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Fate and behaviour of dissolved organic matter in a submerged anoxic-aerobic membrane bioreactor (MBR)

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Abstract

In this study, the production, composition and characteristics of dissolved organic matter (DOM) in an anoxic-aerobic submerged membrane bioreactor (MBR) were investigated. The average concentrations of proteins and carbohydrates in the MBR aerobic stage were 3.96±0.28 and 8.36±0.89 mg/L, respectively. After membrane filtration, the values decreased to 2.9±0.2 and 2.8±0.2 mg/L, respectively. High performance size exclusion chromatograph (HP-SEC) analysis indicated a bimodal molecular weight (MW) distribution of DOMs and that the intensities of all peaks were reduced in the MBR effluent compared to the raw influent. Three-dimensional fluorescence excitation emission matrix (FEEM) indicated that fulvic and humic acid-like substances were predominant DOMs in biological treatment processes. Precise identification and characterization of low-MW DOMs were carried out using employed by gas chromatography- mass spectrometry (GC-MS). The GC-MS analysis indicated that the highest peak numbers (170) were found in the anoxic stage, and 54 (32%) compounds were identified with a similarity greater than 80%. Alkanes (28), esters (11) and aromatics (7) were the main dominant compounds detected. DOMs exhibited both biodegradable and recalcitrant characteristics. There were noticeable differences in the low-MW DOMs present down the treatment process train in terms of numbers, concentrations, molecular weight, biodegradability and recalcitrance.

Keywords: Dissolved organic matter (DOM); anoxic-aerobic MBR; fluorescence excitation emission matrix (EEM); gas chromatography - mass spectrometry (GC-MS)

1.   Introduction

With continuing depletion of fresh water resources, the focus of many researchers has shifted more towards resource recovery than treatment. Enhanced wastewater reclamation is gaining considerable attention due to the fast-growing demand for water reuse, and more stringent compliance regulations for wastewater discharge. Membrane bioreactor (MBR) technology, which combines biological treatment and membrane separation, is an increasingly attractive option for the treatment and reuse of industrial and municipal wastewater. Compared to the conventional activated sludge (CAS) process, MBRs have various distinct advantages such as lower sludge production, prolonged biomass retention, smaller footprint and better effluent quality (Meng et al., 2009a, Stuckey, 2012).

Dissolved organic matter (DOM) is a heterogeneous mixture of aromatic and aliphatic organic compounds, which contains humic substances, hydrophilic acids, proteinss, lipids, carbohydrates, carboxylic acids, amino acids, and hydrocarbons (Wang et al., 2009). It is ubiquitous in surface water and sewage, and has been a major concern in water and wastewater treatment processes for a long time (Tang et al., 2010). DOM not only plays a critical role in affecting microbial activity, pollutant degradation, and transport of metals, but also can potentially be converted to toxic by-products during treatment (Imai et al., 2003). In the biological wastewater treatment processes, it has been reported to affect both the kinetic activity and flocculating properties of activated sludge, and can react with disinfectants resulting in the formation of various disinfection by-products (DBPs) (Kunacheva and Stuckey, 2014). Since DOMs usually exhibit colloidal properties, e.g., a large surface area, mobility and an electronic double layer (Seo et al., 2007), they encompass a broad MW distribution, from smaller than 1 kDa to over 100 kDa (Liang et al., 2007). On the one hand, DOM is composed of various types of non-biodegradable organic compounds, and these recalcitrant compounds may be retained in the system and discharged in the effluent (Trzcinski et al., 2016); on the other hand, while with cell lysis some DOMs may be biodegraded into small molecules (Aquino and Stuckey, 2004,

Meng et al., 2009b). Therefore, the great structural complexity and chemical stability of DOM in wastewater biological treatment processes impose challenges on MBR operation and performance.

