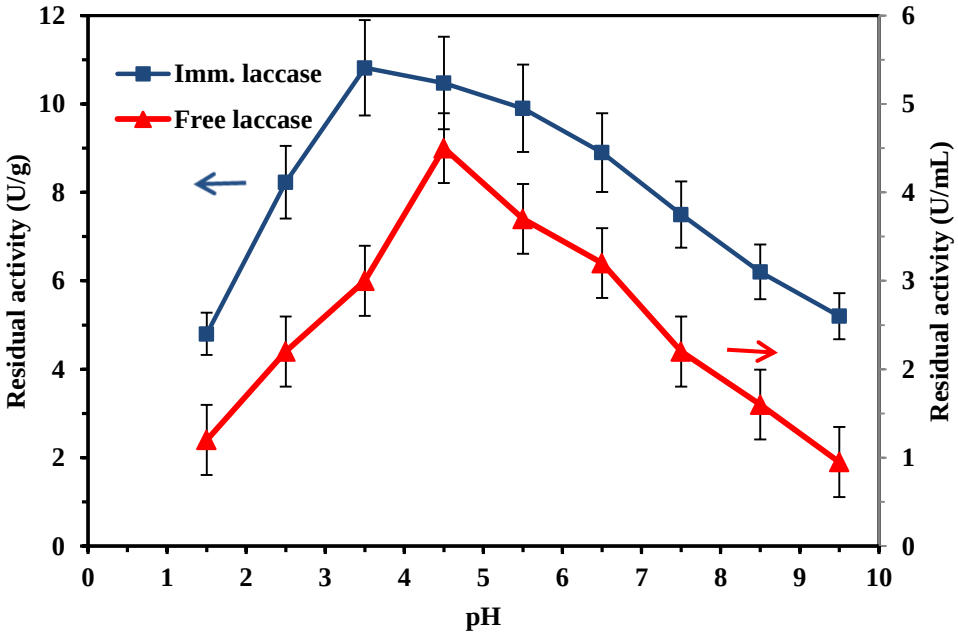

Figure 3.2.5 Effect of pH on activity of free and immobilized laccases

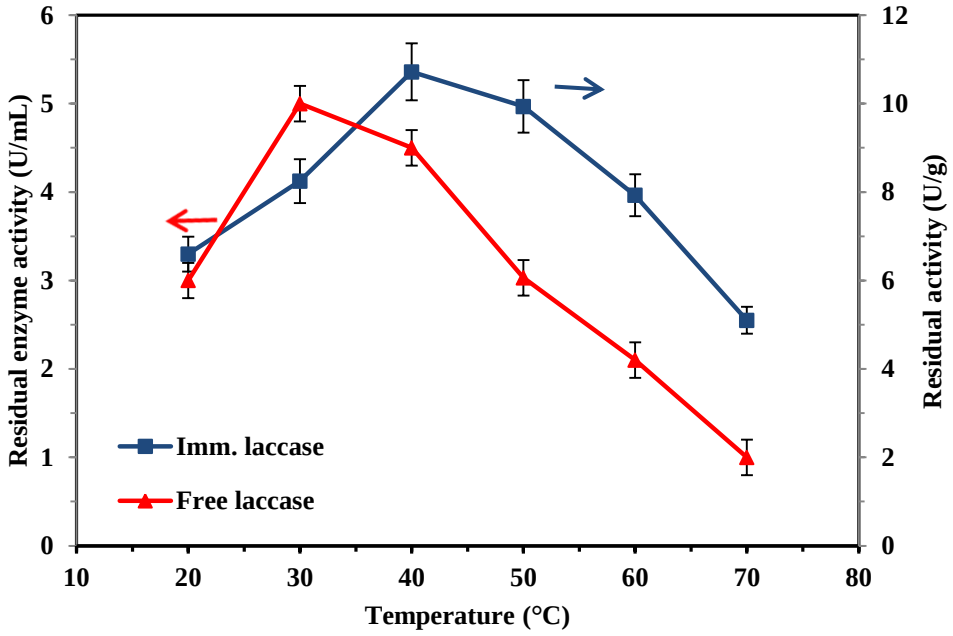

Figure 3.2.6 Effect of temperature on residual activity of free and immobilized laccases

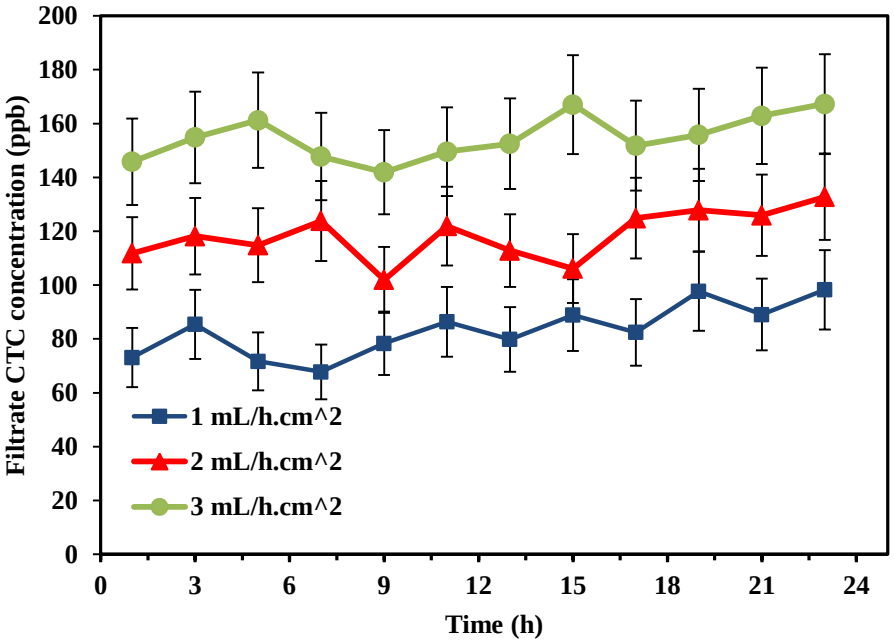

Figure 3.2.7 Degradation of chlortetracycline in continuous mode

Part 3

Covalent Immobilization of laccase onto nanofibrous membrane for degradation of pharmaceutical residues in water

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Résumé

La dégradation enzymatique en utilisant un enzyme ligninolytique, ici la laccase, est une solution verte pour l'élimination potentielle de composés pharmaceutiques qui sont libérés dans l'environnement par le rejet des eaux usées. Cependant, plusieurs inconvénients de l'utilisation d'enzyme dans ses formes libres ont été mis en évidence telles que la réutilisabilité et la stabilité qui devraient être abordés avant leur application industrielle. Dans cette étude, la laccase a été immobilisée sur une membrane à base de nanofibres fabriquée à partir d'un mélange polyacrylonitrile-biocharbon. Le biocatalyseur obtenu a été utilisé pour l'élimination du chlorotétracycline (CTC), de la carbamazépine (CBZ) et du diclofénac (DCF) à une concentration pertinente pour l'environnement en mode discontinu. Ces composés pharmaceutiques représentaient trois catégories principales, à savoir les antibiotiques, les antidépresseurs et les anti-inflammatoires. Les résultats ont montré que la laccase immobilisée avait une meilleure stabilité au stockage, à la température et au pH par rapport à la laccase libre. En outre, il a maintenu plus de 17% de son activité initiale après 10 cycles d'oxydation ABTS, ce qui améliore significativement sa réutilisabilité. En utilisant la laccase immobilisée pour la dégradation, trois composés pharmaceutiques dans des expériences discontinues présentaient 72,7%, 63,3% et 48,6% d'efficacité de dégradation pour DCF, CTC et CBZ, respectivement, après 8 h de réaction. La tendance à la baisse de l'adsorption au cours du temps de réaction pour tous les composés a confirmé l'effet régénérateur de la laccase sur les sites d'adsorption du biocharbon.

Mots clés

Laccase, Biodégradation, Eaux usées, Composés pharmaceutiques

Abstract

Enzymatic degradation with ligninolytic enzyme e.g. laccase is a potential green solution for removal of pharmaceutical compounds that are released into the environment through wastewater effluent. However, the deficiencies of using the enzyme in its free forms, such as reusability and stability should be addressed before industrial applications. In this study, laccase was immobilized onto tailor-made Polyacrylonitrile-biochar composite nanofibrous membrane through covalent bonding and the parameters of immobilization were optimized. The obtained biocatalyst was utilized for removal of chlortetracycline (CTC), carbamazepine (CBZ) and diclofenac (DCF) at an environmentally-relevant concentration in batch mode. These pharmaceutical compounds represented three main categories of pharmaceutical compounds i.e. antibiotics, antidepressant, and anti-inflammatory. The results showed that the immobilized laccase has improved storage, temperature and pH stability compared to free laccase. Also, it maintained more than 17 % of its initial activity after 10 cycles of ABTS oxidation which indicated improved reusability of the enzyme. Using immobilized laccase for degradation of three pharmaceutical compounds in batch experiments exhibited 72.7%, 63.3% and 48.6% degradation efficiency for DCF, CTC, and CBZ, respectively after 8 hours of reaction. The decreasing trend of adsorption extent during reaction time for all compounds confirmed the regenerative effect of laccase on adsorption sites of biochar.

Keywords

Laccase, Biodegradation, Wastewater, Pharmaceutical compounds

Introduction

The occurrence of pharmaceutically active compounds (PhACs) in different environmental compartments has attracted attention in the recent decade due to their potential effects on humans and other organisms even at very low concentrations [1]. For example, there are reports on histological alteration in gills of fish caused by tetracycline compounds [2]. Also, there is evidence on the development of antibiotic resistance among bacteria antibiotics [3, 4]. After the intake of PhACs by human or animals, they leave the body as intact substances or metabolites through urine and feces. Since wastewater treatment plants (WWTPs) are not able to inefficiently remove all of these micropollutants, they enter the environment by releasing the effluent into the surface waters [5]. Therefore, an increasing interest was shown by researchers and industries in recent years to develop methods to achieve efficient removal of these compounds. Till date, versatile methods e.g. physical, biological and chemical processes have been proposed and investigated for removal of PhACs from water and wastewater. However, several issues, such as the production of toxic by-products and generation of a waste stream need to be addressed before implementing these methods at large scale [6, 7].

White-rot fungi (WRF) proved to have the capability of breaking organic pollutants into less harmful products under mild conditions. They attack these compounds through the production of intracellular enzymes (cytochrome P450) and extracellular ligninolytic enzymes e.g. laccases and peroxidases [8]. The extracted ligninolytic enzymes, especially laccases, for treatment of wastewater make a promising option due to their oxidizing capability towards a wide range of micropollutants and their lower environmental impact [9]. There are numerous reports on the efficient degradation of different classes of PhACs e.g. antibiotics, anti-inflammatory, psychiatric, etc. by using laccase in its free form [9-11]. However, using free laccase involves reusability problem and also lower stability which increases the cost of operation [1]. Immobilization of enzyme onto porous membranes is a promising approach to push back the mentioned problems and achieve longer shelf-life and stability against pH and temperature [12, 13].

There are many research works in the literature reporting immobilized laccase for removal of pollutants but in most of them, purified laccase was used while using

crude can be more economical. Furthermore, the reported concentrations of micropollutants in the literature (>20 mg/L) is far higher than their real concentrations in municipal wastewater.

The main objective of this research was to evaluate the potential of immobilized laccase on the degradation of a mixture of three representatives of PhACs, namely antibacterial chlortetracycline (CTC), anti-inflammatory diclofenac (DCF) and antiepileptic carbamazepine (CBZ) at an environmentally relevant concentration. These PhACs were selected due to their high consumption and widespread occurrence in WWTPs and also their different physicochemical properties (Table 3.3.1) that affect their fate in WWTPs. The membrane for immobilization of enzyme was a composite of Polyacrylonitrile (PAN) and biochar adsorbent particles which were processed onto nanofibrous membrane through electrospinning. Using biochar for treatment of wastewater is of high interest due to its interesting physico-chemical properties, availability, low cost and role in value-addition to wooden residues. In this work, the activated biochar was entrapped into the nanofibers to create the adsorption capability in the membrane and increase the contact time between the immobilized enzyme and target pollutants [14]. The employed laccase was produced by growing a strain of WRF on a cost-effective substrate and it was covalently bonded to the membrane through a method that has not been reported before for laccase immobilization.

Materials and methods

Chemicals

2, 2'-azino-bis (3-ethyl-benzothiazoline-6-sulphonic acid: ABTS) was provided by Sigma-Aldrich (Oakville, Canada). Tween 80, methanol and glutaraldehyde (purity > 99%) were purchased from Fisher scientific (Ottawa, Canada). HPLC grade water was produced in the laboratory using milli-Q/Milli-Ro system (Millipore, USA). Polyacrylonitrile (PAN), with an average molecular weight of 1.5×10^5 (g/mol), was purchased from Scientific Polymer Product Company (USA). Sodium hydroxide, hydrogen chloride and N, N'-Dimethyl-Formamide (DMF) of analytical grade was purchased from Fisher Scientific (USA). Chlortetracycline (CTC, purity > 97%) was provided by Toronto Research Chemicals (TRC-Canada). Carbamazepine (CBZ, purity ≥99%) was purchased from Sigma-Aldrich (Oakville, ON, Canada). Diclofenac sodium salt (DCF, purity ≥98%) was purchased from Fisher Scientific (Ottawa, ON,

Canada). Apple pomace was provided by Vergers Paul Jodoin Inc., (Quebec, Canada). Biochar was provided by Pyrovac Inc. (Canada) and it originated from 80% of pine white wood and 20% of spruce and fir. This biochar was produced at 525±1 °C under atmospheric pressure for 2 minutes. Biochar was activated through alkali treatment at 800 °C. The detailed process and the properties of activated biochar have been discussed elsewhere [16].

Preparation of inoculum

Fungus, *Trametes versicolor* (ATCC 20869) was grown aerobically in potato dextrose broth (PDB) liquid medium inoculated with lyophilized strain and then the flask was incubated in an orbital shaker at 30±1 °C and 150 rpm for 7 days. Later, 100 µL of PDB medium was used to inoculate the potato dextrose agar (PDA) plates and the plates were kept in a static incubator at 30±1 °C for 9 days.

