

## Performance of a membrane bioreactor in extreme concentrations of Biphenyl A

Yassine Ouarda, Mehdi Zolfaghari, Patrick Drogui, Brahim Seyhi, Gerardo Buelna and Rino Dubé

### ABSTRACT

In this study, a submerged membrane bioreactor was used to study the effect of low and high BPA concentration on the sludge biological activity. The pilot was operated over 540 days with hydraulic retention time and solid retention time of 5.5 hours and 140 days, respectively. As hydrophobic compound, BPA highly adsorbed by activated sludge. In lower concentration biodegradation rate remained low, since BPA concentration in the sludge was lower than 0.5 mg/g TS; yet, at influent concentration up to 15 mg/L, biodegradation rate was increasing, resulting in 99% of BPA removal efficiency. The result on chemical oxygen demand removal showed that BPA concentration has no effect on the heterotrophic bacteria which were responsible for the organic carbon degradation. In higher concentration, up to 16 mg of BPA was used for each gram of sludge as a source of carbon. However, the activity autotrophic bacteria, including nitrifiers, were completely halted in the presence of 20 mg/L of BPA or more. Although nitrification was stopped after day 400th, ammonia removal remained higher than 70%, due to air stripping. Assimilation by bacteria was the only removal pathway of phosphorus which resulted in average 35% of P-PO<sub>4</sub> removal efficiency.

**Key words** | ammonia, Bisphenol A, chemical oxygen demand, membrane bioreactor, phosphorus

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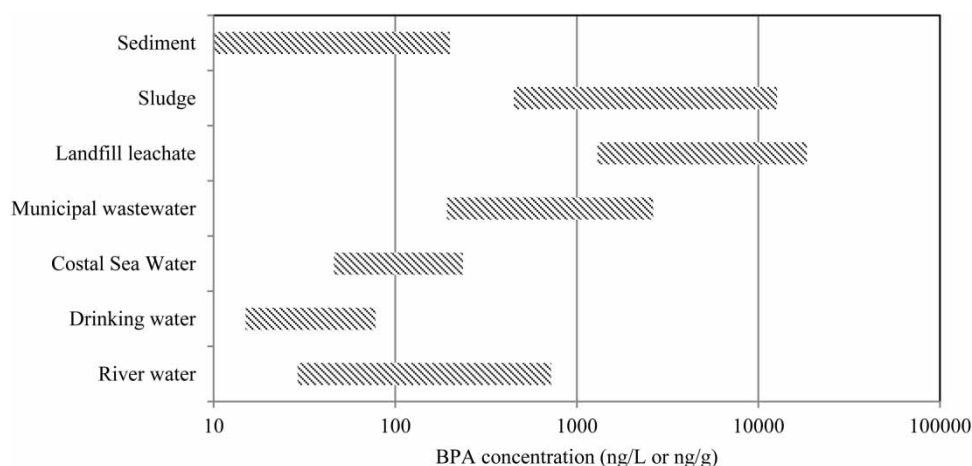
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### INTRODUCTION

Emerging contaminants, especially endocrine disrupting compounds (EDCs) are in the center of attention, due to their ever increasing concentration in the environment (Seyhi *et al.* 2013; Zhang *et al.* 2016). Wastewater with high concentration of EDCs was the main source of contaminants water body. The presence of EDCs in wastewaters must be taken into account owing to their potential toxicity for humans (brain cancer, affection of immune and reproductive systems, e.g.) (Vandenberg *et al.* 2009) and aquatic species (feminization, toxicity to fish and invertebrates, e.g.). Bisphenol-A (BPA) is one of the most prominent EDC, extensively used for many industrial applications such as, production of polycarbonate plastics, Epoxy resins, flame retardant and PVC (Staples *et al.* 1998; Chen *et al.* 2008). As the carcinogenic and mutagenic effect of BPA has been proven, The US and Canadian federal ministry of health and environment imposed strict regulation on its release into the environment (Mohapatra *et al.* 2010). Due to its high production (5 million tons per year) and

resistance toward biodegradation, BPA has been found in many environmental matrices around the world, especially in the effluent of wastewater treatment plants (Mohapatra *et al.* 2010; Huang *et al.* 2012). As illustrated in Figure 1, constant introduction of BPA from landfill leachate, municipal wastewater and sewage sludge increase its concentration in surface water, sediment, and even drinking water (Staples *et al.* 1998; Fromme *et al.* 2002; Fent *et al.* 2003; Sharma *et al.* 2009; Mohapatra *et al.* 2010; Huang *et al.* 2012).