During the biological treatment, a part of the bound extracellular polymeric substances (EPS) can be hydrolysed or excreted to produce DOMs (Jarusutthirak and Amy, 2006, Liang et al., 2007). However, despite the fact that low-MW DOMs are dominant in secondary effluents, very little information is available about the precise composition of DOMs produced in biological processes (Barker and Stuckey, 1999). Furthermore, although several major components of DOMs such as proteins, carbohydrates, lipids, nucleic acids and humic acids have been frequently identified, little is known about the precise composition of DOMs in biological processes (Liang et al., 2007). There have been several analytical methods developed to distinguish the characteristics of DOM in wastewater, however, few researchers have analysed their composition using sophisticated instruments such as gas chromatography- mass spectrometry (GC-MS) to determine the molecular weight (MW), structures, biodegradability and recalcitrance of specific compounds quantitatively and qualitatively (Kunacheva and Stuckey, 2014, Zhang et al., 2016). Zhang et al. (2016) investigated the behaviour and characteristics of DOMs in an aerobic-anoxic submerged MBR using synthetic wastewater and GC-MS results revealed that aromatics, long-chain alkanes and esters were the predominant DOMs. Kunacheva et al. (2017) investigated the DOMs produced at different hydraulic retention times at both steady state and under transient load conditions in a submerged anaerobic MBR using synthetic wastewater, and reported that 120 compounds were identified in the effluent at steady state, and the predominant DOMs were alkanes (39), esters (11), phenols (11), nitrogenous compounds (11), alcohols (7), and others. Trzcinski et al. (2016) compared the performance of the A-stage and B-stage sludge in terms of anaerobic biodegradability and the low-MW compounds present in the supernatant using GC-MS, and revealed that the main DOMs in A-stage and B-stage included aromatics (27.9% and 21%), alcohols (25.6% and 15%, and acids (30.2% and 15%). To better understand where these compounds originated from and why they are generated, it is important to evaluate the production and composition of DOMs in biological treatment processes, so that we can

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find an appropriate solution for attenuating the levels of these compounds in wastewater treatment effluent.

The main objective of this study was to investigate the fate and behaviour of DOMs in a submerged anoxic-aerobic MBR during their degradation and transformation. More specifically, the objectives of this study were to i) investigate the MW distribution of DOMs; ii) explore the main chemical composition of the DOMs; and iii) identify low-MW (< 580 Da) DOMs produced in the biological treatment processes.

2. Materials and methodologies

2.1. Bench-scale MBR

A bench-scale MBR system consisting of an anoxic compartment (2 L) and an aeration compartment (5 L) was operated in series at room temperature (24-25°C) (Figure S1). A hollow fibre ultrafiltration (UF) membrane (ZeeWeed 500, GE Singapore), made of polyvinylidene fluoride, was submerged inside the aerobic compartment, and its effective membrane surface area was 565 cm^2 with a nominal pore size of 0.04 μm. To control the MBR process, 3 min of filtration was followed by 1 min of relaxation was achieved using fully automated SCADA software (IFIX).

The MBRs were inoculated with biomass obtained from Ulu Pandan Wastewater Reclamation Plant (WRP), Singapore. Synthetic wastewater was used in this study to simulate domestic sewage (~640 mg COD/L), and its chemical composition is given in Table S1. This procedure allows us to understand which molecules are produced by bacteria and end up as dissolved organics under controlled conditions. The influent was prepared in a 70-L glass tank. The concentration of mixed liquor suspended solid (MLSS) in the aeration tank was maintained at around 3-6 g/L with an average sludge retention time (SRT) of 25 d. The hydraulic retention time (HRT) was approximately 10 h, and a permeate flux of 13 - 15 L/m^2 h (LMH) was maintained. Level sensors were installed in the MBR to control the feeding of influents and production of membrane permeates. The MBR was fitted with a

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gas diffuser located on the bottom of the aeration tank to maintain the dissolved oxygen (DO) concentration in the sludge at about 3-4 mg/L for biological oxidation and to achieve membrane scouring. The transmembrane pressure (TMP) was monitored automatically using a digital pressure gauge (Ashcroft). General parameters, such as membrane flux, pH, DO, and temperature were automatically recorded using a data logger. The MBR was operated continuously for a period of 6 months after 60 days of acclimatisation.

2.2. Analytical methods

2.2.1 Detection of water quality parameters

Influents, anoxic mixed liquors, aerobic mixed liquors, and membrane effluents were collected twice a week from the MBR for measurement of conventional parameters. The measurement of mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), chemical oxygen demand (COD), ammonium (NH_4^+-N) and phosphate (PO_4^{3-}) was in accordance with Standard Methods (APHA, 2012).

2.2.2 Determination of proteins and carbohydrates concentrations

The extraction of DOMs and bound EPS from the mixed liquor in the MBR anoxic and aerobic stage followed the method described by Zhang et al. (2017). Briefly, the mixed liquor of activated sludge was centrifuged at 12,000 g for 15 min; the resulting supernatant represented the DOMs. Next, the dewatered sludge pellet was washed with saline water (0.9% NaCl solution) twice prior to extraction. The mixed liquor was then subjected to sonication at 20 kHz for 2 min, and centrifuged at 12,000 g for 15 min. The phenol-sulfuric acid and Lowry methods were used for the determination of proteins and carbohydrates concentrations, respectively.