Solid-state fermentation

For the production of laccase by *T. versicolor*, apple pomace (with 70 wt% moisture and pH 4.5) was used as a cost-effective solid substrate. About 40 grams of apple pomace was added to a 500 mL flask along with 0.5% v/w Tween 80 as inducer, mixed thoroughly and autoclaved at 121±1 °C for 30 min. Later, the substrate was inoculated with the biomass content of one Petri plate obtained in the previous step, and it was kept in a static incubator at 30±1 °C for 15 days.

Enzyme extraction and assay

One gram of fermented sample, prepared in the previous step, was added to 20 mL of sodium phosphate buffer (50 mM, pH 4.5), mixed thoroughly for 1 h at 35±1 °C and then centrifuged at 7,000 × g for 30 min. Then, the supernatant was collected and its relative laccase activity was analyzed by monitoring the oxidation of ABTS at 420 nm (ε_{420} = 36,000 M^{-1} cm^{-1}) at pH=4.5 and 45±1 °C using a Varian Cary-50 UV-VIS spectrophotometer. Each unit of laccase was defined as the amount of enzyme required to oxidize one micromole of ABTS within one minute under assay conditions. To determine the laccase activity of the immobilized enzyme, 20 mg of the immobilized sample was mixed with 4 mL sodium phosphate buffer (pH 3.5) containing 0.5 mM ABTS and after 3 min of incubation at 45 °C, the absorbance at

420 nm was recorded. The measurement was performed in triplicate and the averages have been illustrated along with the related standard deviations.

Preparation of PAN-biochar membrane

About 2 g of PAN was dissolved in DMF (12%, w/v) and stirred on a magnetic stirrer for one day. Activated biochar at the ratio of 1.5 % (w/w) of PAN was introduced to the solution and the mixture was stirred for another day. PAN-biochar nanofibrous membrane was produced through electrospinning process by using a rotary drum collector (length= 25 cm, diameter= 10 cm) at 25 °C and 35% relative humidity. The electric field strength, flow rate and rotational speed of collector were adjusted to 1.4 KV/cm, 3 mL/h, and 400 rpm, respectively. Also, the distance of the needle tip to the center of collecting drum was adjusted to 18 cm and the needle gauge was 22. The process continued for 8 h and the fabricated mat was washed with methanol and distilled water for removal of residual DMF. Later, the nanofibrous membrane was dried for 10 h at 50±1 °C.

Immobilization of laccase onto PAN-biochar membrane

The membrane sample was cut into pieces of 9 cm^2 and precisely weighed. They were immersed in 1M NaOH (15 mL) and kept at 40 °C for 1 h. The samples were washed with ultrapure water until they reached neutral pH. The samples were then immersed in 50 mL of 10% v/v HNO_3/H_2SO_4 (50:50 v/v) and kept at 25 °C for 2 hours. In this process, COOH groups are formed onto PAN and biochar and the number of these groups were quantified by using a method described elsewhere [1]. The functionalized PAN/biochar samples were treated with 30 mL of 10% (v/v) ethylenediamine at 25 °C for 1 h and then washed with MQ water. The modified samples were equilibrated with 10 mL of sodium phosphate buffer (pH 7.0) for 4 h, rinsed and transferred to a 20 mL solution containing different amounts of glutaraldehyde (2, 4, 6, 8, 10% v/v) for 1 hour at 25 °C. The modified samples containing glutaraldehyde were washed thoroughly with 0.1 M sodium phosphate buffer (pH 7.0). Finally, the activated samples were treated with 20 mL of enzyme solution (1 g/L) for varied time periods (2, 5, 10, 15, 20 h), pH values (2, 3, 4, 5, 6, 7) and temperatures (5, 10, 15, 20, 25 °C). The immobilized laccase samples were then taken out and thoroughly rinsed with 0.1 M solution of phosphate buffer. In Figure

3.3.1**Error! Reference source not found.**, the processes involved in immobilization f laccase are illustrated.

Optimal pH and temperature

For evaluation of optimal pH, 50 µL of free laccase and 20 mg of immobilized samples were added to separate tubes containing 3 mL of buffers at different pH levels (2 to 9) and incubated at 25 °C and 200 rpm for 24 h. Later, the laccase activities of free and immobilized samples were measured at same pH. For optimal temperature, samples were incubated at different temperatures (20–70 °C) for 1 h at constant pH of 3.5 and then the activities were measured at the same temperature.

Enzyme stability

To evaluate the storage stability of the immobilized laccase, samples of free and immobilized laccase were kept at 4 °C and 25 °C for two months and their laccase activity was measured for two months period. The measuring procedure was performed in triplicates and the average along with the standard deviation is presented in figures. The ANOVA, provided by Excel software, showed p-value less than 0.01 for each graph which confirmed that the changes in the laccase activity are directly related to changes in studied parameters i.e. pH, temperature, time and number of cycles.

Characterization of fabricated NFM

The surface morphology of the fabricated NFM was examined using an EVO-50 (Zeiss, Germany) scanning electron microscope (SEM). The acceleration voltage was 10 kV and prior to analysis, the sample was coated with a thin layer of gold using an SPI Module sputter coater.

Biodegradation test

The efficiency of immobilized laccase on PAN-biochar nanofibrous membrane for degradation of three PhACs as representatives of different categories of pharmaceutical compounds was investigated in batch mode. A 3×3 cm^{-2} of prepared biocatalyst along with 50 mL aqueous solution containing CTC, CBZ and DCF concentration of 200 ppb at different pH values (4, 5, 6 and 7) was added to a 100 mL flask and incubated at room temperature. Samples for measuring the

concentrations of compounds in solution were taken at each 2-hour interval for 8 hours. Also, samples of biocatalyst were collected and a number of compounds adsorbed onto membrane were measured through desorption into methanol after 24 h of incubation at room temperature. The concentration of compounds was estimated by using Laser Diode Thermal Desorption (LDTD) (Phytronix technologies, Canada) coupled with an LCQ Duo ion trap tandem mass spectrometer (Thermo Finnigan, USA). The details of the analytical methods are described elsewhere [17-19].

Results and discussion

Functionalization and immobilization

In the first step of functionalization (Figure 3.3.1), carboxylic groups were formed on the surface of PAN-biochar nanofibrous membrane and the amount of these groups was quantified to be 5.6 mmol/g. Figure 3.3.2 demonstrates the SEM micrograph of PAN nanofibers with and without laccase immobilization. The surface of nanofibers before laccase immobilization is quite smooth but after immobilization, they showed a rough texture which may be due to the effect of acid treatment as well as binding of enzyme macromolecules. We already demonstrated that the entrapment of biochar particles into the nanofibers can increase the specific surface area of nanofibrous membrane to more than 12 m^2/g. These adsorbent particles can adsorb the micropollutants from solution and prepare enough time for degradation with laccase [14].

Effect of process variables on immobilization

An illustrative scheme for laccase immobilization on PAN-biochar nanofibrous membrane is shown in Figure 3.3.1. During the process, one of the amine groups in ethylenediamine reacts with the carboxylic group to make amide group and then the other amine group reacts with one of the aldehyde groups in glutaraldehyde. Finally, the other free aldehyde group reacts with amino groups in the enzyme to form imino group (–CH=N–). The properties of the immobilized enzyme are significantly affected by the immobilization parameters and therefore it is important to discuss the importance of main variables in laccase immobilization. Laccase was immobilized for different times varying from 2 h to 20 h and the effect of time on the activity of

immobilized laccase is illustrated in Figure 3.3.3-A. In this experiment, the temperature, pH and glutaraldehyde concentration were fixed at 5 °C, 4 and 6% (v/v), respectively. According to Figure 3.3.3-A, the maximum activity was obtained after 2 hours of reaction and then a sharp decline was observed in the activity. Jiang *et al.* immobilized laccase on chitosan microspheres and found that 2 hours of immobilization gave the highest activity which is accordance with the results of this research. They explained the sharp decline in activity after 2 hours by reducing the accessibility of the active sites to the substrate due to excessive immobilized enzyme molecules [20]. In a similar research, Trivedi *et al.* found that after two hours of contact time between enzyme and substrate, the activity of the immobilized enzyme is reduced and related this behavior to shear forces [21]. Also, Bayramoglu *et al*. observed a reduction in activity of chloroperoxidase after 9 hours of immobilization onto magnetic particles. They concluded that multipoint attachment of enzyme onto carrier and overcrowding of surface with enzyme molecules are the main reasons for activity reduction after a certain time [22].

To understand the effect of temperature on immobilization, laccase was immobilized at different temperatures, varying from 5 °C to 25 °C. In this experiment, the values of glutaraldehyde concentration, pH and immobilization time were fixed at 6 % (v/v), 4 and 2 h. The effect of temperature on laccase immobilization in terms of activity unit per gram support is shown in Figure 3.3.3-B. According to this figure, by increasing the temperature from 5 °C to 25 °C, the immobilization efficiency decreased continuously from 12.7 U/g to 5.5 U/g. Trivedi *et al*. [21] and Liao and Chen [23] observed same behavior for immobilization of alcohol dehydrogenases which indicated that low temperature was less detrimental to the enzyme during immobilization.

To determine the effect of glutaraldehyde concentration on immobilization, laccase was fixed on PAN-biochar nanofibrous membrane with different concentrations of glutaraldehyde ranging from 2% to 10% (v/v). In this experiment, the temperature, pH and immobilization time were fixed at 5 °C, 4 and 2 h. As shown in Figure 3.3.3-C, the activity of immobilized enzyme increased from 5.39 U/g to 12.79 U/g by increasing the concentration of glutaraldehyde from 2% to 6% (v/v). It can be due to the insufficient aldehyde groups bonded to the surface and poor mechanical strength for keeping immobilized laccase at low concentrations of glutaraldehyde [20]. However, the activity of immobilized enzyme showed a decrease to 2.45 U/g by

further increasing the concentration of glutaraldehyde to 10% (v/v). It is reported that the extensive interaction of single enzyme macromolecules with aldehyde groups on the surface of the carrier can change the conformation of enzyme and decrease its activity [20].

Electrostatic interaction between enzyme and carrier, which in turn is affected by solution pH, can influence the immobilization of laccase onto PAN-biochar nanofibrous membrane. In order to study the pH effect, immobilization was performed at different pH values, varying from 2 to 7. In this experiment, values of temperature, glutaraldehyde concentration and immobilization time were fixed at 5 °C, 6% (v/v) and 2 h and the results are illustrated in Figure 3.3.3-D. According to this figure, by increasing the pH from 2 to 4, the activity was increased from 5.8 U/g to its maximum level i.e. 12.19 U/g. By further increasing the pH level to 6, the activity decreased continuously to 9.07 U/g and it remained constant by increasing pH to 7. The values of the isoelectronic point – the pH at which the molecules carry no net electrical charge– for fungal laccase and PAN nanofibers are reported to be around 4 and 3.6, respectively [24, 25]. Considering the values of isoelectronic points, one can conclude that at pH levels lower than 3.6 and greater than 4, electrostatic repulsion happens between laccase and nanofibrous membrane and can reduce the enzyme loading on support [26]. Therefore, the maximum level of activity should be expected at pH range of 3.6-4.

Effect of temperature and pH on laccase activity

For evaluating the effect of pH and temperature, a sample was fabricated at optimized immobilization conditions discussed in previous section. The pH dependence of activity of free and immobilized laccase under optimized conditions is shown in Figure 3.3.4-A. The optimal pH for free laccase was between 4 and 5 while for immobilized laccase, it was between 3 and 4. The unequal partitioning of OH^- and H^+ concentrations in the microenvironment of the immobilized enzyme and the bulk solution often causes a displacement in the optimal pH toward more acidic values [27]. In this work, it seemed that biochar and the cross-links affected the partitioning of H+ and OH^- and shifted the optimum pH for immobilized enzyme to lower values. Wang *et al.,* reported that the optimum pH for maximal activity of laccase shifted from 3.5 for free laccase to 4 for immobilized laccase onto PAN membrane with metal chelation due to electrostatic interactions between career and

enzyme [28]. In contrast, Xu *et al.* [29] and Catapane *et al.* [30] observed no changes in optimum pH of immobilized laccase onto PAN nanofibers through amidination and diazotization processes and related it to the neutral nature of cross-links and zero surfaces electric charge of PAN. Furthermore, the immobilized laccase in this work showed a lower sensitivity to pH in the acidic range which can be due to the multi-point attachments of enzyme and nanofibers which protect the enzyme from deactivation [31].

The effect of temperature on the activity of free and immobilized laccase is illustrated in Figure 3.3.4-B. The optimal temperature for both free and immobilized enzyme was around 35 °C. However, immobilized laccase showed higher stability at temperatures higher than optimal temperature so that at 70 °C, free laccase retained 19% of its maximum activity, while immobilized laccase retained 50% of its maximum activity. The multipoint attachment and interactions between laccase and membrane reduced the conformational changes at a higher temperature and protected laccase from deactivation. Therefore, the optimum temperature can be shifted to higher values [13]. The reported effects of temperature on the stability of laccase in literature are not in accordance with each other. For instance, although many researchers reported a slight improvement in thermal stability of immobilized laccase (20-45%) [28, 32], Jiang *et al.* observed higher sensitivity of laccase after immobilization onto chitosan compared to free laccase (up to 65 %) [20].

Storage Stability of immobilized laccase

The denaturation of the enzyme is a natural phenomenon that happens over a period of time and results in the depletion of enzymatic activity. Immobilization can restrict the macromolecules and increase their stability [33]. Figure 3.3.5-A illustrates the residual activity of free and immobilized enzymes stored at 25±1 °C and 4±1 °C. Accordingly, the immobilized enzymes showed more than 10% and 94% of their initial activities after 30 days, while the free enzymes showed only 1% and 32% of their initial activities. It indicated that laccase immobilization on PAN-biochar nanofibrous membrane can enhance the storage stability of biocatalyst. This enhancement is due to the restriction of enzyme conformational changes [13]. Similar results for immobilized enzymes on PAN after 20 days of storage at 4±1 °C were reported by Feng *et al.* (52%) [13], Wang *et al.* (50%) [28] and Zhang *et al.* (60%) [34]. Xu *et al.* immobilized laccase on chitosan nanofibers and observed 60%

residual activity after 10 days of storage at ambient temperature, while free laccase showed no activity after 10 days [35]. In a related study, Jiang *et al.* immobilized laccase onto chitosan microspheres and reported 70% of initial activity after 30 days storage at 4±1 °C, while the free enzyme retained 30% of its initial activity [20].