Utilization of advanced processes, such as chemical oxidation (Dudziak & Burdzik 2016), membrane filtration (Chen *et al.* 2008; Seyhi *et al.* 2013; Yang *et al.* 2013) and adsorption (Liu *et al.* 2009) have been recommended for efficient removal of BPA. However, complete oxidation of BPA without production of byproduct is still challenging and requires high chemical consumption and treatment cost. Due to its specific characteristics, membrane bioreactor (MBR) seems to be a promising treatment option for wastewater treatment with high concentration of BPA. Firstly,



**Figure 1** | average Bisphenol A concentration in different media (ng/g refers to solid matrices, while ng/L refers to aquatic matrices).

high concentration of biomass (over 15 g/L) (Ahmed & Lan 2012) increases the biosorption of hydrophobic compounds including BPA with log Kow of 3.32 (Seyhi et al. 2011). Secondly, high solid retention time (SRT) helps to develop the microorganisms strain, specialized in BPA removal through enzymatic reaction (Yang et al. 2013; Zhu & Li 2013; Zhang et al. 2016). In previous studies, the long-term performance of a laboratory submerged MBR system for the treatment of synthetic effluent was investigated with BPA concentration in the range of 0.6 to 1.2 mg/L (Seyhi et al. 2013). In the present study, the performance of MBR for removal of macro-pollutants (carbon, nitrogen and phosphorus) was investigated. Synthetic wastewater used in this study was spiked by very low or very high BPA concentration. The mechanisms of removal in these two extreme conditions were investigated by analyzing BPA concentration in the sludge, influent and effluent.

## MATERIAL AND METHODS

### Chemical

All the organic solvents, included methanol, dichloromethane, were of analytical grade with the highest purity commercially available. Silica-based bonded C18 (Sep-Pak, 6 mL, 500 mg) cartridges were supplied by Waters Ltd. (Mississauga, ON, Canada). BPA, glucose,  $(\text{NH}_4)_2\text{SO}_4$ ,  $\text{KH}_2\text{PO}_4$ , and the deuterated bisphenol A (BPA-d16), used as internal standard, were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada). Stock solutions of BPA were prepared in methanol at 2,000 mg/L and kept at 4 °C.

### MBR setup

The study was conducted at a laboratory scale submerged MBR. The pilot was composed of 6 L biological reactor equipped with hollow-fiber membrane (ZW-1, Zenon Environmental Inc., Canada) with pore diameter of 40 nm and 0.047 m<sup>2</sup> of total filtering surface area, assembled vertically. Filtration and feeding of pilot was achieved by two peristaltic pumps. For controlling the fouling of membrane, the pump was filtrating for 5 min with 30 seconds of pause. Whenever the transmembrane pressure was increased more than -40 KPa, 30 second of backwash was carried out. By increasing the transmembrane pressure more than -69 KPa (the maximum limit of vacuum pressure), the chemical wash was performed on the membrane. Firstly, the sludge cake was removed by tap water, following by circulation of 1,000 mg/L of NaClO for 2 h to eliminate biofouling. In order to remove inorganic compound, citric acid solution with concentration of 3.0 g/L was also used for 30 min. Air was supplied through an extended aeration tube located inside the aeration basin and membrane module.

### Experimental design and operation

The MBR was inoculated with sludge taken from Quebec City municipal wastewater treatment plant. For start-up, it was feed by synthetic wastewater with glucose and BPA as the carbon source. The mineral composition of the synthetic effluent was made, in order to provide the best environment for the growth of microorganism. As indicated in previous studies (Seyhi et al. 2012), C/N/P ratio was kept at around

100/5/2 by  $C_6H_{12}O_6/(NH_4)_2SO_4/KH_2PO_4$ . During startup which took almost 40 days, desirable concentration of sludge was developed (14 g/L of total suspended solid), resulting in steady carbon and nitrogen removal. MBR operation was carried out in two main phases. During the first period (A), the concentration of BPA in the synthetic solutions was increasing from 0.050 to 0.400  $\mu\text{g/L}$  and finally 20  $\mu\text{g/L}$  in the period of 200 days. In the second period (B), the performance of MBR was investigated in higher BPA concentration by gradual increase in concentration from 1,000  $\mu\text{g/L}$  to 40,000  $\mu\text{g/L}$ . During startup and operating period, hydraulic retention time (HRT) and SRT were 5.5 h and 140 days, respectively; while, temperature and pH were maintained around  $20 \pm 2^\circ\text{C}$  and  $7 \pm 1$ , respectively.