2.2.3 The bioelimination rate (BER) and membrane rejection rate (MRR)

The net bioelimination can be used to characterize the degradation of DOM during the biological processes, which was obtained by comparing DOM in the influent and sludge supernatant (Meng et al., 2009b). The bioelimination rate (BER) and membrane rejection rate (MRR) of DOMs were calculated by using the following equations:

$$BER\ (\%) = \frac{DOM_{influent} - DOM_{sludge}}{DOM_{influent}} \times 100\% \tag{1}$$

$$MRR\ (\%) = \frac{DOM_{sludge} - DOM_{permeate}}{DOM_{permeate}} \times 100\% \tag{2}$$

Where, $DOM_{influent}$ is the DOM concentration in the influent (mg/L); DOM_{sludge} is the DOM concentration in the aerobic supernatant (mg/L); and $DOM_{permeate}$ is the DOM concentration in membrane permeate (mg/L).

2.2.4 Molecular weight (MW) distribution of DOMs

A high performance size exclusion chromatograph (HP-SEC) (Agilent Technologies, 1260 LC system) equipped with the PL Aquagel-OH 8 lm MIXED-M column was used for the analysis of MW distribution of DOMs. A 10 mL sample was first centrifuged at 10,000 rpm for 10 min and then filtered with a 0.22 mm PTFE syringe filter (SLFG013NK, Millipore, Millex-FG). Milli-Q water was used as the mobile phase at a flow rate of 1 mL min^{-1}. Polyethylene glycols (PEGs) and polyethylene oxide standards with molecular weights of 500 kDa, 70 kDa, 4 kDa, 600 Da and 106 Da were used for the calibration. MW of DOMs was calculated according to the calibration curve and a linear relationship was derived between the log of MW (Da) and retention time (Rt: min) as shown in Eq. (3).

$$Log\ (MW) = 9.8823 - 0.6748\ (Rt) \tag{3}$$

2.2.5 Main components of DOMs

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Three-dimensional EEM fluorescence spectra were measured using a luminescence spectrometry (Perkin Elmer LS55 Fluorescence Spectrometer). The spectrometer slits were set at 10 nm for both excitation and emission and excitation wavelengths were increased from 220 nm to 600 nm in 10 nm steps; for each excitation wavelength the emission was detected from 300 nm to 550 nm in 10 nm steps. The X-axis represents the emission spectra while the Y-axis represents the excitation wavelength, and the contour line is used to express the fluorescence intensity. The software FL Winlab Version 4.00.03 (Perkin Elmer) was employed for handling the EEM data, which were plotted as elliptically shaped contours.

2.2.6   Identification of low-MW DOMs (< 580 Da)

In the present study, identification of low-MW DOMs (< 580 Da) was carried out using Gas chromatography - mass spectrometry (GC-MS), which allows for the detection of non-polar, volatile and thermo-stable compounds. Prior to the GC-MS analysis, liquid-liquid extraction was performed on a 100 mL filtered supernatant (< 0.45 μm) using 70 mL dichloromethane (GC-MS grade, Merck) (Wu and Zhou, 2010), this solvent was selected because it had been used by previous researchers for SMP analysis using GC-MS (Wu and Zhou, 2010). All glassware was washed with acetone prior to the procedure. Mixing was for 3 minutes by manually inverting the extraction funnel and separation of the 2 phases occurred over 5 minutes. Traces of water were removed by mixing the solvent phase with 2 spoons (5 mL) of Na_2SO_4. The solvent was evaporated at 50°C under vacuum until 1 mL of solvent remained.

The eluted samples were then analysed using a gas chromatograph (5890 Series) equipped with a QP2010Ultra Mass Spectrometry Detector (GCMS-QP2010ULTRA, Shimadzu, Japan). The analytes were separated using an Rtx-5MS column (30 m x 0.25 mm with a film thickness of 0.25 µm). ~~The GC-MS oven temperature program was: 50 °C, hold 7 min, rate 7 ºC min^-1 and then thereafter increased to 325°C and hold 14 min.~~ Helium was the carrier gas at a flowrate of 1 mL/min. The

