ABSTRACT

In this study, a submerged membrane bioreactor was used to study the effect of low and high bisphenol A (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 a hydrophobic compound, BPA was highly adsorbed by activated sludge. In lower concentrations, the biodegradation rate remained low, since the BPA concentration in the sludge was lower than 0.5 mg/g TS; yet, at an influent concentration up to 15 mg/L, the biodegradation rate was increasing, resulting in 99% BPA removal efficiency. The result for chemical oxygen demand removal showed that BPA concentration has no effect on the heterotrophic bacteria that were responsible for the organic carbon degradation. In higher concentrations, up to 16 mg of BPA was used for each gram of sludge as a source of carbon. However, the activity of autotrophic bacteria, including nitrifiers, was completely halted in the presence of 20 mg/L of BPA or more. Although nitrification was stopped after day 400, ammonia removal remained higher than 70% due to air stripping. Assimilation by bacteria was the only removal pathway for phosphorus, which resulted in an average 35% of P-PO₄ removal efficiency.

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 for water bodies. The presence of EDCs in wastewaters must be taken into account owing to their potential toxicity for humans (brain cancer, effects on the immune and reproductive systems, etc.) (Vandenberg et al. 2009) and aquatic species (feminization, toxicity to fish and invertebrates, etc.). Bisphenol A (BPA) is one of the most prominent EDCs, extensively used for many industrial applications such as production of polycarbonate plastics, epoxy resins, flame retardants 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 have 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 byproducts is still challenging and requires high chemical consumption and treatment cost. Due to its specific characteristics, the membrane bioreactor (MBR) seems to be a promising treatment option for wastewater treatment with high concentrations of BPA. Firstly,
the 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 solids 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 concentrations in the range of 0.6 to 1.2 mg/L (Seyhi et al. 2013). In the present study, the performance of the MBR for removal of macro-pollutants (carbon, nitrogen and phosphorus) was investigated. Synthetic wastewater used in this study was spiked with a very low or very high BPA concentration. The mechanisms of removal in these two extreme conditions were investigated by analyzing the BPA concentration in the sludge, influent and effluent.

**MATERIAL AND METHODS**

**Chemical**

All the organic solvents, which included methanol and 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, (NH₄)₂SO₄, KH₂PO₄, 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 a 6 L biological reactor equipped with a hollow-fiber membrane (ZW-1, Zenon Environmental Inc., Canada) with pore diameter of 40 nm and 0.047 m² of total filtering surface area, assembled vertically. Filtration and feeding of the pilot was achieved by two peristaltic pumps. To control the fouling of the membrane, the pump was filtrating for 5 min with a 30 second pause. Whenever the transmembrane pressure was increased more than −40 KPa, 30 second of backwash was carried out. By increasing the transmembrane pressure to 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 compounds, citric acid solution with a 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 fed 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 microorganisms. As indicated in previous studies (Seyhi et al. 2012), the C/N/P ratio was kept at
around 100/5/2 by C₆H₁₂O₆/(NH₄)₂SO₄/KH₂PO₄. During startup, which took almost 40 days, a desirable concentration of sludge was developed (14 g/L of total suspended solids), 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 increased from 0.050 to 0.400 μg/L and finally 20 μg/L in a period of 200 days. In the second period (B), the performance of the MBR was investigated in higher BPA concentrations by a gradual increase in concentration from 1,000 μg/L to 40,000 μg/L. During the startup and operating period, the hydraulic retention time (HRT) and SRT were 5.5 h and 140 days, respectively, while temperature and pH were maintained around 20 ± 2 °C and 7 ± 1, respectively.

**Analytical methods**

Twice per week, samples were collected at the inlet, outlet and mixed liquor for the analysis of total suspended solids (TSS), total volatile suspended solids (TVSS), chemical oxygen demand (COD), ammonium (N-NH₄), nitrate (N-NO₃), orthophosphate (P-PO₄), pH, and BPA. All parameters were determined in conformity with standard 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 °C. The pellet was kept for drying at 105 °C for 24 h. Subsequently, TVSS was measured by burning the dried pellet at 550 °C for 30 min according to MA-104-S.S.1.1 method. Ammonia (N-H₄-N) and phosphorus (PO₄-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 samples using the Visiprep system on C-18 cartridges (Seyhi et al. 2013). The samples were filtered through a glass fiber membrane (Whatman GF-C, porosity: 0.7 μm) 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 °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 μg/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, which 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 ± 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 μg/L) did not affect the ability of microorganisms to degrade the organic matter. However, increase in BPA concentration up to 10,000 μg/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 μg/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).
**N-NH₄ and P-PO₄ removal**

The removal performance of inorganic pollutants including nitrogen and phosphorus was also monitored to have an idea of reactor stability. The initial ammonium concentration (N-NH₄) fluctuated between 22 and 82 mg/L, with an average concentration of 58 mg/L. In aerobic condition, autotrophic ammonia-oxidizing bacteria (AOB) transform NH₄⁺ into NO₂⁻/NO₃⁻ by means of HCO₃⁻/CO₃²⁻, followed by nitrite-oxidizing bacteria (NOB), which oxidize toxic nitrite ions into 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).