### Analytical methods

Twice per week, samples were collected at the inlet, outlet and mixed liquor for the analysis of total suspended solid (TSS), total volatile suspended solid (TVSS), chemical oxygen demand (COD), ammonium ( $N-NH_4$ ), nitrate ( $N-NO_2$ ), nitrate ( $N-NO_3$ ), orthophosphate ( $P-PO_4$ ), pH, and BPA. All parameters were determined in conformity with standards methods. COD was measured by colorimetric method in the presence of potassium dichromate and the absorbance was measured at 600 nm using a UV spectrometer (Cary 50, Varian Canada Inc., Mississauga, ON, Canada) according to MA-315-DCO 1.0 method, proposed by CEAEQ. TSS measurement was conducted by centrifugation of 100 mL of sample from sludge mixed liquor at 8,000 rpm for 20 min at  $4^\circ\text{C}$ . The pellet was kept for drying at  $105^\circ\text{C}$  for 24 h. Subsequently, TVSS was measured by burning the dried pellet at  $550^\circ\text{C}$  for 30 min according to MA-104-S.S.1.1 method. Ammonia ( $NH_3-N$ ) and phosphorus ( $PO_4-P$ ) were analyzed simultaneously by QuikChem LACHAT Instrument, based on 10-107-06-2-B and 10-115-01-1-B analytical method.

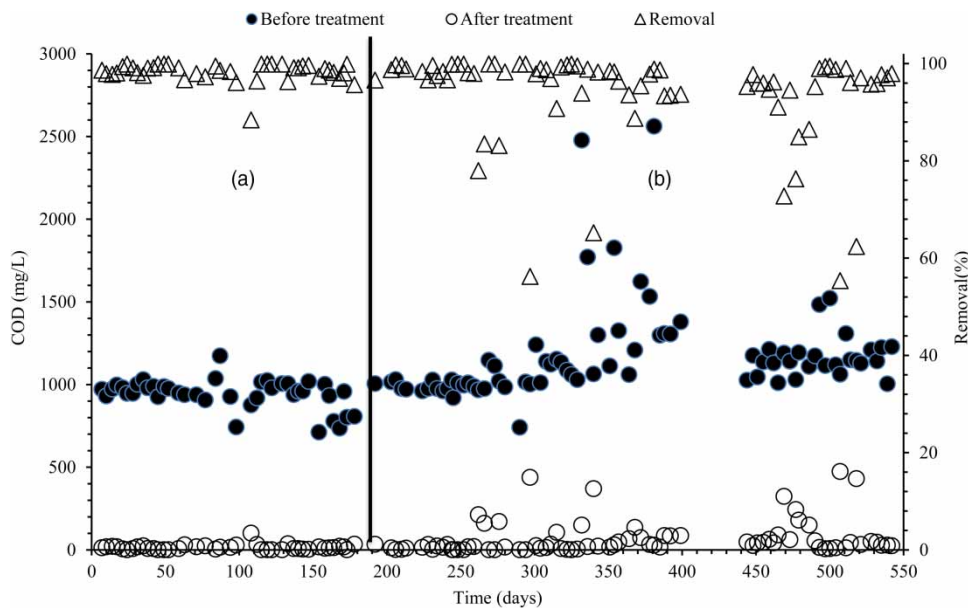
Solid Phase Extraction (SPE) was used for BPA extraction from solid sample using Visiprep system on C-18 cartridges (Seyhi *et al.* 2013). The samples were filtered through glass fiber membrane (Whatman GF-C, porosity: 0.7  $\mu\text{m}$ ) and the retained particles were rinsed with 0.5 mL of methanol. Prior to sample loading, the SPE cartridges were pre-conditioned with 13 mL of methanol and 13 mL of ultrapure water. Later on, the cartridges were rinsed 12 times with 8 mL of ultrapure water to remove impurities having fewer interactions with the adsorbent. The cartridges were dried by a vacuum pump. Elution was performed twice

with 5 mL of methanol/dichloromethane (9: 1, v/v) during 5 minutes. The extracts were evaporated under nitrogen flux at  $40^\circ\text{C}$  and then resuspended with 1 mL methanol. Liquid chromatography in a reversed-phase hypersil Gold column equipped with mass spectrophotometer was used for the BPA analysis (LC-MS). An isocratic flow of 0.2 mL/min of a water/methanol (5:95, v/v) was used at room temperature for elution. Detection was carried out in a MS system using an electrospray interface in negative ionization mode with detection limit to 0.020  $\mu\text{g/L}$ .