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injector temperature was set at 270°C, and the MS was operated in the electron impact ionisation mode (70 eV). The total runtime per sample was 60 min, and the GC-MS oven temperature program was: 50 °C, hold 7 min, rate 7 °C min^{-1} and then thereafter increased to 325°C and hold 14 min. The temperature program was modified based on the alkane standards (C_{10}-C_{40}, 50 mg/L, Sigma-Aldrich). Mass spectra were acquired from *m/z* 30 to 580 after a 10 min solvent cut time. The chromatograms were analysed using the NIST11 library (National Institute of Standards and Technology, Gaithersburg, MD, USA, http://www.nist.gov/srd/mslist.htm), and the compound was considered identified if the match percentage was higher than 80%. Compounds that had a match percentage below 80% were mentioned as unknown peaks. Similarity index, mass spectrum and retention index were all used as selection criteria for compound identification of the NIST library list of suggested compounds. Method blanks (deionised water) were run through the same pre-treatment and analysis, while feed samples were also run to identify compounds in the feed.

Alkanes were selected for the approximate quantification of SMPs, since alkanes have widely variant chain lengths 9C10-C40), and hence are able to cover most of the volatility range of the RTX®-5MS column. The calibration curve for each compound was plotted with concentration points 0.1, 0.25, 0.5 and 1 mg/L. Quantification was done separately for each unknown compound using the alkane with the closest retention time. A set of standards was run in and between every batch of analyses to minimise instrumental error. The instrumental identification limit (IDL) of alkane standards was evaluated for each compound based on maximum blank concentration, and the signal-to-noise ratio of 3. Although there may be a considerable degree of uncertainty surrounding the concentration of identified compounds beside alkanes, it is a useful tool to ~~gain~~ understand~~ing what~~ which compounds we~~re~~ produced as SMPs and their approximate concentrations.

3.   Results and discussion

3.1.   Process performance

The general performance of the MBR in terms of MLVSS, MLSS, COD, NH_4^+-N, and PO_4^{3-} are summarized in Table 1. Throughout the experiment, the average concentrations of MLSS and MLVSS were 4.7 g/L and 4.1 g/L, respectively. As expected, almost complete degradation of organic substrates and nitrification were seen during the experimental period. The average COD concentration in the effluent was 15.4±2.2 mg/L, resulting in high removals of 97.6%, which shows the substantial potential of the MBR in wastewater treatment. With respect to NH_4^+-N, an average removal efficiency of 94.8% was observed in the MBR.

3.2.   ~~Variations of p~~Proteins and carbohydrates concentrations in the MBR process

The variations in proteins and carbohydrates concentrations in the MBR treatment processes are shown in Figure 1a. The average concentration was 4±0.3 mg/L for proteins and 8.4±0.9 mg/L for carbohydrates in the aerobic stage, respectively. After membrane filtration, the values decreased to 2.9±0.2 mg/L and 2.8±0.2 mg/L, respectively. Our finding is consistent with Liang et al. (2007) who reported a proteins concentration of 7.2 mg/L in the sludge supernatant and 3.64 mg/L in the MBR effluent, respectively, while the values for carbohydrates were 10.14 mg/L and 4.93 mg/L, respectively. In addition, in the present study, the levels of proteinaceous EPS were 41.8±3.7 mg/g VSS and 38.2±4.7 mg/g VSS in the MBR anoxic and aerobic stage, while the values for carbohydrates were 18.7±3.9 mg/g VSS and 17.1±2.7 mg/g VSS, respectively (Figure 1b).

The origin of DOMs in biological wastewater treatment is very complex, while the majority of the DOMs in the feed are biodegradable over time in both aerobic and anaerobic processes (Barker and Stuckey, 1999). In the present study, 82 % of proteins and 42% of carbohydrates in the influent could be removed through bioelimination. Moreover, the rejection rate of DOMs by the membrane was 30% for proteins and 73% for carbohydrates, on average. This was attributed to the smaller size of proteins compared to carbohydrates, resulting in their permeation into the MBR effluent more easily. This finding is consistent with Drews et al. (2007) who reported that the rejection rate of DOM by a PAN membrane ranged from 20% to 70% for proteins, and from 75% to 100% for carbohydrates.

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3.3.   Molecular weight (MW) distribution of DOMs

The HP-SEC chromatograms representing different treatment stages are shown in Figure 2. Five representative peaks in the chromatograms were identified. Peak 1 and Peak 2 demonstrate high-MW SMPs (313.4 kDa and 196.6 kDa, respectively), while Peak 3 (14.1 kDa) and Peak 4 (10.3 kDa) showed the presence of an intermediate-MW fraction. Peak 5 (3.8 Da) indicating low-MW compounds (< 1 kDa) were also detected.