\[
\begin{align*}
NH_4^+ + 1.98 \text{HCO}_3^- + 1.83 \text{O}_2 & \rightarrow 0.021 \text{C}_5\text{H}_7\text{NO}_2 + 0.98 \text{NO}_3^- + 2.92 \text{H}_2\text{O} + 1.88 \text{CO}_2 \\
\end{align*}
\]

(1)

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-NH₄ of ammonia. Since both AOB and NOB have a biological half-life of more than 10 days, it takes more than a month for development of the nitrifier culture and start of the 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 means of nitrogen removal. In the alkaline pH of the aeration basin, almost 5% of ammonium transformed into the dissolved ammonia (Equation (2)).

\[
\begin{align*}
NH_4^+ + \text{H}_2\text{O} & \leftrightarrow \text{NH}_3 + \text{OH}^- \\
\end{align*}
\]

(2)

Intense aeration in the MBR gradually stripped the dissolved NH₃, leading to unbalancing of Equation (2) and production of more dissolved ammonia; hence, even more than 20% of ammonia removal was observed in the absence of nitrifier bacteria. The MBR with high SRT provides exceptional conditions for maximum development of nitrifiers, along with a high kinetic constant of biological nitrification, complete ammonia oxidation was expected for different organic load rates and sludge retention time higher than 30 days (Zolfaghari et al. 2013). After treatment, the residual concentrations of N-NH₄ in the treated water fluctuated between 0.1 and 17 mg/L, with an average residual concentration of 3 mg/L, corresponding to 94 ± 4% of ammonia removal efficiency (Figure 3). A 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 the nitrifier. Absence of nitrate in the effluent indicated the complete halt of biological nitrification (Figure 4). Nitrifying bacteria are extremely sensitive toward low concentrations of oxygen and high concentrations of toxic compounds. Long term exposure at 40 mg/L of BPA could change the quality and quantity of *Nitrosomonas* and *Nitrobacter* in the aeration basin (Zielinska et al. 2014). In the absence of an anoxic process for transformation of nitrate into nitrogen gas (Henze et al. 2001), air stripping of ammonia is the only removal pathway for total nitrogen removal in period B, which remained below 15% during the operating period.

Unlike ammonia, phosphorus removal requires a combination of anaerobic and aerobic processes 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 an SRT of 140 days, an average of 35% of phosphorus was assimilated by the sludge, which later withdrew from the aeration basin. During the monitoring period, orthophosphate (P-PO₄) 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 ± 16% of PO₄ removal efficiency (Figure 5). It is worth
mentioning that the phosphorus concentration in the effluent was highly fluctuating, due to the sudden release of soluble microbial product into the effluent (Seyhi et al. 2012). Previous study on an aerobic MBR 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 2015; Zielinska et al. 2014). In this study, therefore, HRT remained constant during the 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 40 to 110), the MBR was fed by a synthetic solution with an average BPA concentration of 0.400 μg/L. The BPA influent concentration in the MBR varied between 0.240 – 0.480 μg/L. As shown in Figure 6, the residual BPA concentration was relatively near to or below 0.02 μg/L, with an average removal efficiency of 90%. From day 130 to day 190, the concentration of BPA was then increased to 20 μg/L. In the vast majority of treated water samples, the residual BPA concentration 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 at around 480 μg/g dry sludge. According to Figure 7, the BPA mass balance showed that almost half of 0.72 mg of BPA was degraded in the sludge per day. Biosorption 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·mol⁻¹) (Chen et al. 2008). Furthermore, the size of BPA was much smaller than the 40 nm of the ultrafiltration pore size; therefore, membrane retention has no effect on the BPA removal pathway (Zhu & Li 2013).

Performance of MBR for high BPA concentration

From day 200 to day 550, the 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, the glucose concentration decreased 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 the MBR). After increasing the BPA concentration in each stage, the performance of the MBR was dramatically decreased (Figure 8), yet the BPA removal efficiency recovered over a period of two weeks to reach a rate of 97% or more. Indeed, increasing the initial concentration of BPA put the biomass into a state of stress, due to the toxic character of BPA. The results obtained in this study indicated that after the adaptation period, the MBR effectively
treated an effluent with initial BPA concentrations up to 40,100 μg/L (Figure 8). The BPA mass balance study for the high influent concentration showed that the rate of biodegradation rapidly increased up to 16 mg/g/day, resulting in 87% of BPA removal by biodegradation. As also reported in the literature (Chen et al. 2018), development of specialized bacteria for BPA removal in the aeration basin increased the portion of biodegradation ratio for total removal. As these bacteria are retained by the membrane, their concentration is continuously increased, resulting in high BPA removal efficiency in higher concentrations of BPA. In higher BPA concentrations, only adapted microorganisms could survive, resulting in 560 times increase in the daily biodegradation rate. Enzymatic reaction could be also responsible for the 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 resistant toward further biodegradation, and could be the main reason for the increase in residual COD in the effluent of the MBR.

CONCLUSION

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

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