Operational stability of immobilized laccase

Free laccase is soluble in aqueous reaction media and consequently, its discharge with product flow increases the cost of operation. In contrast, immobilized laccase can be reused which in turn decreases the operational cost for practical application. However, denaturation during the process decreases the enzyme activity during repeated usage of the enzyme. Hence, understanding the behavior of immobilized laccase during repeated usage is important for its industrial application. In this research, the operational stability of immobilized laccase was determined by reusing and monitoring the activity of the same immobilized laccase sample for 10 successive cycles of ABTS oxidation. Each run was performed for 3 min and the activity of the sample was measured in each cycle and the results are illustrated in Figure 3.3.5-B. From first to the third run, there is a sharp reduction of activity (52 %) and in next 7 cycles, 30% reduction was observed in the activity. The sharp decrease in the activity of laccase is expected due to leaching of some enzyme molecules or denaturation of enzymes as many researchers reported the same behavior [22, 34]. For example, Xu *et al.* immobilized laccase onto PAN nanofibers through covalent bonding (amidination) and reported around 40% of activity reduction after 10 cycles [29]. In a related research, Feng *et al*. immobilized laccase onto PAN nanofibers and observed 35% reduction in laccase activity after 10 cycles of usage [32]. Denaturation of laccase may happen due to the formation of radicals during the reaction with ABTS which can block the enzyme active sites [36].

Biodegradation of pharmaceutical compounds

Laccase is an oxidoreductase enzyme known for non-specific oxidation of organic compounds. The generation of free radicals through four copper ions in laccase is considered as the main mechanism for attacking the organic molecules [10]. In this research, the immobilized laccase onto composite electrospun membrane was employed for degradation of a mixture of three pharmaceutical compounds CTC, CBZ and DCF in different pH values. According to the values of octanol water

partitioning coefficients (Kow), CTC, CBZ, and DCF with Kow values of -3.6, 2.45 and 4.51 are highly hydrophilic, moderately hydrophilic and hydrophobic, respectively which affect their interaction with membrane and biochar. The concentration of each compound in solution was set to 200 ppb since the reported concentrations in literature ranged from several ppb levels in municipal wastewater [37] to several mg/L in pharmaceutical industries effluent [38]. The results of biodegradation tests are presented in Figure 3.3.6. Accordingly, the concentrations of three compounds were reduced gradually during the reaction and the maximum removal of all compounds appeared at pH 5. It indicates that such an enzymatic treatment can be useful for neutral to slightly acidic water and wastewater sources. In drinking water treatment plants and municipal wastewater treatment plants, the pH value of influent streams falls within the range of 6-8 and hence this system has a potential to treat these sources of water.

DCF had the maximum degradation efficiency of 72.7% followed by CTC with 63.3% and CBZ with 48.6% degradation efficiency. Sathishkumar *et al.* and Xu *et al.* used immobilized laccase for degradation of DCF in batch mode and observed complete removal after 5h and 6 h respectively[39, 40]. Also, De Cazes *et al.* employed immobilized laccase onto the ceramic membrane and observed 56% degradation efficiency for tetracycline after 24 h [12]. However, they used high concentration (>12 mg/L) of target compound which is far beyond the environmentally relevant concentrations of pharmaceutical compounds and it is hardly possible to extrapolate the data to lower concentrations. There are also several reports in the literature on integration of adsorbents such as montmorillonite and graphene oxide into nanofibers for laccase immobilization to degrade the micropollutants. It was confirmed that addition of adsorbent improved the removal efficiency [41, 42]. However, the capacity of adsorbents and the regenerative effect of laccase were not investigated in those research works. In this study, comparing the adsorption of target compounds on the membrane showed that CTC and CBZ, which are considered hydrophilic, did not significantly adsorb onto membrane (<3%) while DCF which is a hydrophobic compound was significantly adsorbed (up to 21%) in the beginning of reactions (p-value<0.01). However, the decreasing trend of adsorption extent during reaction time was observed for all compounds and almost for all pH values which confirmed the regeneration of adsorption sites of biochar by the action of laccase. In real world applications, such as tertiary treatment stage of the

wastewater treatment plants, this regeneration capability is very important due to the continuous large flow of the micropollutants through the stage. Furthermore, the capacity of adsorption, the blockage of adsorption sites by non-degradable species and the kinetics of regeneration in real wastewater effluent should be investigated before considering this treatment method for large scale application.

Conclusion

Laccase, obtained from *Trametes versicolor,* was covalently immobilized onto a nanofibrous membrane made by entrapment of activated biochar into PAN nanofiber through electrospinning. The obtained biocatalyst showed the enzyme loading of 12.7 U/g and it was used for degradation of three representative pharmaceutical compounds, namely i.e. CTC, CBZ, and DCF. Compared to free laccase, the stability of immobilized laccase in terms of tolerating high temperature, acidic pH, and long-term storage was improved by up to 32 %, 43% and 66 %, respectively. Employing immobilized laccase for degradation of three pharmaceutical compounds exhibited 72.7%, 63.3% and 48.6% degradation efficiency for DCF, CTC, and CBZ, respectively after 8 hours of reaction. The decreasing trend of adsorption extent during reaction time for all compounds confirmed the regenerative effect of laccase on adsorption sites of biochar.

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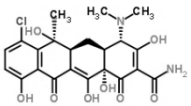
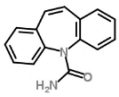
Table 3.3.1 Properties of pharmaceutical compounds investigated in this research

Compound	Molecular structure	Classification	Log Kow	pKa	Characteristics	Ref.
Chlortetracycline (CTC)		Antibacterial	−3.60	3.3, 7.4 & 9.3	toxicity for environment, antibacterial resistance	[15]
Carbamazepine (CBZ)		Anticonvulsant Antiepileptic	2.45	-	Stable structure, low removal by activated sludge	[5]
Diclofenac (DCF)		Anti-inflammatory	4.51	4.15	Worldwide presence in treatment plants, low removal by activated sludge	[5]

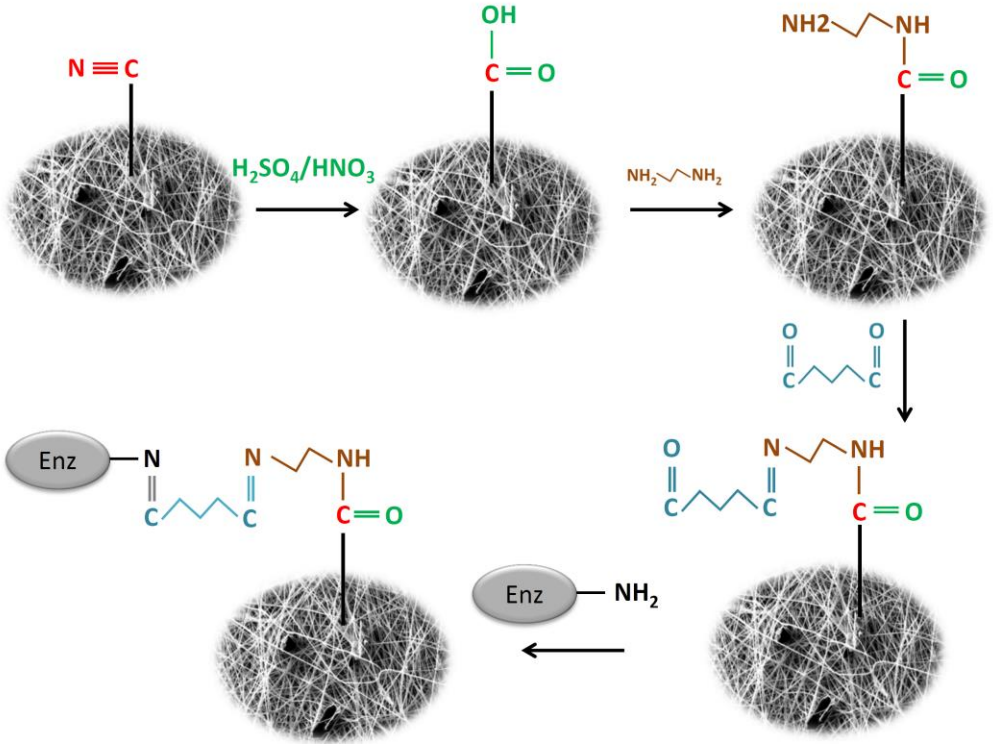


Figure 3.3.1 Immobilization of laccase on PAN-Biochar nanofibrous membrane

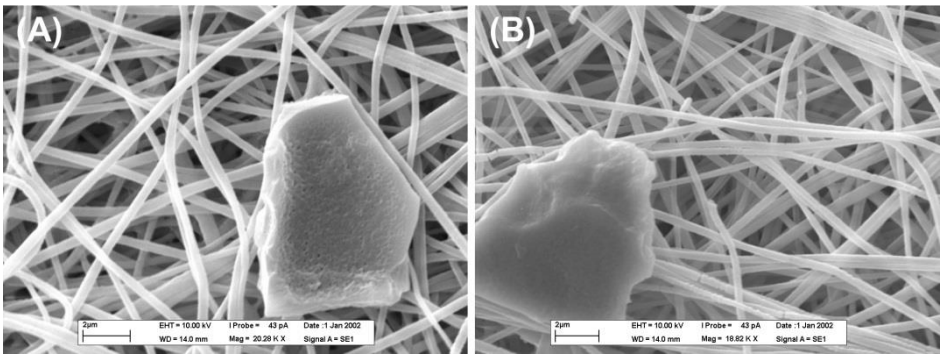


Figure 3.3.2 SEM micrographs of PAN nanofibers A: before laccase immobilization and B: after laccase immobilization

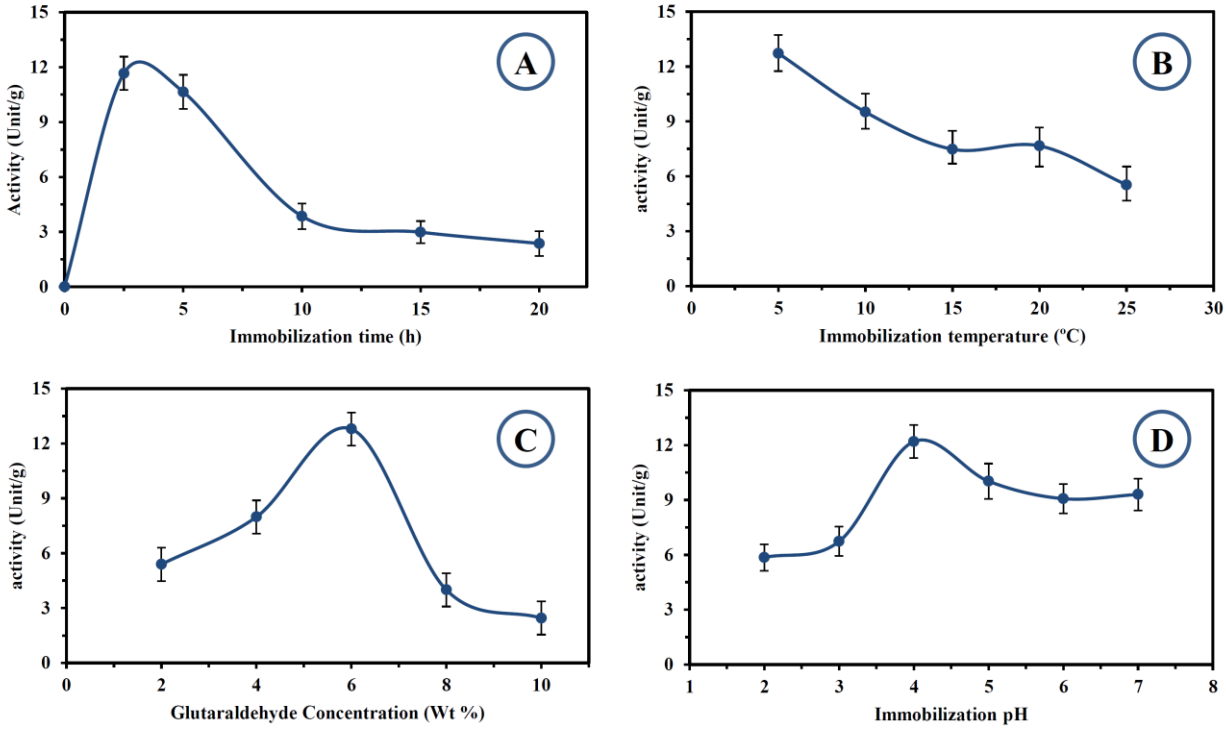


Figure 3.3.3 Effect of: a) time, b) temperature, c) glutaraldehyde concentration and d) pH on immobilization of laccase onto Polyacrylonitrile/biochar nanofibrous membrane

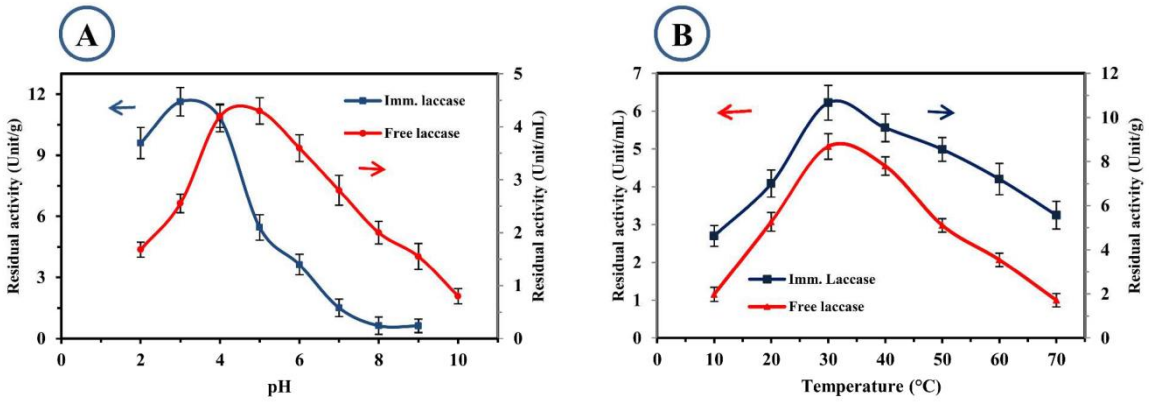


Figure 3.3.4 Effect of: A) pH; and B) temperature on activity of the free and immobilized laccase