The peak intensity of HP-SEC chromatograms can be used to express relative concentration of DOMs (Wang et al., 2009). Although the chromatograms indicated similar retention times for the collected samples, their peaks and locations along with the treatment process were different. During the operation of the MBR, the high-MW (313.4 and 196.6 kDa) fractions, which are mainly derived from the decay of bacterial cells during the endogenous stage (Aquino et al., 2006, Shin and Kang, 2003), did not exhibit remarkable changes in the MBR aerobic stage compared to the influent. High-MW compounds usually exhibited refractory characteristics and remained in the system until discharged (Jarusutthirak and Amy, 2006, Namour and Müller, 1998). Due to membrane rejection, DOMs with high-MW are usually accumulated and retained in the bioreactor, which can consequently result in severe membrane fouling (Rosenberger and Kraume, 2002, Shin and Kang, 2003). Moreover, dynamic transformation of DOMs could be observed from Peak 3 and Peak 4 presenting intermediate-MW (14.1and 10.3kDa) fractions, in which their intensities reduced in the anoxic stage, but increased in the MBR aerobic stage, implying these compounds had been firstly biodegraded into smaller fractions and then transformed into larger fragments. In addition, the intensities of all peaks were reduced in the effluent, compared to the aerobic stage, suggesting an efficient removal of DOMs by membrane filtration.

3.4.   Fluorescence Excitation Emission Matrix (FEEM) contours

The composition of DOMs was monitored by three-dimensional EEM spectroscopy, and representative fluorescence spectra are shown in Figure 3. Five fluorescence peaks could be identified from the FEEM fluorescence spectra. Peak A was detected at the excitation/emission wavelengths (Ex/Em) of 265-275/300-315 nm, while Peak B was located at the Ex/Em of 290-305/345-360 nm. These two peaks have been ascribed to SMP-like substances in which the fluorescence was associated with the tyrosine (Peak A) and tryptophan (Peak B) proteins, respectively (Chen et al., 2003, Wang et al., 2009). Tyrosine and tryptophan substances are labile organics, which are associated with microbial activity and predominant in wastewater (Ishii and Boyer, 2012). Two strong peaks at the Ex/Em of 265-275/445-455 nm (Peak C) and 300-350/410-425 (Peak D) were detected, which have been reported to be related to polyaromatic- and polycarboxylate- type humic acids, respectively (Chen et al., 2014, Chen et al., 2003, Wang et al., 2009, Wang and Zhang, 2010). A minor peak (Peak E) at the Ex/Em of 220-230/390-420 nm associated with fulvic acid-like substances (Chen et al., 2003, Wang et al., 2009) was also observed in MBR effluent.

Fluorescence parameters, including peak locations and peak intensity, can be used to analyse DOM characteristics. In general, an intensity reduction of the fluorescence peak between raw water and the sludge supernatant is an indication of degradation of the fluorescing material. Among all the samples tested, the intensities of Peak C, Peak D and Peak E were higher than those of other peaks, implying that humic acids and fulvic acid-like substances were dominant in the samples. Fulvic and humic acids are hydrophobic fractions of DOMs, which are metabolized by natural or biological degradation, and their structures are known to be rich in aromatic carbon and carboxyl groups (Ma et al., 2001). Moreover, compared with the EEM spectra in the influent, the location of Peak D in the MBR effluent was blue-shifted by 20 nm along the excitation axis, and by 10 nm along the emission axis, and the location of Peak E was blue-shifted by 30 nm along the emission axis, indicating that the structure and component of fulvic and humic acid-like substances in the MBR effluent were different from those in the influent. A blue shift is associated with the decomposition of condensed aromatic moieties and the break-up of large molecules into smaller fragments, such as a decrease in the number of aromatic rings, a reduction of conjugated bonds in a chain structure, a conversion of a linear ring

system to a non-linear system, or the elimination of particular functional groups including carbonyl, hydroxyl and amine (Wang et al., 2009).

In order to better understand the similarities and differences between the samples collected from different treatment units, the fluorescence regional integrating (FRI) method was also used to analyse the excitation-emission regions, as described by previous researchers (Chen et al., 2003, Wang et al., 2009) (Figure 4). Regions I, II, III and IV represent tyrosine-like proteins, tryptophan-like proteins and soluble microbial by-product-like substances, respectively. Regions IV, V and VI represent fulvic acid-like, polyaromatic humic acid-like and polycarboxylate humic acid-like substances, respectively. Compared to other treatment units, a noticeable increase in the fraction of compounds in Regions I and II, which were associated with tyrosine-like proteins and tryptophan-like proteins, was observed in the MBR effluent. In contrast, Regions V and VI, representing polyaromatic- and polycarboxylate-type humic acids, showed a decrease in the MBR effluent. DOM contains large quantities of aromatic structures and unsaturated fatty chains with various types of functional groups (Sheng et al., 2010). This finding indicated that significant changes in the functional groups, the conjugated bonds in a chain structure, and condensed aromatic moieties of the aromatic amino acids and tryptophan proteins might have occurred after MBR biological treatment.