## RESULTS AND DISCUSSION

### COD removal

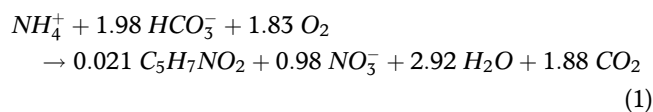
The performance of MBR in terms of COD reduction was monitored simultaneously with BPA. During the first monitoring period that lasted for about 200 days (Figure 2), COD concentration at the inlet had fluctuated between 710 and 1,170 mg/L, with an average value of 940 mg/L. As highly biodegradable glucose was used as the source of carbon, the residual COD concentration in the treated effluent was only 5 and 37 mg/L (removal efficiency of  $98 \pm 2\%$ ). Like reported in literature, presence of high concentration of heterotrophic bacteria in aeration basin results in biological oxygen demand removal efficiency higher than 95% independent of the operating condition (Ahmed & Lan 2012; Seyhi *et al.* 2013; Zhang *et al.* 2016). The relatively low concentrations of BPA (0.4 and 20  $\mu\text{g/L}$ ) did not affect the ability of microorganisms to degrade the organic matter. However, increase in BPA concentration up to 10,000  $\mu\text{g/L}$  during the second period slightly increased the effluent COD concentration. During 350 days of monitoring period, influent COD concentration fluctuated between 741 and 1,241 mg/L. Presence of BPA byproducts in the effluent decreased the average of COD removal efficiency; yet, it was still higher than 96% (Figure 2). Increase in BPA concentration up to 40,000  $\mu\text{g/L}$  destabilized MBR operating performance, increasing the COD of effluent between 444th to 490th days. Three reasons might explain this unstable condition: firstly, adsorption of extra BPA could be toxic to microorganism, caused their lysis and increase of extracellular polymer in the effluent; secondly, adopted microorganism adsorbed BPA as source of carbon instead of glucose; and finally, high concentration of BPA biodegradation byproduct release into aeration basin, increased the COD concentration in the effluent (Seyhi *et al.* 2012).



**Figure 2** | Evolution of COD concentration before and after treatment (BPA initial concentration ( $\mu\text{g/L}$ ) (a) = 0.4–20, (b) = 1,000–40,000; COD initial concentration = 1,000 mg/L; HRT = 5.5 h; SRT = 140 days).

### N-NH<sub>4</sub> and P-PO<sub>4</sub> removal

The removal performance of inorganic pollutants included nitrogen and phosphorus were also monitored to have an idea of reactor stability. The initial ammonium concentration (N-NH<sub>4</sub>) was fluctuated between 22 and 82 mg/L, with the average concentration of 58 mg/L. In aerobic condition, autotrophic ammonia-oxidizing bacteria (AOB) transform NH<sub>4</sub><sup>+</sup> into NO<sub>2</sub><sup>-</sup> by means of HCO<sub>3</sub><sup>-</sup>, followed by nitrite-oxidizing bacteria (NOB) that oxidize toxic nitrite ions into the nitrate by carbonate ions (nitrification). As the nitrogen was assimilated or used to produce energy by nitrifier bacteria, the biological reaction was very complicated. Based on experimental consumption of alkalinity and oxygen, the following reaction is the overall biological nitrification (Henze *et al.* 2001).

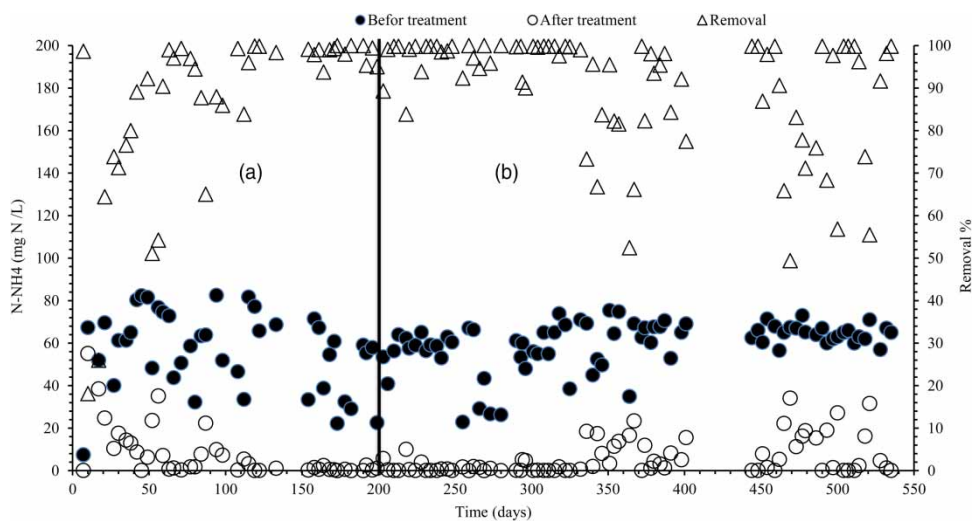


As shown in Equation (1), approximately 14 g of total alkalinity and 4.2 g of oxygen was required for total nitrification of 1 gN-NH<sub>4</sub> of ammonia. Since both AOB and NOB have biological half-life more than 10 days, it takes more than a month for development of nitrifier culture and start of nitrification process (Ahmed & Lan 2012); therefore