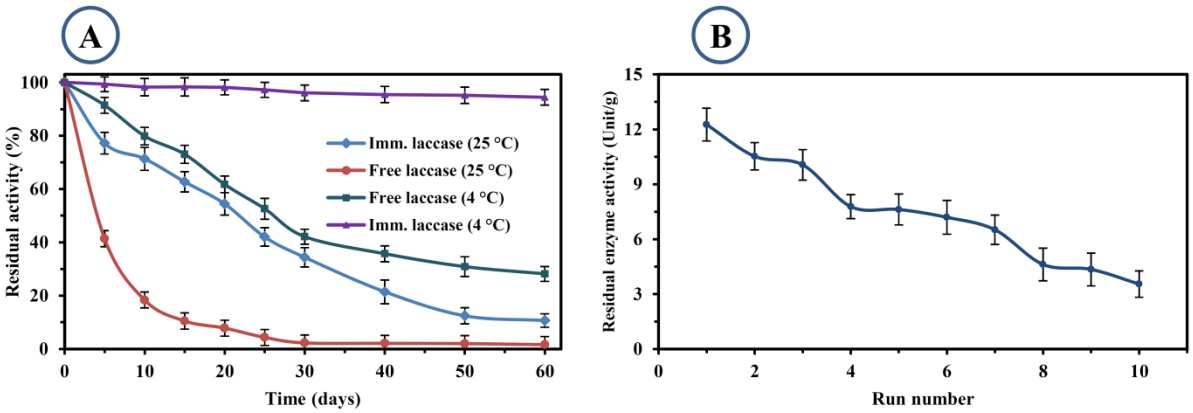


Figure 3.3.5 A) Storage stability of free and immobilized laccases at different temperatures and; B) reusability of immobilized laccase

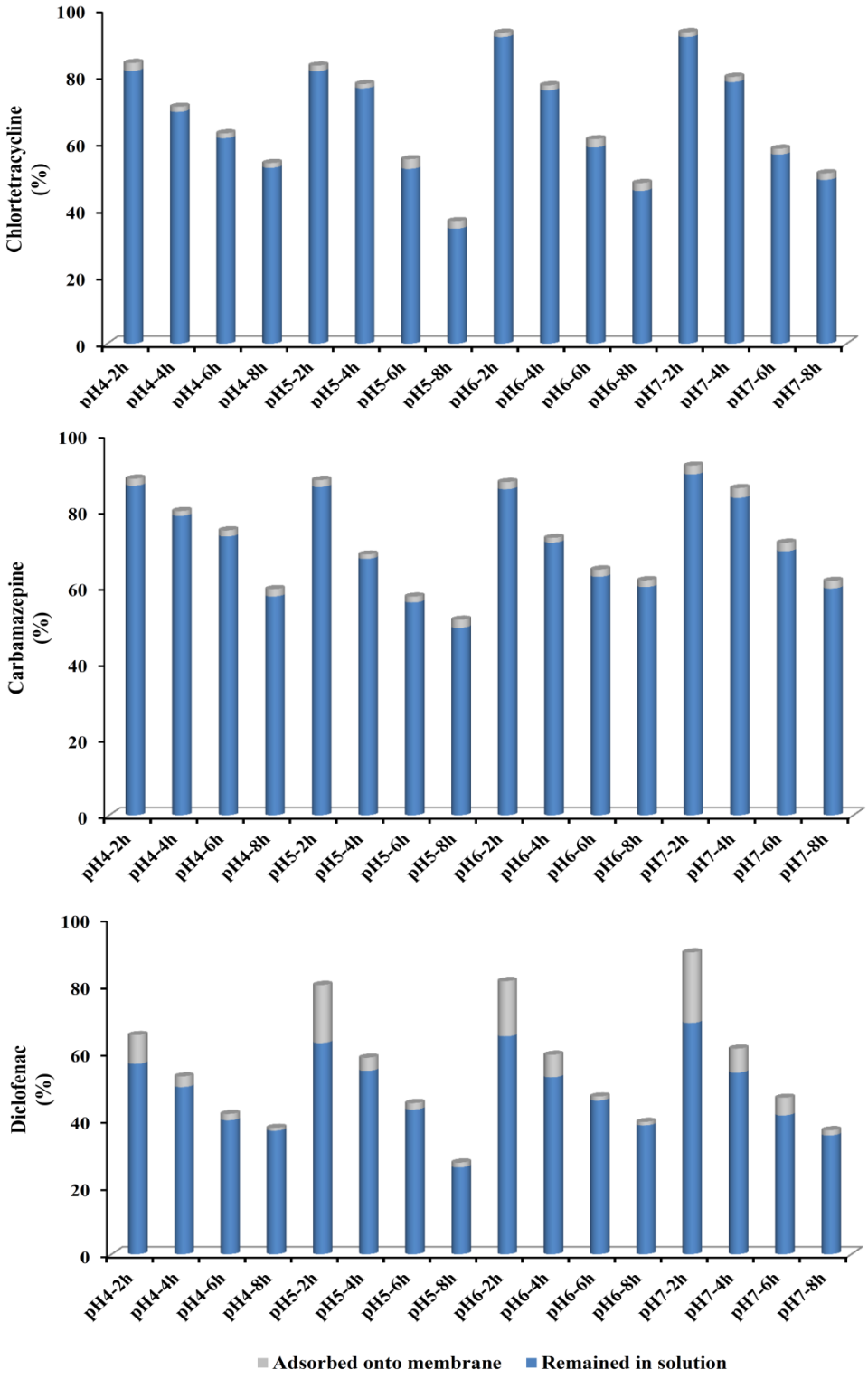


Figure 3.3.6 Biodegradation of pharmaceutical compounds using immobilized laccase

CHAPTER 4

Application of BiMeMS for the removal of PhACs from effluents

Part 1

Development of a multi-functional membrane for removal of pharmaceutical residues from water

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Résumé

La libération de composés pharmaceutiques actifs (PhACs) dans l'environnement par l'effluent d'une usine de traitement des eaux usées soulève des problèmes majeurs à cause de leurs effets potentiels sur la santé des organismes vivants. Le traitement de ces effluents avec l'enzyme laccase est une solution verte potentielle si nous adressons profondément les questions de réutilisabilité et de stabilité de cet enzyme. Dans cette étude, la membrane composite polyacrylonitrile-chitosane (PAN-CTN) a été produite par la méthode d'inversion de phase et la laccase a été immobilisée sur la membrane par une liaison covalente. La membrane d'ultrafiltration-biocatalyseur fabriquée a été utilisée pour l'élimination de trois grandes catégories des PhACs: anti-inflammatoire, antidépresseur et antibiotiques, à savoir le diclofénac (DCF), la carbamazépine (CBZ) et la chlorotétracycline (CTC) en concentration discontinue. Les résultats ont montré que la laccase immobilisée sur la membrane PAN-CTN avait une meilleur stabilité à la température et au pH par rapport au laccase libre. De plus, il n'y a pas eu de perte d'activité qu'après 15 cycles d'oxydation ABTS, ce qui démontre la stabilité remarquable de l'enzyme immobilisé. À température ambiante, la laccase immobilisée a montré une efficacité de dégradation de 61%, 56% et 48% pour DCF, CTC et CBZ dans l'eau pure et 75%, 68% et 57% dans l'effluent secondaire de la station d'épuration.

Mots clés

Laccase, Composés pharmaceutiquement actifs, traitement de l'eau, Carbamazépine, Chlortétracycline, Diclofénac.

Abstract

The release of pharmaceutically active compounds (PhACs) into the environment through the effluent of wastewater treatment plants (WWTPs) has raised concerns over their potential impacts on the health of human and other organisms. Treatment of these effluents with laccase is a potential green solution if the problems with reusability and stability can be addressed. In this study, polyacrylonitrile-chitosan (PAN-CTN) composite membrane was produced through phase-inversion method and laccase was immobilized onto it through covalent bonding. The fabricated membrane-biocatalyst was employed for removal of three representatives of main PhACs: anti-inflammatory, antidepressant and antibiotics namely diclofenac (DCF) carbamazepine (CBZ) and chlortetracycline (CTC) at an environmentally-relevant concentration in batch mode. The results showed that the immobilized laccase onto PAN-CTN membrane had improved temperature and pH stability compared to free laccase. Also, it did not have activity loss after 15 cycles of ABTS oxidation which indicated the improved operational stability of the enzyme. At room temperature, the immobilized laccase showed 61%, 56% and 48% degradation efficiency for DCF, CTC and CBZ in distilled water and 75%, 68% and 57% in the secondary effluent of WWTPs. The results are promising for removal of PhACs from wastewater at large scale in the future.

Keywords

Laccase, Pharmaceutically active compounds, water treatment, Carbamazepine, Chlortetracycline, Diclofenac

Introduction

A significant portion of prescribed pharmaceutically active compounds (PhACs) leave the body of patients as either metabolites or intact compounds through urine and feces and find their way into wastewater treatment plants (WWTPs) [1]. Since WWTPs are not efficient in removing these micropollutants, they end up into surface and ground waters [2]. The occurrence of these compounds affects the human and ecosystem [3]. For example, development of antibiotic resistance among bacteria has been already evidenced which can lead to a great risk for curing fatal infectious diseases [4, 5]. Therefore, lot of attention has been drawn from researchers toward this issue to develop new techniques to retrofit current technologies of wastewater treatment. Some techniques, such as osmosis membranes, advanced oxidation, adsorption onto carbonaceous materials and biological treatment showed high capabilities for removal of these pollutants from water and wastewater [2]. However, they are not still commercialized due to their related issues, such as formation of toxic by-products and waste streams [6, 7]. Using ligninolytic enzymes, such as laccases (EC 1.10.3.2) for treatment of wastewater is an interesting option since they are able to oxidize a wide range of organic pollutants without environmental impacts [8]. Immobilization of enzymes on different supports e.g. nanosized particles and membranes have been investigated since free enzymes are difficult to recover and suffer from low stability [3].

There are numerous researchers who reported laccase immobilization on different supports for removal of micropollutants, but purified laccase was used in most of them while crude form can reduce the costs. Also, the tested concentrations of target compounds (>20 mg/L) were beyond their real concentrations in the environment (<1 mg/L). Furthermore, if these systems are intended to retrofit tertiary treatment stage of WWTPs, they need to resist against bacterial attack. In our previous study, we immobilized laccase onto fabricated polyacrylonitrile-biochar electrospun membrane in order to degrade several PhACs [9]. However, the immobilization method included several costly and time-consuming steps and the final product did not have antibacterial property. Furthermore, the efficacy of biocatalyst was not studied for real WWTP effluent and at different operational temperature levels.

The present study was intended to investigate the potential of a polyacrylonitrile-chitosan (PAN-CTN) composite membrane for immobilization of laccase and its

performance in degradation of three different PhACs at an environmentally relevant concentration. The degradation tests were performed in ultrapure water real as well as WWTP effluent and at different temperature levels. Different concentrations of CTN were mixed with PAN to fabricate composite membranes (PAN-CTN). Using CTN into membrane is of high interest due to its role in facilitating the immobilization through elimination of further steps of membrane functionalization as well as protecting the membrane against bacteria. The representative PhACs i.e. diclofenac (DCF), carbamazepine (CBZ) and chlortetracycline (CTC) were selected due to their widespread occurrence in WWTPs, high consumption and also their versatile physicochemical properties (Table 4.1.1) that determine their fate in WWTPs. The required laccase in this research was obtained by growing *Trametes versicolor* (*T. versicolor*) fungi on apple pomace and it was immobilized onto composite membrane through covalent bonding.

Materials and methods

Chemicals and Microorganisms

PAN, with an average molecular weight of 1.5×10^5 (g/mol), was provided by Scientific Polymer Product Company (USA). CTN (600–800 KDa and 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid: ABTS) were purchased from Sigma-Aldrich (Oakville, Canada). Glutaraldehyde, methanol and Tween 80 (purity > 99%) were provided by Fisher scientific (Ottawa, Canada). Ultrapure water was produced using milli-Q/Milli-Ro system (Millipore, CA, USA). N, N'-Dimethyl-Formamide (DMF), hydrogen chloride, and sodium hydroxide with analytical grade were supplied by Fisher Scientific (Hampton, NH, USA). CBZ (purity ≥99%) was provided by Sigma-Aldrich (Oakville, Canada). CTC (purity > 97%) was purchased from Toronto Research Chemicals (Toronto, Canada). DCF (purity ≥98%) was supplied by Fisher Scientific (Ottawa, Canada). Apple pomace was supplied by Vergers Paul Jodoin Inc., (Quebec, Canada). Biochar was provided by Pyrovac Inc. (Quebec, Canada) and it composed of 80% (w/w) of pine white wood and 20% of fir and spruce. This biochar was produced by heating at 525±1 °C under atmospheric pressure for 2 min. Biochar was activated by alkali treatment at 800 °C for 2 h. The detailed method for biochar activation have been discussed elsewhere [10]. White rot Fungus, *T.*

versicolor (ATCC 20869) and Bacterium *Escherichia coli* (*E. Coli,* ATCC 10798) were donated by Northern Region Research Laboratories (NRRL) (Peoria, IL, USA).

Production of crude laccase

T. versicolor was aerobically grown on apple pomace (with 70% (w/w) moisture and pH 5). In brief, 40 grams of apple pomace was mixed with 0.5% (v/w) Tween 80 as inducer and autoclaved at 121±1 °C for 20 min. Later, the substrate was inoculated with the biomass and kept in a static incubator at 30±1 °C for 15 d.

Enzyme extraction and assay

One gram of fermented sample was mixed with 20 mL of sodium phosphate buffer (50 mM, pH 4.5) for 1 h at 35±1 °C and then centrifuged at 7,000 × g for 30 min. The supernatant was separated and the laccase activity was analyzed by monitoring the ABTS oxidation at 420 nm (ε_{420} = 36,000 M^{-1} cm^{-1}) at pH=4.5 and 45±1 °C using a Varian Cary-50 spectrophotometer. Each unit of laccase was defined as the required amount of enzyme to oxidize one micromole of ABTS in one minute under assay conditions. To determine the laccase activity of immobilized laccase, 20 mg of the immobilized sample was mixed with sodium phosphate buffer (4 mL, pH 3.5) containing 0.5 mM ABTS. After incubation of the mixture for 3 min at 45 °C, the absorbance at 420 nm was recorded. The measurement was carried out in triplicates and the averages have been depicted along with the related standard deviations.