3.5.   Characterization of low-MW DOMs using GC-MS

3.6.1   DOMs in the raw influent

In the present study, GC-MS was employed to precisely identify and characterize low-MW DOM fractions in biological treatment processes. As seen in Figure S2, GC-MS chromatograms revealed that the raw influent had the largest number of spectra registering high organic component loading with greater response abundance, while the effluent of the MBR had smaller number of spectra with much less response abundance. There had was an obvious decrease in the response abundance between raw influent and MBR effluent with the removal of DOMs. The identification of DOM

fractions in the raw influent are shown in Table S2. The total peaks detected were 184, but only 52

(29%) compounds were identified with a similarity greater than 80% (Figure 5a). Among the

compounds identified, alkanes accounted for 37% of the total DOM, followed by alcohols (19%),

aromatics (19%) and esters (19%), respectively (Figure 6a).

3.6.2   DOMs in the MBR anoxic stage

In the MBR anoxic stage, the total peaks detected were 170 and 54 (32%) compounds were

identified with a similarity greater than 80% (Figure 5b). Among the compounds identified, alkanes

accounted for 52% of the total DOMs, followed by esters (20%) and aromatics (13%) (Figure 6b).

This finding is consistent with Wu and Zhou (2010) who investigated DOMs in anaerobic wastewater

treatment using GC-MS, and reported that the predominant DOMs were long-chain alkanes (21%),

esters (18%), acids (17%) and aromatic compounds (12%).

Nearly half of the compounds detected in the MBR anoxic stage, such as alkanes (e.g., 1-

Heptadecene, Nonadecane, and Heneicosane, etc.), esters (e,g., 3,7-Dimethyl-6-nonen-1-ol acetate,

Pentafluoropropionic acid, octadecyl ester, and 9-Octadecenoic acid (Z)-, methyl ester, etc.), alcohols

(e.g., Ethanol, 1-(2-butoxyethoxy)-, n-Heptadecanol-1, and Phytol, etc.), and aromatics (e.g., 10-

Cyclohexylnonadecane, etc.), were not the same as in the influent (Tables 2 and 3), implying that

these compounds might belong to DOMs generated in the biological treatment processes, and were

most probably the results of a direct transformation of the original substrate (Barker and Stuckey,

1999). In general, SMPs typically accumulate during the start-up stage in biological wastewater

treatment, and are partially degraded rapidly in the initial hours, and serve as substrate for a

generation of new biomass (Huang et al., 2000, Shin and Kang, 2003). In contrast, some compounds

identified in the influent, such as alcohols (e.g., Behenic alcohol, and n-Tetracosanol-1, etc.), esters

(e.g., Hexacosyl heptafluorobutyrate, and Methyl stearate, etc.,), and aromatics (e.g., 1,3-

Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, etc.) disappeared in the MBR anoxic stage. This

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finding indicated that slowly biodegradable matter and inert matter in the feed could result in generation and accumulation of DOM in the subsequent biological processes.

3.6.3   DOMs in the aerobic stage of the MBR

Compared to the total number of DOMs generated in the MBR anoxic stage (170), only 96 compounds were detected in the aerobic stage (Figure 5c). 33 compounds (34%) were identified with a match percentage greater than 80%. Alkanes still accounted for the largest percentage of DOM (43%), followed by alcohols (18%), aromatics (12%) and esters (12%) (Figure 6c). In comparison, Zhang et al. (2016) investigated the behaviour and characteristics of SMP in an anoxic-aerobic MBR for treating municipal wastewater containing pharmaceutical compounds, and found that the dominant compounds identified were alkanes (51%), aromatics (20%) and esters (17%) in the aerobic stage. In particular, these long-chain alkanes and esters are frequently reported in biological treatment effluent and are known to be the main components of low-MW SMPs in aerobic reactors (Janga et al., 2007, Liang et al., 2007). Alkanes can be produced by bacterial metabolism and bacteria appear to be able to degrade alkanes under both aerobic and anaerobic conditions (Rojo, 2009).