during the first 40 days of operation, no nitrate was detected in the effluent and ammonia removal efficiency was lower than 18%. Air stripping is the second way of nitrogen removal. In alkaline pH of aeration basin, almost 5% of ammonium transformed into the dissolved ammonia (Equation (2)).



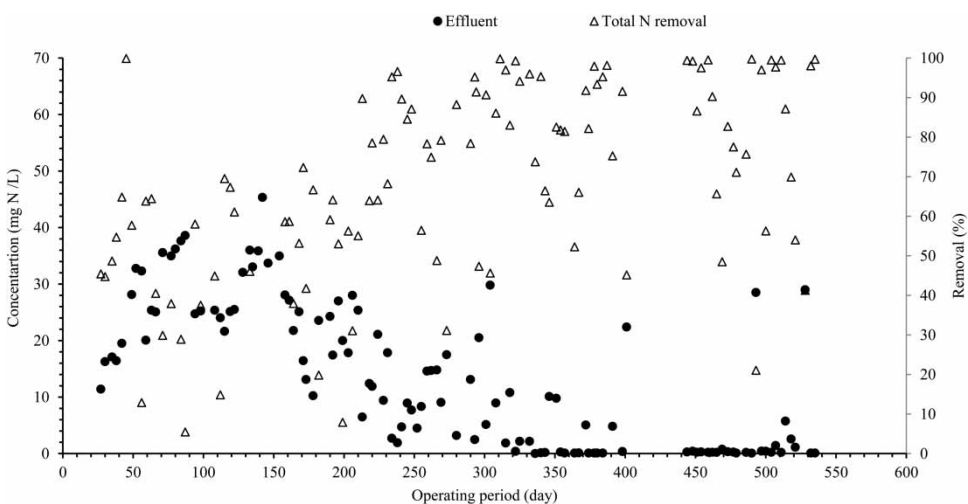
Intense aeration in MBR, gradually stripped the dissolved NH<sub>3</sub>, leading to unbalancing the Equation (2) and production of more dissolved ammonia; hence, even more than 20% of ammonia removal was observed in the absence of nitrifier bacteria. MBR with high SRT provides exceptional condition for maximum development of nitrifier; along with high kinetic constant of biological nitrification, complete ammonia oxidation was expected for different organic load rate and sludge retention time higher than 30 days (Zolfaghari *et al.* 2015). After treatment, the residual concentrations of N-NH<sub>4</sub> in the treated water fluctuated between 0.1 and 17 mg/L, with average residual concentration of 3 mg/L, corresponding to 94 ± 4% of ammonia removal efficiency (Figure 3). Low concentration of BPA has no effect on the performance of nitrification, as autotrophic bacteria use carbonate and bicarbonate as a source of carbon. Yet, increasing the BPA concentration more than 40 mg/L after day 400, dramatically decreased



**Figure 3** | Evolution of influent and effluent concentration of N-NH<sub>4</sub> (BPA initial concentration ( $\mu\text{g/L}$ ) (a) = 0.4–20, (b) = 1,000–40,000; COD initial concentration = 1,000 mg/L; HRT = 5.5 h; SRT = 140 days).