Preparation of PAN-CTN membrane

CTN (8 g) was dissolved in 200 mL of water with 6 g methane sulfonic acid at room temperature for 1 h. The solution was then freeze-dried at −50 °C for 2 d. The polymeric solutions (20 %, w/v) with PAN to CTN weight ratio of 100:0, 95:5, 90:10, 85:15, and 80:20 were prepared in DMF/DMSO solvent mixture (50:50, v/v) at 50 °C for 6 h. The films were made using an applicator on a glass plate (20 cm × 20 cm) and then dipped into methanol at room temperature for 30 min followed by dipping in a 1% (w/w) sodium hydroxide (NaOH) solution for 60 min. Finally, the samples were thoroughly rinsed with distilled water.

Antibacterial test on fabricated membrane

For growing *E. coli*, a liquid nutrient broth medium (1 g/L beef extract, 2 g/L yeast extract, 5 g/L peptone and 5 g/L NaCl) was prepared. Bacterial strain was grown in liquid medium at 37 °C for 24 h in an incubator with the rotational speed of 150 rpm. The colony forming units (CFU) of *E. coli* per mL of liquid medium was measured using solid medium containing 15 g/L agar-agar and above mentioned nutrient broth at 37 °C for 24 h. One mL of the liquid culture of *E.coli* was subjected to serial dilution with 9 mL of saline water and incubated through streak-plate method at their related conditions. The total bacterial count was 4.1 10^9 CFU/mL.

The PAN/CTN membrane samples were challenged with *E. coli* to evaluate their antibacterial properties through AATCC Test Method 100-2004 [11]. Aliquots of bacterial suspension (200 µL) were added to 4 cm^2 of the samples swatch and they were sandwiched with a second swatch for maximum contact between the bacterial cells and the membrane. The samples were kept at room temperature for 30 min and then they were quenched with 5.0 mL of sterile saline solution to release the viable bacteria. The CFU counting was performed for quenched samples as mentioned earlier. The number of viable bacteria was determined in triplicates and the averages were reported.

Immobilization of laccase onto PAN-CTN membrane

About 100 mg of PAN-CTN sample was reacted with 20 mL of a 5% (w/w) glutaraldehyde aqueous solution for 8 h and washed several times to remove excess glutaraldehyde. In the next step, the sample was incubated with phosphate buffer (pH 7.0, 0.1 M) for 10 h and then added into 20 mL of phosphate buffer (pH 7.0) containing laccase at 1 g/L concentration. The mixture was incubated at 25 °C and for 1 h followed by incubation at 4 °C for 3 h. The samples were thoroughly washed with phosphate buffer (pH 7.0, 0.1 M) until no laccase could be detected in the washing liquid. The immobilized laccase was kept in phosphate buffer at 4 °C.

Optimal pH and temperature

The temperature-activity profile of free and immobilized laccase on PAN-CTN was determined at pH 3.5 in the temperature range of 20 °C to 60 °C. Similarly, the pH-activity profile was determined in the pH range of 2.5–8.5 at 45 °C.

Enzyme operational stability

Consecutive ABTS oxidizing cycles were carried out to evaluate the operational stability of the immobilized laccase. At the end of each cycle, the immobilized laccase was thoroughly washed with phosphate buffer, and the procedure was repeated with fresh ABTS. The measurement was performed in triplicates and the average along with the standard deviation is presented in Figures 1, 4, 5, and 6. The ANOVA, provided by Excel software, showed p-value less than 0.01 for each graph which confirmed the direct relation between the changes in the laccase activity and changes in studied parameters i.e. pH, temperature, time and number of cycles.

Characterization of membrane

An EVO-50 (Zeiss, Jena, Germany) scanning electron microscope (SEM) was employed for examination of the morphology of fabricated membranes. The acceleration voltage was 10 kV and, the sample was coated with a thin layer of gold prior to analysis. Fourier transform infrared-attenuated total reflectance (FTIR-ATR) spectra were recorded using a Nicolet iS50 spectrometer (Thermo Scientific, Waltham, MA, USA) with resolution of 0.04 cm^{-1} in the range of 400–4000 cm^{-1}.

Biodegradation test

The efficiency of immobilized laccase on PAN-CTN membrane for degradation of three PhACs as representative of different categories of pharmaceutical compounds was investigated in batch mode. A 4×4 cm^2 of prepared biocatalyst along with 50 mL pure water or secondary effluent of WWTP spiked with CTC, CBZ and DCF at 200 ppb was added to a 100 mL flask and incubated at different temperatures (10 °C, 25 °C and 40 °C). Sampling for measuring the concentrations of three compounds in solution was performed at each 6 h interval for 24 h. A positive control (samples with pharmaceutical compound and without biocatalyst) and a negative compound (distilled water and WWTPs' effluent without pharmaceutical compounds and biocatalyst) was considered in the experiments. The experiments were performed in triplicate and the average results were reported. The concentrations of compounds were determined using Laser Diode Thermal Desorption (LDTD) (Phytronix technologies, Quebec, Canada) coupled with triple quadrupole tandem mass spectrometer (Thermo Scientific, Waltham, MA, USA). The details of the analytical methods are described elsewhere [12-14].

Results and discussions

Antibacterial activity of PAN-CTN membranes

The antibacterial efficacy of PAN/CTN membranes was determined through AATCC standard method and the results are shown in Figure 4.1.1. It is proved that CTN can disrupt the barrier properties of the outer membrane of bacteria [15]. This disruption comes from the linkage between the anions on the bacterial surface and the polycations of CTN [16]. As expected, the membrane made of pure PAN (the control sample) had very little effect on reduction of bacterial population but the PAN/CTN membranes with CTN loading of 5% to 20% (w/w)revealed 1.6 to 3.6 log reductions against *E.coli* after 30 min of contact time. These efficacies are equivalent to 98.7% to 99.9% reduction in the number of viable bacteria. In a related work, *Kim et al.* obtained 83% reduction in number of *E.coli* after 60 min for PAN/CTN at 85:15 mass ratio [11]. The results are comparable with silver nanoparticle-filled nanofibers which showed 2-4 log reduction after 12-24 h [17, 18]. This property is very important for filtration of surface water and WWTP effluent which are known for fouling and deterioration of membranes as a result of bacterial activity.

The cross-sectional area surfaces of the membrane made from PAN and PAN-CTN (85:15, w/w) were explored by electron microscopy (Figure 4.1.2). The micrograph for PAN membrane exhibited a denser structure compared to PAN-CTN membrane which can be due to the different coagulation rate between the surface and inner position of the films [11]. No phase separation is visible in the micrograph in Figure 4.1.2-B which indicated that CTN was uniformly blended in the PAN matrix. Also, the FTIR spectra (Figure 4.1.3) of immobilized laccase onto PAN-CTN membrane (85:15, w/w) showed the charactristic peaks of the three constituents, namely 1450 cm^{-1} ($–CH_2$ in PAN), 1655 cm^{-1} (amide I in CTN), 2245 cm^{-1} ($C\equiv N$ in PAN), 1735 cm^{-1} (C=O in laccase) and 3350 cm^{-1} (NH_2 in laccase and CTN) [19, 20]. It indicated the uniform blending of PAN and CTN as well as uniform immobilization of laccase onto the composite membrane.

Immobilization of laccase onto PAN-CTN membranes

During the immobilization reaction, the amino group of enzyme interacted with aldehyde group on the surface of CTN to form imino group (–CH=N–). Also, it is possible to immobilize some enzyme through electrostatic adsorption which is

governed by pH of the enzyme solution. In this research, immobilization process was carried out at pH 7 which was already reported as the optimum pH [21]. The results of laccase immobilization on PAN/CTN membranes at different CTN loadings are illustrated in Figure 4.1.1. According to this figure, by increasing the CTN loading from 5% to 20% (w/w), the laccase activity increased from 2.25 U/g to 5.77 U/g. This is due the increasing of amine groups on the surface of membrane which enables the formation of more imino groups for possible immobilization of enzyme. For pure PAN membrane, around 0.4 U/g of activity was observed which can be attributed to the entrapment of enzyme molecules in the macropores of membrane. The sample with 15% (w/w) of CTN was selected for further experiments since at higher loadings of CTN, the membrane was brittle and difficult to handle.

Effect of pH and temperature on the activity of Immobilized laccase

The pH dependence of enzymatic activity of immobilized and free laccase is shown in Figure 4.1.4. The optimal pH for both free and immobilized laccase was around 5. The difference in partitioning of OH^- and H^+ concentrations in the surrounding microenvironment of the enzyme in free and immobilized forms often causes a shift in the optimal pH toward acidic values [22]. However, in this study, the optimal pH of the immobilized laccase was not shifted towards more acidic values as predicted from the presence of cations in CTN. Similar results have been observed by other researchers [21, 23]. Xu *et al.* and Catapane *et al.* immobilized laccase onto PAN nanofibers and observed no changes in optimum pH due to the zero surface electric charge of PAN and the neutral nature of cross-links i.e. amidination and diazotization [24, 25].

Furthermore, the immobilized enzyme showed lower sensitivity to pH variation in the acidic range due to the multi-point attachments between the enzyme and the membrane which protect the enzyme from denaturation [26].

The effect of temperature on the activity of immobilized and free laccase is illustrated in Figure 4.1.5. The immobilized laccase exhibited higher stability at higher temperatures so that at 70 °C, immobilized laccase preserved 52% of its maximum activity while free laccase preserved only 19% of its maximum activity. The higher stability at high temperatures can be attributed to the interactions and multi-point attachment between the membrane and the enzyme which reduce the conformational changes and protect the enzyme from denaturation. Therefore, the

optimum temperature can be displaced to higher values [27]. In this study, the optimal temperature for free laccase was around 35 °C, while for immobilized system, it was around 50 °C. The reported effect of immobilization on the laccase optimal temperature and thermal stability in literature are not in accordance with each other. For example, Jiang *et al.* observed -5 °C shift in optimal temperature and higher sensitivity after immobilization of laccase [21] while Feng *et al.* reported +5 °C shift in optimal temperature and a slight improvement in thermal stability of immobilized laccase [28]. However, having higher thermal stability enables the biocatalytic system to work at higher temperature (>40 °C) which brings increased activity and consequently enhanced removal efficiency.

Operational stability of immobilized laccase

The discharge of free laccase with product flow of enzymatic reactor increases the cost of operation while reusability of immobilized laccase can lead to decreasing the cost for practical application. However, even in the case of immobilized enzymes, deactivation can happen during repeated utilization of the enzyme. Therefore, acquiring knowledge on the behavior of immobilized enzyme during repeated utilization is essential for industrial application. The operational stability of immobilized laccase in this research was investigated by reusing the same sample of immobilized laccase for 15 successive ABTS oxidation cycles. In each cycle, the sample was in contact with ABTS for 3 min and then the activity was measured and the results are presented in Figure 4.1.6. Normally, immobilized enzymes show a sharp decrease after first cycle due to leaching or denaturation of enzymes and after the first cycle, depleting the catalytic activity happens continually [29, 30]. For example, Xu *et al.* and Feng *et al*. reported 40% and 35% of reduction in activity after 10 cycles for immobilized laccase onto PAN nanofibers [24, 28]. However, in this research, from the first cycle to the seventh cycle, the laccase activity increased from 4.46 to 10.62 U/g and then in the next eight cycles, it was gradually reduced to 6.8 U/g. Increasing the laccase activity up to seventh cycle indicated negligible leakage or deactivation of enzyme. This increase is due to the fact that some enzymes may occur in different forms i.e. native intermediate, resting oxidized and partly reduced and after catalytic cycles, some enzyme molecules pass from the resting to the catalytic state [31]. Similar results were observed for lipase and laccase immobilized on carbon nanotubes and coconut fiber, respectively [32, 33].

Biodegradation of pharmaceutical compounds

Laccase is known for its non-specific oxidative behavior towards organic compounds. It produces free radicals using four copper ions which can attack organic molecules [34].

As discussed in section 3.3, temperature has a significant effect on degradation efficiency and generally higher temperature is in favor of enzymatic reactions to a certain extent. The working temperature of WWTPs in northern countries, such as Canada is reduced below 10 °C in winter and increased to around 22 °C in summer [35]. Therefore, it is important to have concrete data on the performance of new enzyme-based treatment systems if they are designed to be implemented in WWTPs. In this research, the immobilized laccase onto composite PAN-CTN membrane was used to degrade a mixture of three representative pharmaceutical compounds, namely, CBZ, CTC and DCF at different temperatures (10-40 °C). CTC, CBZ and DCF are highly hydrophilic, moderately hydrophilic and hydrophobic, respectively based on the values of octanol water partitioning coefficients (Table 4.1.1). This property is very important in conventional WWTPs and the treatment systems that require the compound to be adsorbed on the surface. Since the reported concentrations of these compounds in literature ranged from several ppb levels in municipal WWTPs [36] to several mg/L in pharmaceutical industries effluent [37], the concentrations of each compound was set to 200 ppb. The results of biodegradation tests in pure and municipal WWTP effluent are presented in Figure 4.1.7. According to Figure 4.1.7, the concentrations of CTC, CBZ and DCF declined during the reaction and the maximum removal for three compounds happened at 40 °C in WWTP effluent and the minimum removal happened at 10 °C in pure water. The higher removal in WWTP effluent compared to pure water can be related to the presence of different ions, especially copper, which is a cofactor for laccase and can facilitate the oxidation of target compounds [38]. In a similar study, Rong *et al*. showed that addition of copper ions to a solution containing immobilized laccase can enhance the oxidization ability of laccase [39]. Also, the presence of different organic acids that can act as the mediators for oxidoreductase enzymes, may affect the performance of the enzymatic system. In pure water, the final degradation efficiencies were in the range of 40-71%, 36-64 % and 26-59 % for DCF, CTC and CBZ, respectively. In WWTP effluent, the ranges of final degradation efficiency were enhanced to 56-82 %, 47-73 % and 37-65 % for DCF, CTC and CBZ, respectively. In

a related research work, De Cazes *et al.* observed 56% degradation efficiency for tetracycline after 24 h by using immobilized laccase onto ceramic membrane [40]. Similarly, Xu *et al.* and Sathishkumar *et al.* used immobilized laccase to degrade DCF and observed complete removal after 6 h and 5h, respectively [41, 42]. However, the concentration of target compounds (>12 mg/L) were far beyond their environmentally relevant concentrations and the extrapolation of data to lower concentrations was hardly possible. To sum-up, the results of this research indicated that the immobilized laccase onto bacterial resistant PAN-CTN membrane can remove the pharmaceutical compounds at low concentrations with average removal efficiency (>55% at room temperature). Also, the removal efficiency is significantly affected by matrix nature and temperature that need to be considered for large-scale implementation.