Both biodegradable and refractory DOMs may be released into the biological treatment systems. Nearly half (16) of the compounds identified in the MBR anoxic stage, such as aromatics (e.g., Phenol, 2,4-bis(1,1-dimethylethyl)- and 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, etc.), alkanes (e.g., Heptadecane, 10-Methylnonadecane, and 1-Nonadecene, etc.), esters (e.g., Nonadecyl pentafluoropropionate), and alcohols (e.g., 1-Heneicosanol, etc.), were the same as in the MBR aerobic stage, implying the incomplete biodegradation across a range of biological catabolic pathways (Tables 3 and 4). In contrast, some compounds identified in the MBR anoxic stage, such as alkanes (e.g., 1-Tridecene, Octadecane), and esters (e.g., 3,7-Dimethyl-6-nonen-1-ol acetate, and 9-Octadecenoic acid (Z)-, methyl ester), were not found in the MBR aerobic stage. This finding indicated that these simple compounds might have been completely biodegraded, especially in the

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aerobic stage, where biodegradability is enhanced by increased bacterial activity, and the aerobic degradation of organics might be more complete.

3.5.3 DOMs in MBR effluent

By identifying the recalcitrant compounds in the treated effluent, operators could take an informed decision on the level and type of advanced post-treatment (chlorination, ozone, UV light or reverse osmosis) required to protect water sources. There has been an increase in the release of pharmaceuticals, drugs, personal care products, pesticides, emerging contaminants in raw sewage, and it is important to understand which molecules are likely to accumulate in the hydrosphere. Knowing which compounds escape the MBR under controlled conditions, and knowing their biodegradability can help to select appropriate tertiary treatment and fine-tune them. From a scientific point of view, it is important to understand which type of compounds constitute the final effluent of a MBR.

Fewer compounds were detected in the MBR effluent (60) compared to the MBR aerobic stage (96), resulting in a DOM removal of 37.5% induced by membrane rejection. Only 22 (37%) compounds could be identified with a match percentage greater than 80% (Figure 6d). The main compounds detected were aromatics (32%) and alcohols (32%), followed by alkanes (23%) (Figure 6d).

In the present study, DOMs exhibited both biodegradable and recalcitrant characteristics. Some compounds such as alkanes (e.g., Heptadecane, 10-Methylnonadecane, Eicosane, 5-Butyl-5-ethylheptadecane, and 2-methyltetracosane), esters (e.g., Hexadecanoic acid, methyl ester, Nonadecyl pentafluoropropionate), and aromatics (e.g., Phthalic acid, di(6-methylhept-2-yl) ester), which were detected in both the MBR anoxic and aerobic stages, were not found in the MBR effluent. In contrast, recalcitrant compounds such as alkanes (1-Nonadecene, Tetracosane and 2-methyloctacosane), alcohols (1-Heneicosanol), acids (Octadecanoic acid), and aromatics (Phenol, 2,4-bis(1,1-dimethylethyl)-, Octadecanoic acid, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, and 1,2-

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Benzenedicarboxylic acid, butyl 2-methylpropyl ester), which were detected in the influent, remained in the MBR effluent; these results indicates that these compounds were resistant to biodegradation in the MBR processes (Tables 3 and 4). Furthermore, the MW of the compounds detected in the MBR effluent ranged from 150-408 Da, while this value was 206-450 Da in the MBR aerobic stage, suggesting that high MW fractions could be efficiently retained by membrane filtration. In the present study, GC-MS was used for the identification of low-MW, volatile and thermos-stable DOMs (< 580 Da) which limits the interpretation of results to a very narrow group of compounds. From Table 4, the total concentration of analytes amounts to only 107 µg COD/L (assuming a COD equivalence of 3.46 g O_2/g docosane), which contrast with the COD concentration of 15.4 mg/L (Table 1), clearly indicating that high MW compounds make up the bulk of permeate COD. These high MW compounds were also more likely to have caused fouling on the membrane as indicated by the TMP that gradually increased to 90 mbar over the period of the study (data not shown).