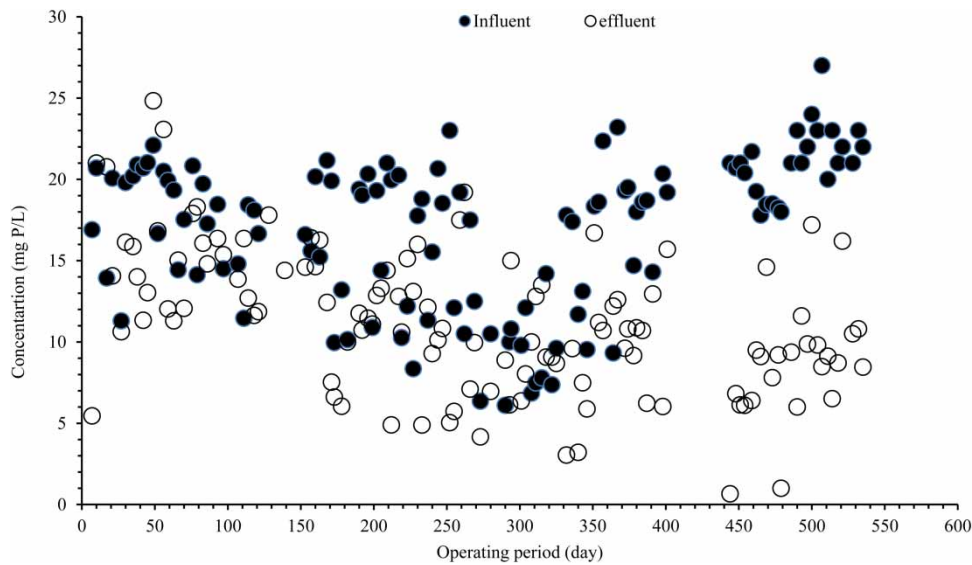
the nitrifier. Absence of nitrate in the effluent indicated the complete halt of biological nitrification (Figure 4). Nitrifying bacteria are extremely sensitive toward low concentration of oxygen and high concentration of toxic compounds. Long term exposure of 40 mg/L of BPA could change the quality and quantity of nitrosomonas and nitrobacter in the aeration basin (Zielińska *et al.* 2014). In the absence of anoxic process for transformation of nitrate into the nitrogen gas (Henze *et al.* 2001), air stripping of ammonia is the only removal pathway of total nitrogen removal in period B, which remained below 15% during operating period.

Unlike ammonia, phosphorus removal requires the combination of anaerobic and aerobic process for its efficient removal. Phosphorus uptake for new cell synthesis was the only removal pathway (6 mg P for each gram of sludge) (Zolfaghari *et al.* 2015). For the MBR with SRT of 140 days, the average of 35% of phosphorus was assimilated by the sludge, which later withdrew from the aeration basin. During the monitoring period, orthophosphate (P-PO<sub>4</sub>) concentrations in the feeding solution have fluctuated between 11 and 39 mg/L. After treatment, the residual concentration of phosphorus was between 5 and 24 mg/L, indicated  $31 \pm 16\%$  of PO<sub>4</sub> removal efficiency (Figure 5). It is worth



**Figure 4** | Evolution of N-NO<sub>3</sub> concentration in the effluent of MBR (BPA initial concentration ( $\mu\text{g/L}$ ) (A) = 0.4–20, (B) = 1,000–40,000; COD initial concentration = 1,000 mg/L; HRT = 5.5 h; SRT = 140 days).





**Figure 5** | Evolution of P-PO<sub>4</sub> concentration before and after treatment (BPA initial concentration (μg/L) (A) = 0.4–20, (B) = 1,000–40,000; COD initial concentration = 1,000 mg/L; HRT = 5.5 h; SRT = 140 days).

mentioning that the phosphorus concentration in the effluent was highly fluctuated, due to sudden release of soluble microbial product into the effluent (Seyhi *et al.* 2012). Previous study on aerobic MBR had also reported similar results with low biological phosphorus reduction rates (47%–70%) (Vera *et al.* 1997; Battistoni *et al.* 2006; Seyhi 2012).

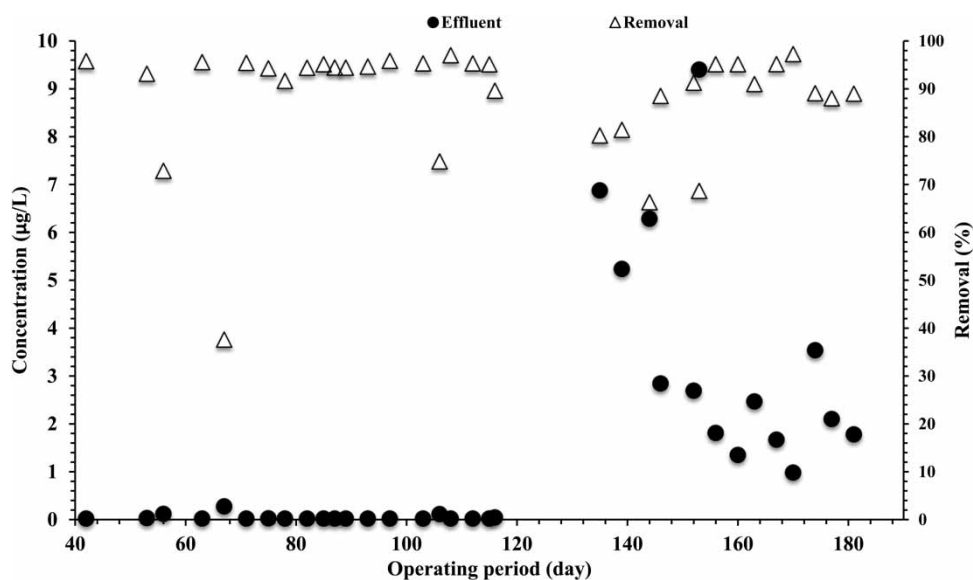
### Performance of MBR for low BPA concentration