Conclusion

Laccase, obtained from *T. versicolor,* was covalently immobilized onto PAN-CTN composite membrane. Increasing the loading of CTN improved the bacterial resistance of the membrane so that the membrane with 15% (w/w) CTN showed 2.8 log reductions against *E.coli* after 30 minutes of contact time. The fabricated biocatalyst, made from membrane with 15% (w/w) CTN, showed the enzyme activity of 4.88 U/g loading and it was used for degradation of three pharmaceutical compounds namely DCF, CTC and CBZ. Compared to free laccase, immobilized laccase showed higher stability in terms of tolerating temperature and pH, variations. At room temperature, the immobilized laccase showed 61%, 56 % and 48 % degradation efficiency for DCF, CTC and CBZ respectively in pure water and 75 %, 68 % and 57 % in the secondary effluent of WWTP. The higher removal rate in the wastewater effluent can be attributed to the presence of laccase cofactors i.e. copper or other organic acids that can act as the mediator for enzyme.

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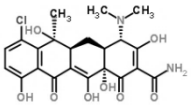
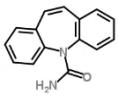
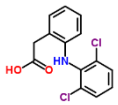
Table 4.1.1 Properties of pharmaceutical compounds investigated in this research

Compound	Molecular structure	Classification	Log Kow	pKa
Chlortetracycline (CTC)		Antibacterial	−3.60	3.3, 7.4 & 9.3
Carbamazepine (CBZ)		Anticonvulsant Antiepileptic	2.45	-
Diclofenac (DCF)		Anti-inflammatory	4.51	4.15

Ref: [9]

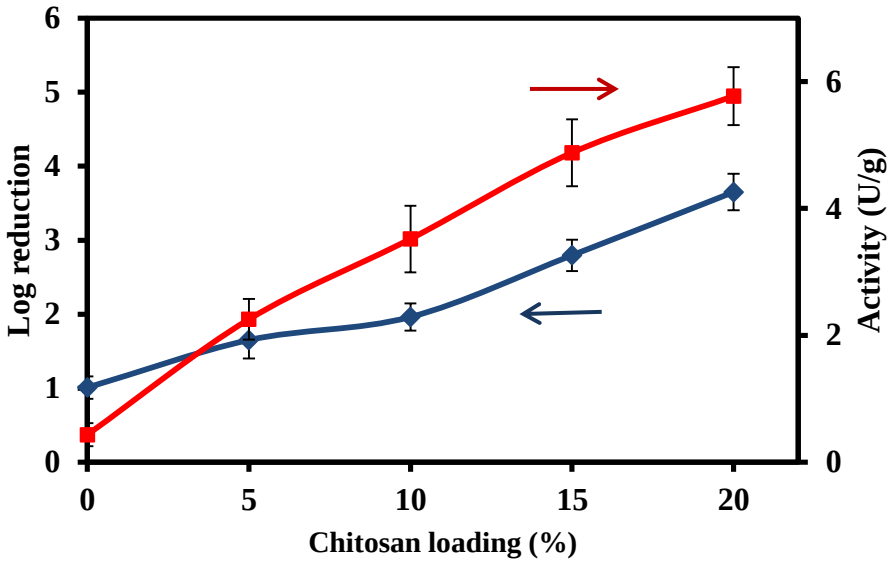


Figure 4.1.1 Antibacterial activity against E.coli and enzyme immobilization capacity of polyacrylonitrile/chitosan membranes (Total bacterial count: 5.2 × 109 CFU/mL)

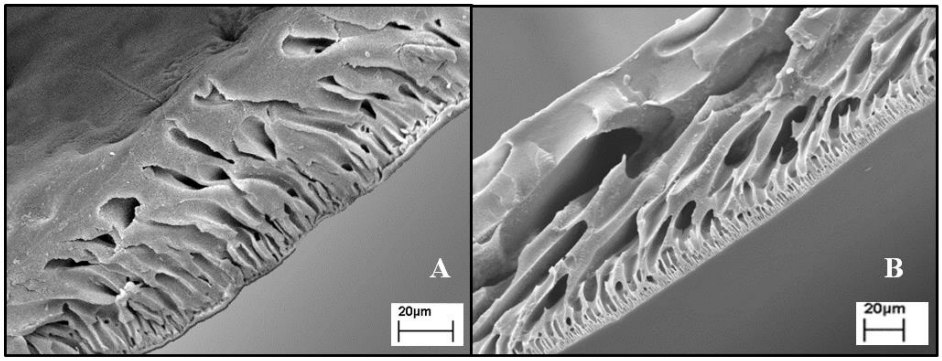


Figure 4.1.2 SEM images of membrane made of: A)polyacrylonitle and; B) polyacrylonitle-chitosan at 85:15 weight ratio

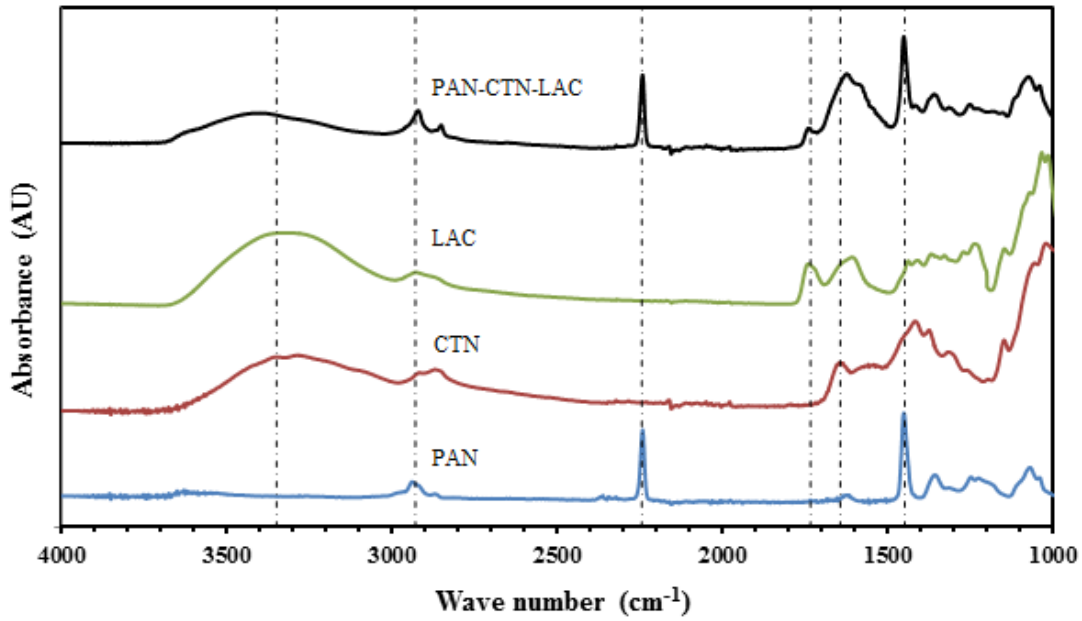


Figure 4.1.3 Infra-red Spectra of polyacrylonitlre (PAN), chitosan (CTN), laccase (LAC) and immobilized laccase onto PAN-CTN membrane (85:15, w/w)

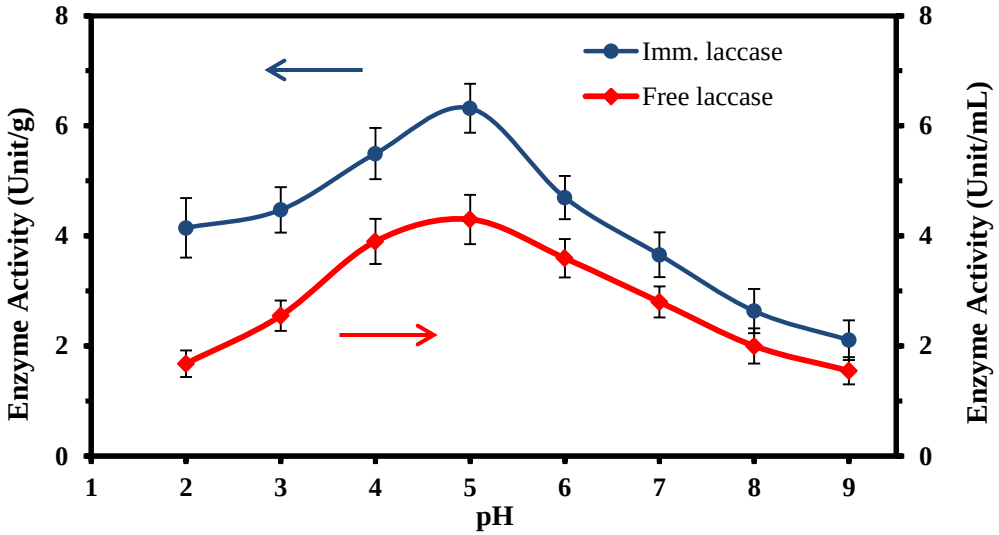


Figure 4.1.4 Effect of pH on the activity of free and immobilized enzyme

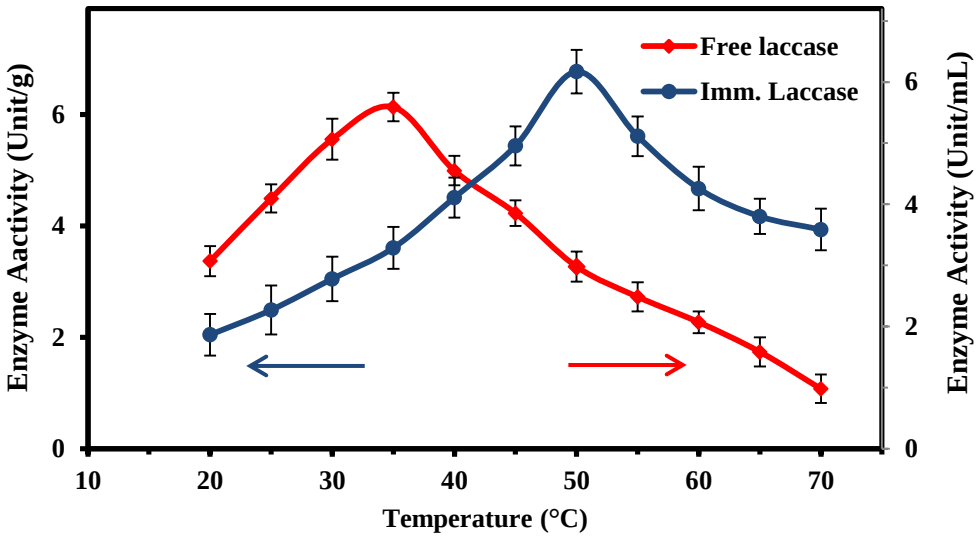


Figure 4.1.5 Effect of temperature on the activity of free and immobilized enzyme

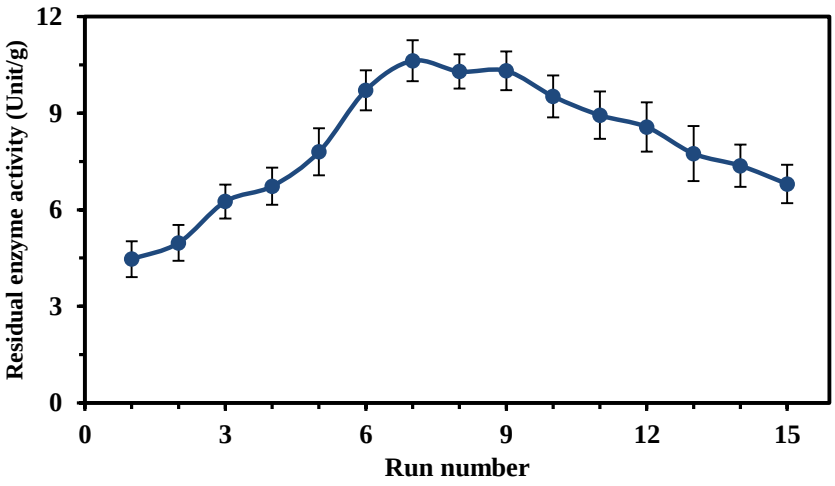


Figure 4.1.6 Reusability of immobilized laccase

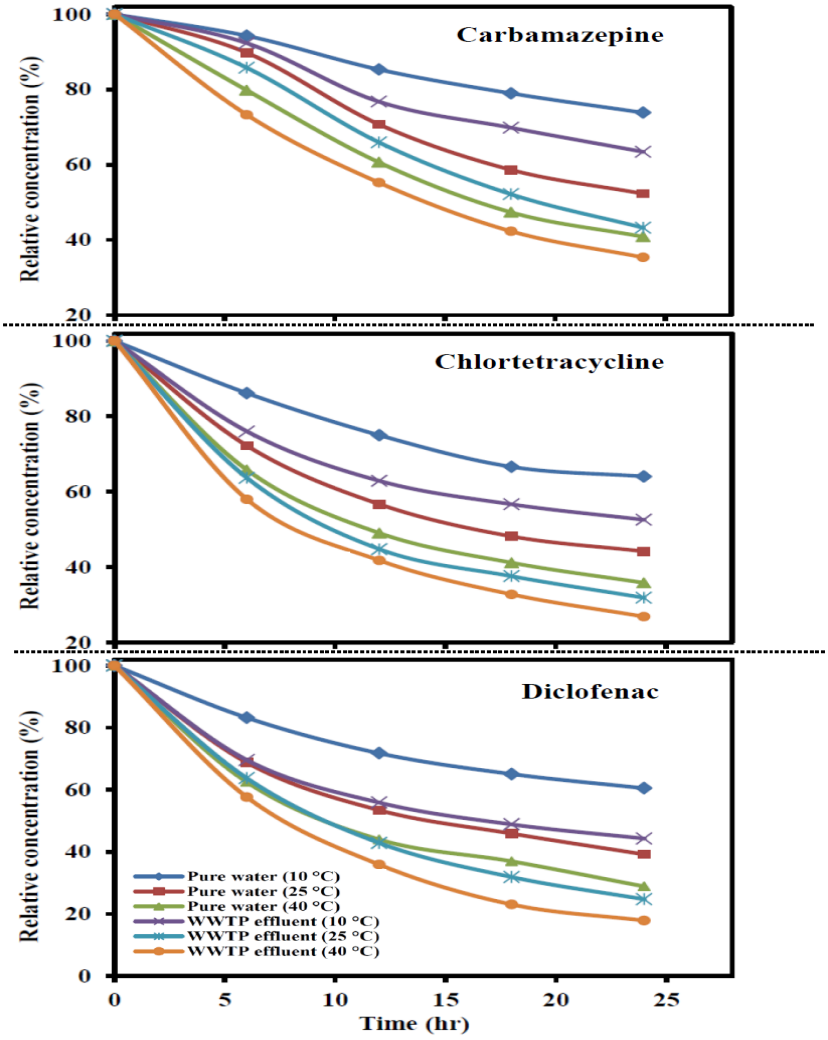


Figure 4.1.7 Biodegradation of pharmaceutically active compounds using immobilized laccase

Part 2

Development of an advanced multifunctional portable water purifier

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Résumé

Le présent travail fournit une nouvelle approche pour fabriquer un dispositif efficace de purification d'eau qui est portatif. Les membranes composites polyacrylonitrile/Chitosane (PAN-CTN) et polyacrylonitrile / Biochar (PAN-BC) ont été réalisées par électrofilage et la laccase a été immobilisée sur membrane PAN-BC (PAN-BC-LAC). Trois membranes composites (PAN-CTN, PAN-BC-LAC et PAN-BC) ont été placées dans une série pour la purification des eaux en termes de micro-organismes, de micropolluants et de turbidité. Le système a montré une performance environ 83% d'élimination des micropolluants, 99% d'élimination des micro-organismes et jusqu'à 77% de réduction de la turbidité en moins de 5 minutes de contact. Cet appareil n'a pas besoin de source d'énergie pour fonctionner et peut éviter l'utilisation de bouteilles d'eau en plastique.