Organic analytes in environmental samples are usually present in the milligram per liter (ppm) to the microgram per liter (ppb) level. Moreover, due to their different polarities and chemical properties, they cannot be analyzed accurately without sample pre-treatment, which is an essential part of chromatography, to both concentrate and also reduce the signal saturation of the chromatogram (Kunacheva et al., 2017b). Due to the wide range and complexities of molecules involved, there is no method capable of extracting all compounds from the water matrix and successfully analyzing them. Nonetheless, liquid/liquid extraction method using dichloromethane is considered suitable to extract a wide range of compounds from moderately polar to non-polar compounds such as aromatics. This method was shown to detect some aromatics, but maybe not all. On the other hand, aromatics may be harder to identify from known compounds in the library compared to aliphatic compounds. With this in mind, some authors have used a combination of solid and liquid phase extraction involving several solvents varying from polar to nonpolar (methanol, acetone, dichloromethane and n-hexane) to maximize the number of compounds peaks (Kunacheva et al., 2017b).

An increase in aromatic compounds was however seen in the MBR effluent (32%), compared to the anoxic (13%) and aerobic stage (12%). This finding is consistent with Liang et al. (2007) who found that the percentage of aromatic compounds in the total SMPs increased after passing through the membrane, and aromatic SMPs seemed much less prone to accumulate in the MBR. This increase in the percentage of aromatics in the MBR effluents from an anoxic-aerobic MBR fed on simple and biodegradable substrates was interesting; aromatic compounds are generally more recalcitrant, and may not be easily degradable during biological treatment, thereby causing the residual COD (Aquino and Stuckey, 2004). It was also observed that certain aromatic compounds with high concentrations, such as Phenol, 2,4-bis(1,1-dimethylethyl)- (17.32 µg/L), exhibited consistently recalcitrance along biological wastewater treatment process; while ~~Phenol, 2,4-bis(1,1-dimethylethyl)~~ phthalic acid, 4,4-dimethylpent-2-yl octyl ester (~~17.32~~ 37.39 µg/L) was only present in the MBR effluent, implying that some compounds possibly transformed into more aromatic structures during biological treatment.

Besides bioelimination, membrane filtration should also play a significant role in the fate of DOMs during an MBR process (Meng et al., 2009b). The reasonable DOM rejection by membrane filtration (37.5%) could be due to the cake gel layer by the physical deposition of large DOM aggregates, which were mainly composed of soluble and colloidal materials (e.g., proteins~~s~~, carbohydrates, and organic colloids), and may stick tightly to the membrane surface. In this study, the flux was maintained at 13-15 LMH with 3 minutes filtration followed by 1 minute relaxation. The TMP gradually increased over time, but remained lower than 100 mbar throughout the study indicating the presence of a biofilm and gel layer on the membrane. During membrane filtration, the cake gel layer formed by DOMs and other organic substances (e.g., colloids and solutes) was found to be an effective secondary filtration layer for organic compounds (Horng et al., 2009, Ren et al., 2010). However, bioelimination and permeate discharge cannot thoroughly elucidate the fate of DOM in the MBR, since DOM production and accumulation (e.g., nonbiodegradable fractions in the influent, cell lysis and bound EPS release in the biological processes) and DOM elimination (e.g., biodegradation, adsorption, membrane filtration etc.) always occurs simultaneously.

4. Conclusions

In this study, we investigated the formation, composition and characteristics of DOMs in an anoxic-aerobic submerged MBR system. Specific conclusions can be drawn as follows:

1) HPLC-SEC analysis indicated a bimodal MW distribution of DOMs including the high-MW (313 and 197 kDa) and low-MW (<1 kDa) fractions. The analysis revealed a dynamic transformation of DOMs in both MBR anoxic and aerobic stage and a reduction in the intensities of all peaks in the MBR effluent compared to the raw influent.

2) Three-dimensional FEEM contours revealed that the MBR system could efficiently retain the predominant compositions of DOMs. i.e., fulvic and humic acid-like substances, and the changes in their location and peak intensities indicated the DOM property alteration.

3) The GC-MS analysis indicated that the highest peak numbers (170) were found in the anoxic stage. Alkanes (52%), esters (20%) and aromatics (12%) were the dominant compounds detected. Besides bioelimination, membrane filtration also played a significant role in the fate of low MW DOMs during an MBR process, contributing to 37.5% of DOM removal. An increase in aromatic fractions was seen in the MBR effluent (32%), suggesting that aromatic DOMs were much less prone to accumulate in the MBR.

4) DOMs exhibited both biodegradable and recalcitrant characteristics. Recalcitrant compounds such as alkanes (1-Nonadecene, Tetracosane and 2-methyloctacosane), alcohols (1-Heneicosanol), acids (Octadecanoic acid), and aromatics (Phenol, 2,4-bis(1,1-dimethylethyl)-, Octadecanoic acid, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, and 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester), which were detected in the influent, remained in the MBR effluent.

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