As indicated in previous studies, HRT has no effect on BPA removal efficiency in the MBR; however, MBR performance was enhanced in higher SRT (Zhu & Li 2013; Zielińska *et al.* 2014). In this study, therefore, HRT was remaining constant during operation period; while, SRT was kept as high as possible. In the first period of MBR, the concentration of BPA remained in the range of municipal wastewater and BPA removal efficiency was evaluated accordingly. During the first 70 days of operation (from day 40th to 110th), MBR was fed by a synthetic solution with the average BPA concentration of 0.400 μg/L. BPA influent concentration in MBR was varied between 0.240–0.480 μg/L. As shown in Figure 6, BPA residual concentration was relatively near to or below 0.02 μg/L with the average removal efficiency of 90%. From day 130th to day 190th, the concentration of BPA was then increased to 20 μg/L. In the vast majority of treated water samples, the residual BPA concentration was remained below 1 μg/L. The highest residual concentration was 10.4 μg/L, corresponding to a removal rate of

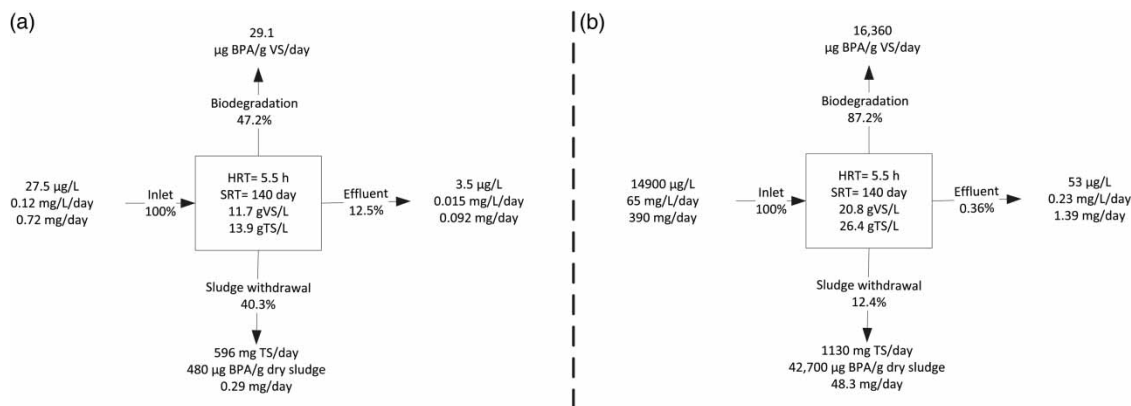
89.5% ± 12%. The BPA concentration in the sludge was estimated around 480 μg/g dry sludge. According to Figure 7, BPA mass balance showed that almost half of 0.72 mg of BPA was degraded in the sludge per day. Bio-sorption was responsible for the remaining 40% of BPA removal. It is worth mentioning that loss of BPA by evaporation could be ignored, because of its low volatility (vapor pressure of  $4 \times 10^{-8}$  mmHg at 25 °C and Henry's constant of  $10^{-5}$  Pa·m<sup>-3</sup>·mol<sup>-1</sup>) (Chen *et al.* 2008). Furthermore, the size of BPA was much smaller than 40 nm of ultrafiltration pore size; therefore, membrane retention has no effect on BPA removal pathway (Zhu & Li 2013).

### Performance of MBR for high BPA concentration

From day 200th to day 550th, COD concentration remained at 1,000 mg/L; while, the BPA concentration was gradually increased from 1 to 40 mg/L in nine stages. As 1 mg/L of BPA was responsible for almost 2.5 mg/L of COD, glucose concentration was decreasing 10% in the presence of 40 mg/L of BPA. The increase in BPA concentration was affected once a steady state was reached (e.g. stability of BPA removal in MBR). After increasing BPA concentration in each stage, the performance of MBR was dramatically decreased (Figure 8); yet BPA removal efficiency was recovered in the period of two weeks to reach a rate of 97% or more. Indeed, increasing the initial concentration of BPA make the biomass in a state of stress, due to the toxic character of BPA. The results obtained in this study indicated



**Figure 6** | Evolution of Bisphenol A concentration in the first period of operation (BPA initial concentration = 0.4–20 µg/L; COD initial concentration = 1,000 mg/L; HRT = 5.5 h; SRT = 140 days).