Mots-clés

Purificateur d'eau portable, micropolluants, micro-organismes, turbidité, membrane, enzyme

Abstract

A novel approach to fabricate an efficient portable water purification device was tested. The polyacrylonitrile/chitosan (PAN-CTN) and polyacrylonitrile/biochar (PAN-BC) composite membranes were made through electrospinning and laccase was immobilized on PAN-BC membrane (PAN-BC-LAC). Three layers of composite membrane (PAN-CTN, PAN-BC-LAC, and PAN-BC) were placed in a series for purification of water measured in terms of microorganisms, micropollutants, and turbidity. The system provided around 83% of micropollutant removal, 99% removal of microorganisms and up to 77% of turbidity reduction within less than 5 minutes of the contact time. This device does not need an energy source for functioning and can prevent using plastic water bottles for activities in remote area.

Keywords

Portable water purifier, micropollutants, microorganisms, turbidity, membrane, enzyme

Introduction

Water is the main constituent of human and other living organisms and consumption of enough water on a daily basis is crucial to stay healthy. World Health Organization (WHO) reported that more than a billion people do not have access to clean water which exposes them to the danger of microbial contamination [1, 2]. Approximately, 6% of global deaths are associated with the contagious water-borne diseases. It is widely accepted that investing in the production of clean drinking water has a direct influence on improving economic productivity and human health [3]. Besides, many people in the third world countries lack safe water and people in developed countries need safe water for many activities in the remote areas. In this case, portable water purifier can help provide safe water from ground and surface water sources, such as rivers and lakes. Different types of portable water purifiers are available in the market with varied efficiency for removal of microorganisms, suspended solids, and organic pollutants. These systems work based on physical (activated carbon, membrane) and chemical purification (chlorine pill, UV light). The chemical-based filters are not attractive due to their energy or chemical requirement and their inability in the reduction of turbidity. In one hand physical-based filters, the membranes that work without pump can remove bacteria but, not dissolved pollutants. It is noteworthy that in recent two decades, the presence of emerging contaminants or micropollutants such as pharmaceuticals, pesticides, personal care products, etc. in the environment raised concerns over their potential adverse effects on human and different organisms and therefore their removal seems important [4]. In the other hand, adsorption media can remove dissolved pollutants but it is inefficient for bacteria. Also, the accumulation and growth of bacteria on both membrane and adsorption media [5, 6] may lead to change in the odor and taste of treated water. Therefore, a robust portable water purifier is required which can remove bacteria, reduce color/turbidity and remove dissolved organic pollutants.

The current research paper investigated a three stages design for portable water purifier which is able to remove bacteria, remove and degrade micropollutants and reduce the color and turbidity. In this design, three membranes i.e. antibacterial, biocatalytic and adsorptive were fabricated through electrospinning and placed in series to be able to remove different types of contaminants.

Materials and methods

Materials

Polyacrylonitrile (PAN) was purchased from Scientific Polymer Product Company (New York USA). N, N'-Dimethyl-Formamide (DMF) with analytical grade were supplied by Fisher Scientific (USA). Chitosan (CTN, 600–800KDa) was provided by Sigma-Aldrich (Oakville, Canada).. Carbamazepine (CBZ, purity ≥99%) was provided by Sigma-Aldrich (Oakville, ON, Canada). Biochar was provided by Pyrovac Inc. (Quebec, Canada). Biochar (BC) was activated by alkali treatment at 800±1 °C for 2 hours. The detailed process of biochar activation has been discussed elsewhere [7]. In brief, 10 g of biochar was mixed with 20 g NaOH and mixed in 100 mL of water at room temperature for 2 h and then dried at 80 ± 1 °C for 24 h. The sample was heated under nitrogen blanket to 800 ± 1 °C and held at this temperature for 2 h before cooling down. The product was washed, neutralized and dried at 60 ± 1 °C for 24 h.

Membrane Production

The details of membrane production and enzyme immobilization were reported in our previous works [8, 9]. In brief, 2 g of PAN was dissolved in DMF at 12% (w/v) and stirred for 24 h. Activated BC or CTN at the relevant loadings were added to the solution and the mixture was stirred for 24 h. The membranes were produced through electrospinning process by using a rotary drum collector at 25°C and 35% relative humidity. The flow rate, electric field strength and rotational speed of collector were adjusted to 3 mL/h, 1.4 KV/cm and 400 rpm, respectively. The fabricated membranes were washed with water for removal of residual DMF. Later, the membranes were dried for 10 h at 50±1 °C.

System design

The designed portable water filter had three compartments which can be replaced with new ones. It is made by attaching 4 pieces of polycarbonate tube (OD: 10 cm, ID: 9 cm, L: 8 cm) with hose clamps as represented in Figure 4.2.1. Three kinds of membranes i.e. antibacterial, biocatalytic and adsorptive were fabricated for three stages of portable filter listed in Table 4.2.1. In brief, the first one contains Polyacrylonitrile-Chitosan (PAN-CTN) at the mass ratio of 85:15 which has

antibacterial activity. The second membrane consisted of immobilized laccase onto a composite of PAN and activated biochar (PAN-BC-LAC) at a mass ratio of 95:05. The composition of the third membrane was similar to the second one except that no enzyme was immobilized onto this membrane (PAN-BC). For purification tests, the top part of the system was filled with raw water to pass through membranes by gravitational force. The first 1.0 L of the filtrate was rejected and then 0.5 L of the filtrate was collected for analysis.

Sampling

Random sampling was performed from the influent streams (before any treatment) of 4 drinking water treatment plants in Quebec city, Canada namely Charlesbourg, Quebec, Ste Foy, and Beauport. Sampling was carried out on March 12, 2018. All water samples were immediately cooled to 4±1 °C and the tests were performed within two days.

Analysis

The turbidity of samples before and after passing through portable water purifier was measured using a 2100N Hach turbidimeter (Hach, USA) and it was expressed in nephelometric turbidity units (NTU).

pH was measured using a pH meter (Fisher Scientific brand, Accumet AR25).

A 50 mL of the samples before and after treatment was filtered through a 0.45 µm glass-fiber filter for Dissolved Organic Carbon (DOC) and UV254 analysis. DOC was estimated using a Shimadzu 5000A analyzer (Shimadzu, Japan). To assess the degree of aromaticity of each sample [10], the absorbance at 254 nm was measured using a Cary 50 UV-VIS spectrophotometer (Varian, Australia).

Carbamazepine (CBZ) was spiked into samples at 10 µg/L to assess the efficiency of the water purifier in the removal of micropollutants. The concentration of CBZ in the treated samples was quantified using Laser Diode Thermal Desorption (LDTD) (Phytronix technologies, Canada) coupled with an LCQ Duo ion trap tandem mass spectrometer (Thermo Finnigan, USA). The daughter ions identified for CBZ in mass spectrometer were 194 and 192 Da. The method reporting limit was 10 ng/L. The calibration curve of CBZ concentration was developed with six standard solutions (10 ng/L to 10 µg/L) and with R2 no less than 0.99. The details of quantification process were described elsewhere [11]. Total alkalinity of samples was determined by

titrating 25.0 mL sample of water with dilute hydrochloric acid in the presence of methyl orange as indicator. The total coliform of water samples was determined by membrane filtration technique (Millipore membrane filter, pore size 0.45 um) according to the standard method [12]. The number of total coliforms was expressed as Colony Forming Unit (CFU) per 100 mL of filtered samples. All the experiments were carried out in triplicates and the average results were reported. Analysis of variance (ANOVA) was performed for the data using Microsoft Excel and the results with P value < 0.05 were considered as significant.

Results and discussion

The results of pH, conductivity, turbidity, color, and UV254 test are listed in Table 4.2.2. Accordingly, the pH and alkalinity of all water samples were increased. Increase in the pH was due to the effect of activated biochar and it is very useful in this case since neutral water is more appropriate for drinking compared to acidic water. The turbidity and apparent color of all samples were decreased up to 77% and 89% respectively. The compound effect membrane and activated biochar resulted in the removal of particulate matters and consequently reduced color and turbidity. In a similar study, El-Harbawi et al. made a five-stage filter media (activated carbon, silica sand, zeolite, bio ball, and mineral sand) and observed maximum 55 % removal in turbidity [13]. Obiora-Okafo et al. used activated carbon made from sawdust and rice-husk-ash for purifying drinking water in rural areas and reported up to 95% removal of turbidity and bacteria [14].

The results for UV254, total coliform, dissolved organic carbon and removal of micropollutants are listed in Table 4.2.3. Accordingly, the light absorbance of samples at 254 nm was reduced for all samples after treatment (63-95%) which meant that the number of aromatic compounds in the raw water samples was substantially reduced through the membranes. Counting the total number of coliform bacteria in a water sample is an indirect method to determine the presence of pathogens. In this study, the designed portable filter removed 99-100 % of coliform which is very important for its application i.e. safe water production. This high removal can be attributed to the filtration properties of the electrospun membrane as well as antibacterial properties of chitosan [15-17]. Chauhan *et al*. used some plant extracts as a filter and observed up to 92% reduction of microbial counts [18]. Similarly, Prasad *et al*. studied the rice-husk ash as filter media and observed nearly

98 to 99% removal in bacteria and turbidity with acceptable filtration rates [19]. Also, Michael *et al*. designed a low cost porous ceramic filter which can filter out the majority of harmful microorganisms, particulates and protozoans [20].

The removal of micropollutants from water sources is important since their adverse effects have been already reported. These pollutants find their way into the environment through releasing the effluents of wastewater treatment plants into surface waters, such as lakes and rivers. In this research, CBZ which is a frequently found and most recalcitrant compound in the effluent of wastewater treatment plants was spiked into raw water samples to assess the performance of portable water purifier. The results of CBZ removal for the fabricated filter showed that for all the samples, 65-83 % of the CBZ, as a representative micropollutant, was removed. The removal of CBZ is attributed to the combined effect of biocatalytic and adsorptive membranes in the second and the third stages of the filter. In our previous research work, laccase immobilized onto biochar exhibited almost same removal efficiency [21]. Also, the removal of CBZ is in accordance with the reduction in DOC levels of all samples after treatment, which represents the total dissolved organic matter in water.

The portable water purifier presented in this research exhibited acceptable results for providing safe water from surface water sources. Therefore, this design is promising to use in different regions in which people have access to surface and ground waters but they are not safe to drink. Further tests on this device using different water sources throughout the world can provide additional data to enhance its credibility or to propose modification in the design.

Conclusion

A design of portable water purifier was proposed which was demonstrated to be efficient in the removal of microorganisms, turbidity, and micropollutants from four lake waters in Quebec, Canada. This system comprised of three membrane systems made of a different combination of polyacrylonitrile, chitosan, biochar, and laccase. In the preliminary tests, this system provided around 99% removal of microorganisms, up to 77% of turbidity reduction and 83% of micropollutants removal within less than 5 min of contact time. More work needs to be done regarding subsequent tests to quantify the purification capacity as well as performance on a

different surface and groundwater sources. This portable purifier has nevertheless, several advantages over other traditional devices, such as not requiring energy source or chemicals and simultaneous removal of bacteria and micropollutants.