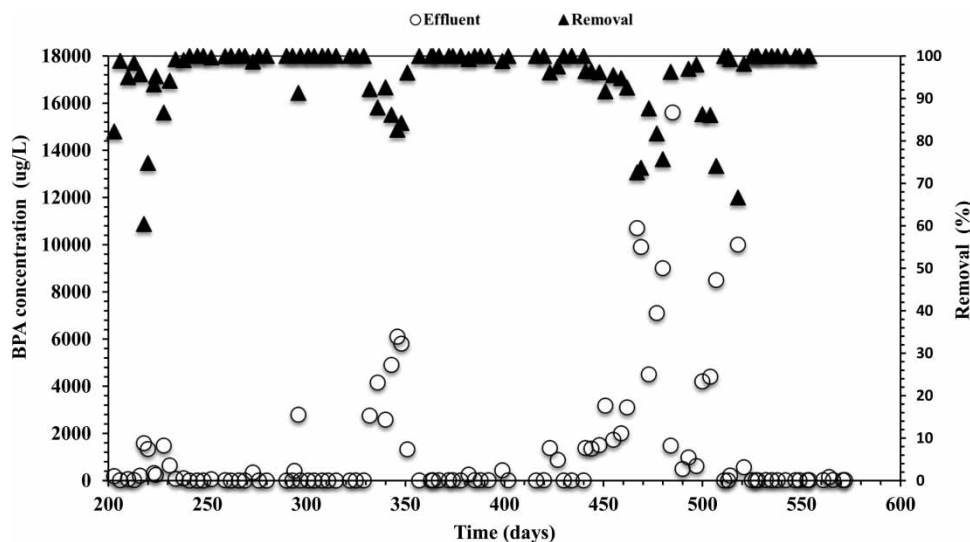
**Figure 7** | Bisphenol A mass balance in the (a) 70th day and (b) 251th day.

that after the adaptation period, MBR treated effectively an effluent with initial BPA concentrations up to 40,100 µg/L (Figure 8). BPA mass balance study for high influent concentration showed that the rate of biodegradation was rapidly increasing up to 16 mg/g/day, resulting in 87% of BPA removal by biodegradation. As also reported in literature (Chen *et al.* 2008), development of specialized bacteria for BPA removal in aeration basin increased the portion of biodegradation ratio for total removal. As these bacteria retained by the membrane, their concentration is continuously increased, resulting in high BPA removal efficiency in higher concentration of BPA. In higher BPA concentration, only adapted microorganism could be survived, resulting in 560 times increase in biodegradation rate.

Enzymatic reaction could be also responsible for high biodegradation rate of BPA. It should be mentioned that high BPA removal efficiency in this stage did not indicate its complete removal. The residual byproducts of BPA biodegradation might be resistance toward further biodegradation and could be the main reason for increase in residual COD in the effluent of MBR.

## CONCLUSION

This study demonstrated the effect of extreme BPA concentration on the performance of a MBR. Due to its hydrophobic characteristic, BPA was adsorbed by high



**Figure 8** | Evolution of Bisphenol A concentration in the second period of operation (BPA initial concentration = 1,000–40,000 µg/L; COD initial concentration = 1,000 mg/L; HRT = 5.5 h; SRT = 140 days).

concentration of activated sludge (11–20 g VS/L) present in the aeration basin, resulted in average BPA removal efficiency more than 90%. In low concentration, BPA mainly accumulated within the sludge without degradation. As its concentration raised more than 1 mg/L in influent, the biodegradation was increasing up to 16 mg/g VS/day responsible for 87% of total BPA removal efficiency. Development of specialized microorganisms for degradation of BPA or triggering enzymatic reaction dramatically increased the biodegradation rate. Despite changing in BPA concentration, heterotrophic bacteria showed high COD removal efficiency (more than 95%); yet, the performance of nitrifying bacteria were significantly reduced under BPA concentration higher than 25 mg/L. Nitrogen mass balance results revealed that biological nitrification was completely stopped after day 400th. In the absence of nitrification, air stripping is responsible for 70% of ammonia removal. As 6 mg of phosphorus was required for the production of a gram of sludge, the average of 37% of phosphate was removed from the influent.

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