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Table 4.2.1 Description of purification steps in portable water purifier

no.	Description	Composition	Target
1	Antibacterial membrane	Polyacrylonitrile+Chitosan at 85:15 mass ratio	Bacteria, particulates
2	Biocatalytic membrane	Laccase (10 unit/g) immobilized onto Polyacrylonitrile+Biochar at 95:05 mass ratio	micropollutants
3	Adsorptive membrane	Polyacrylonitrile+Biochar at 95:05 mass ratio	Polishing (Color and odor)

Table 4.2.2 Properties of water samples before and after treatment

Sample ID	pH		Alkalinity (mg/L $CaCO_3$)		Turbidity (NTU[3])		color (ACU[4])	
Charlesbourg-B[1]	5.7		9.2		0.83		15	
Charlesbourg-A[2]	6.8	⬆	18.6	⬆	0.40	⬇	7	⬇
Quebec-B	6.2		29.2		1.28		19	
Quebec -A	7.1	⬆	43.5	⬆	0.29	⬇	2	⬇
Ste Foy-B	6.5		43.2		1.57		16	
Ste Foy-A	7.3	⬆	77.2	⬆	0.36	⬇	3	⬇
Beauport-B	6.5		12.4		0.60		18	
Beauport-A	6.8	⬆	24.1	⬆	0.39	⬇	3	⬇

[1] Before treatment [2] After treatment [3] Nephelometric turbidity units
[4] Apparent color unit

Table 4.2.3 Properties of water samples before and after treatment

Sample ID	Absorbance (UV254) *10[3]	Total Coliform (CFU[3]/100 mL)	CBZ Conc. (µg/L)	DOC[4] (mg/L)
Charlesbourg-B[1]	93±16	23±4	10.00	18.9±2. 8
Charlesbourg-A[2]	34±12 ⬇	0±0 ⬇	2.32±0.42 ⬇	12.1±2. 2 ⬇
Quebec-B	109±22	198±13	10.00	13.6±1.8
Quebec -A	13±5 ⬇	1±1 ⬇	3.54±0.71 ⬇	6.0±1.3 ⬇
Ste Foy-B	88±15	260±18	10.00	21.7±2.5
Ste Foy-A	10±3 ⬇	2±1 ⬇	3.21±0.57 ⬇	11.5±2.7 ⬇
Beauport-B	93±25	33±8	10.00	32.4±3.1
Beauport-A	4±1 ⬇	0±0 ⬇	1.64±0.32 ⬇	21.1±2.3 ⬇

[1] Before treatment [2] After treatment [3] Colony forming unit

[4] Dissolved organic carbon

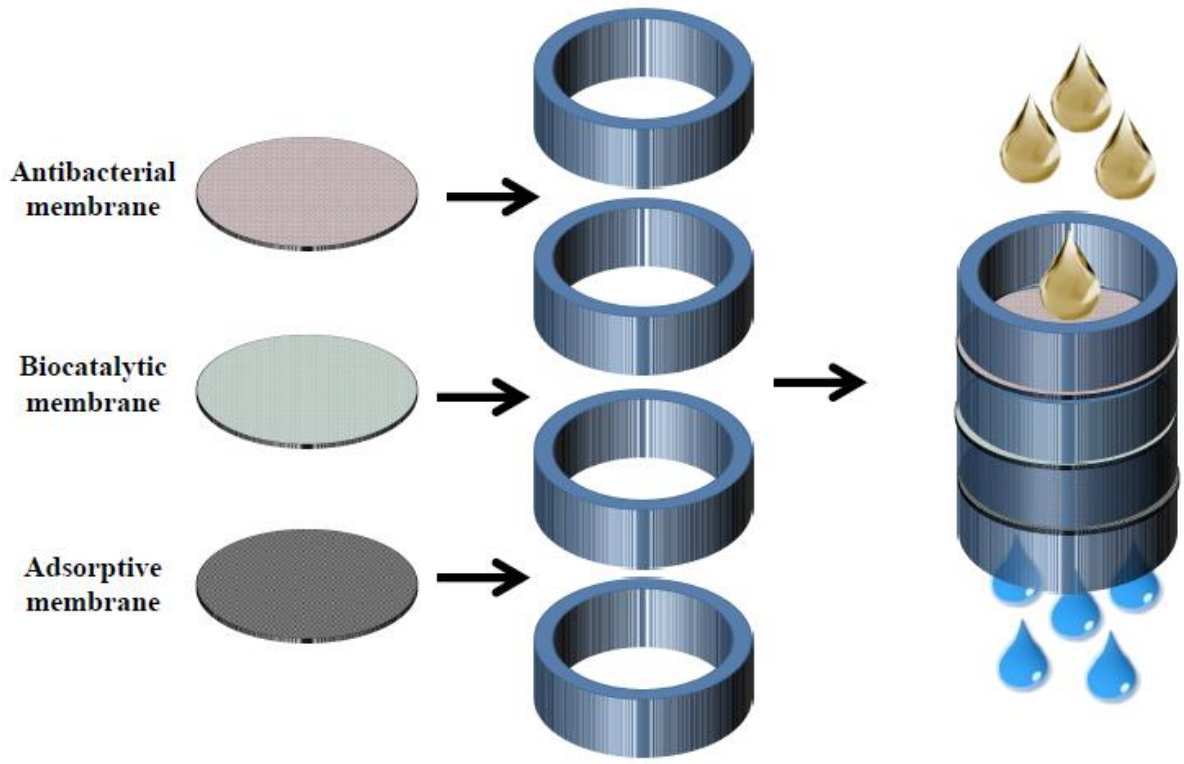

Figure 4.2.1. Illustration of the portable water purifier components

CHAPITRE 5

Conclusions et Recommandations

Conclusions

Les conclusions suivantes peuvent être tirées du travail accompli:

1) La CTC a suivi l'isotherme de Langmuir pour l'adsorption sur biocharbon et charbon activé et que l'adsorption maximale s'est produite à pH 1. La capacité d'adsorption de biocharbon de la CTC peut être augmentée de 2.3 mg/g à 434.78 mg/g ce qui le rend compétitif avec le charbon activé commercial.

2) Une membrane adsorbante en PAN et biochar est capable d'adsorber la CTC à un flux normal (débit par unité de surface) de membranes d'ultrafiltration. Pour la production de la membrane adsorbante par électrofilage, 1,5% de la charge de biocharbon a abouti à une surface spécifique maximale (12,4 m^2/g) due à l'agrégation de particules à des concentrations plus élevées et à la formation de grosses billes qui ont réduit le rapport surface/volume.

3) La laccase libre peut dégrader la CTC jusqu'à 47% en 24 heures de réaction et la transformer en produits moins nocifs. En utilisant l'ABTS, la laccase peut augmenter l'efficacité de dégradation à plus de 95% en moins de 8 heures. La température et le pH sont les paramètres les plus influents dans la dégradation enzymatique de la CTC.

4) La laccase physiquement immobilisée sur la membrane adsorbante a montré une efficacité d'immobilisation élevée (> 70%), une grande stabilité au stockage (90% après un mois) et une grande réutilisabilité (95% après 5 cycles). L'utilisation de la laccase immobilisée pour la dégradation de la chlorotétracycline en mode continu a montré une efficacité d'élimination de 58,3%, 40,7% et 22,6% à 1, 2 et 3 $mL/h.cm^2$ démontrant son application potentielle dans le traitement des eaux usées.

5) L'immobilisation covalente de la laccase sur une membrane nanofibreuse a montré une amélioration de la stabilité de 32%, 43% et 66% en termes de tolérance d'une haute température, d'un pH acide et d'un stockage à long terme. En outre, cette technique a montré comme résultat 72,7%, 63,3% et 48,6% d'efficacité de

dégradation pour le DCF, la CTC et la CBZ, respectivement après 8 heures de réaction. La tendance à la baisse de l'adsorption au cours du temps de réaction pour tous les composés a confirmé l'effet régénérateur de la laccase sur les sites d'adsorption du biocharbon.

6) L'augmentation de la charge de chitosane a amélioré la résistance bactérienne de la membrane de polyacrylonitrile de sorte que la membrane contenant 15% de chitosane présentait des réductions de 2,8 log de la bactérie *E. coli* après 30 minutes de contact. Le biocatalyseur fabriqué à partir de la membrane avec 15% de chitosane a montré une efficacité de dégradation de 61%, 56% et 48% pour le DCF, la CTC et la CBZ respectivement dans l'eau pure et 75%, 68% et 57% dans l'effluent secondaire de la station d'épuration des eaux usées. Le taux d'élimination le plus élevé dans l'effluent d'eaux usées peut être attribué à la présence de cofacteurs de la laccase, c'est-à-dire de cuivre ou d'autres acides organiques qui peuvent jouer le rôle de médiateur pour l'enzyme.

Tableau 5.1.1. Comparaison entre les résultats trouvés dans littérature et les résultats de cette étude

Méthode d'enlèvement	Composés	Conditions	Max. enlèvement (%)	Références
Adsorption (MWCNT*)	Tétracycline	298 K, 72 h, pH 5	67.7	[1]
Adsorption	Chlorotétracycline	298 K, 24 h, pH 1	92.8	Cette étude
Biodégradation	Tétracycline	298 K, 24 h, pH 1	36	[2]
Biodégradation	Chlorotétracycline	298 K, 24 h, pH 7	47	Cette étude
Biodégradation	Tétracycline	308 K, 24 h, pH 7	53	[2]
Biodégradation	Chlorotétracycline	313 K, 24 h, pH 7	64	Cette étude

*MWCNT: nanotube de carbone multi-parois

Recommandations

À partir des résultats obtenus, les recommandations suivantes peuvent être considérées:

1) Les propriétés d'interaction et d'adsorption de toutes les classes de contaminants émergents sur le biocharbon devraient être étudiées.

2) Comme les antibiotiques conduisent au développement d'une résistance antibactérienne, il est nécessaire de développer un nouveau traitement tertiaire pour traiter les effluents des eaux usées avant leur rejet dans l'environnement.

3) De nouveaux polymères tels que le polysulfone devraient être étudiés afin de fournir une charge enzymatique plus élevée, ce qui se traduit par une empreinte plus faible et une efficacité plus élevée.

4) Il est nécessaire d'approfondir les recherches sur les méthodes combinées/hybrides afin d'améliorer leur efficacité par leurs effets synergiques et de les rendre économiquement viables et durables sur le plan environnemental. La combinaison du système membrane-enzyme-biocharbon avec des processus d'oxydation avancés tels que l'ultrasonication pourrait être un système potentiel.

5) Les biocharbons d'autres sources et de différentes méthodes d'activation, telles que l'utilisation de vapeur d'eau peuvent être étudiés.

Références

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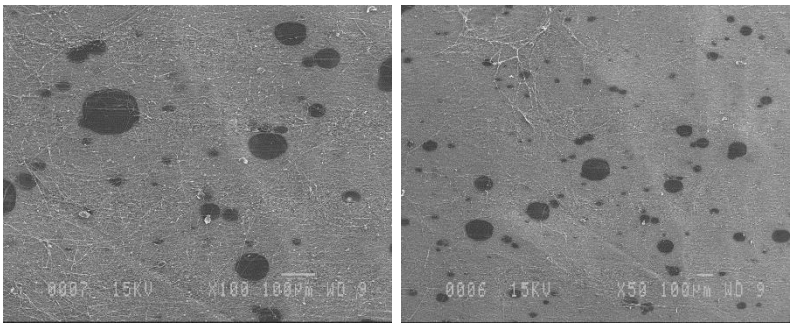
ANNEXES

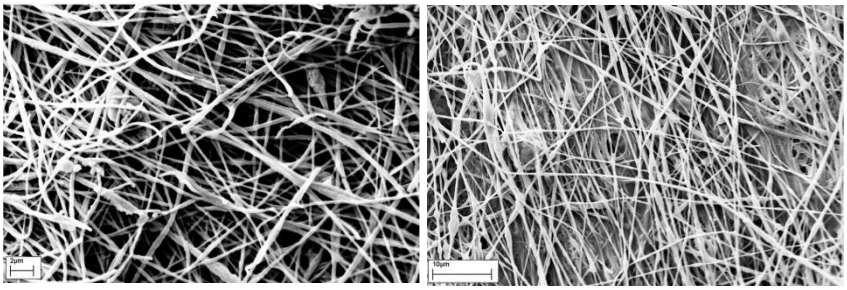
Annex I

CHAPTER 2 PART 4

Data 1: Fabrication of membrane through electrospinning before optimization of conditions showed non uniform distribution of biochar



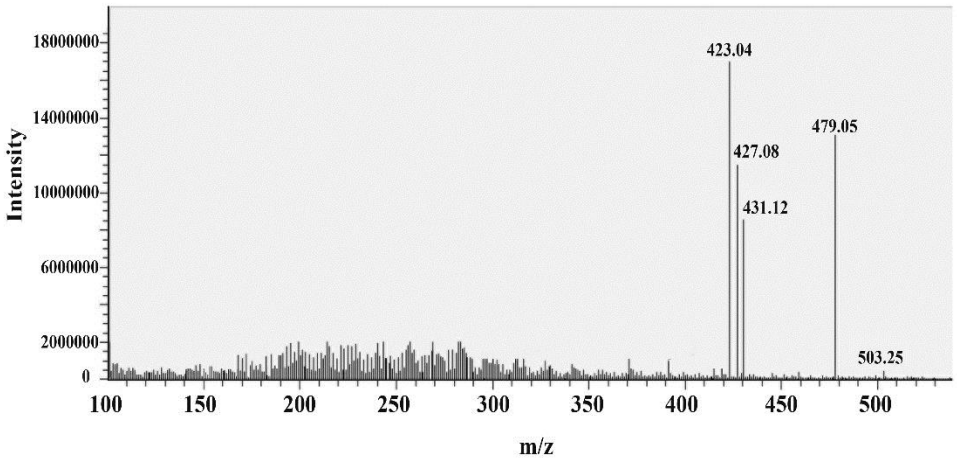
Data 2: Fabrication of membrane through electrospinning before optimization of conditions showed fused and discontinued fibers



Annex II

CHAPTER 3 PART 1

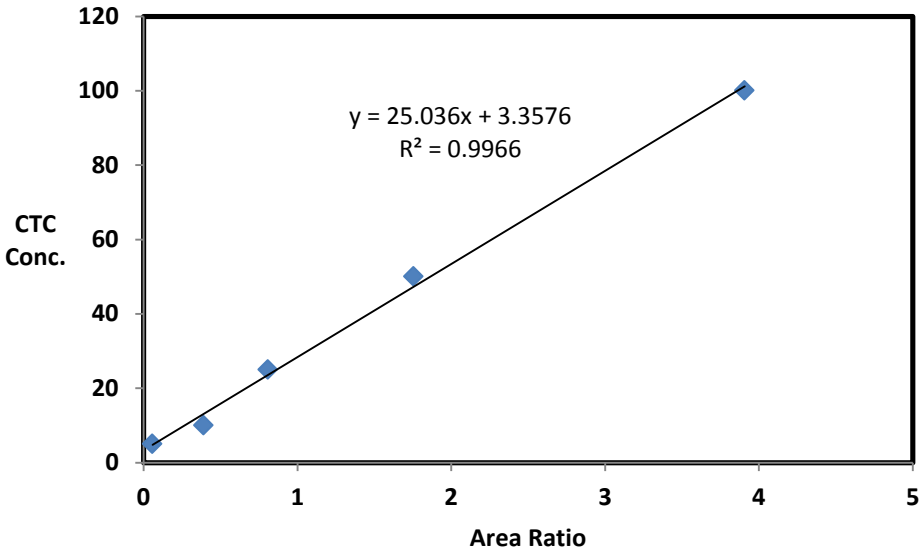
Data: Mass spectrum of treated sample in optimized conditions



Annex III

CHAPTER 3 PART 2

Data: Calibration curve of CTC using LDTD-MS-MS instrument



Annex IV

CHAPTER 3 PART 3

Data: Proposed stage for incorporation of BiMeMS