Environ Sci Pollut Res (2018) 25:4289–4302
https://doi.org/10.1007/s11356-017-0586-x

RESEARCH ARTICLE

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

Dongqing Zhang[1] · **Antoine Prandota Trzcinski**[2] · **Jinxue Luo**[3] · **David C. Stuckey**[4] · **Soon Keat Tan**[1,5]

Received: 19 June 2017 / Accepted: 24 October 2017 / Published online: 27 November 2017

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, these 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 the peaks were reduced in the MBR effluent compared to the influent. Three-dimensional fluorescence excitation emission matrix (FEEM) indicated that fulvic and humic acid-like substances were the predominant DOMs in biological treatment processes. Precise identification and characterization of low-MW DOMs was carried out using 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 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)

Responsible editor: Philippe Garrigues

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s11356-017-0586-x) contains supplementary material, which is available to authorized users.

✉ Dongqing Zhang
dqzhang@ntu.edu.sg

[1] Advanced Environmental Biotechnology Centre, Nanyang Environment and Water Research Institute, 1 Cleantech Loop, #06-10, Singapore 637141, Singapore

[2] School of Civil Engineering and Surveying, Faculty of Health, Engineering and Sciences, University of Southern Queensland, Toowoomba 4350, Australia

[3] Research Center for Eco-Environmental Science, Chinese Academy of Science, Beijing 100085, China

[4] Department of Chemical Engineering, Imperial College London, London SW7 2AZ, UK

[5] School of Civil and Environmental Engineering, Nanyang Technological University, N1-01a-29, 50 Nanyang Avenue, Singapore 639798, Singapore

Introduction

With the 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 contain humic substances, hydrophilic acids, proteins, 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 can also be potentially converted to toxic by-products during treatment (Imai et al., 2003). In 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 electrical double layer (Seo et al., 2007), they encompass a broad molecular weight (MW) distribution, from smaller than 1 kDa to over 100 kDa (Liang et al., 2007). On the one hand, DOMs are 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, 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 biological treatment, part of the bound extracellular polymeric substances (EPS) can be hydrolyzed 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 frequently been 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 analyzed their composition using sophisticated instruments such as gas chromatography-mass spectrometry (GC-MS) to determine the 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 behavior and characteristics of DOMs in an aerobic-anoxic submerged MBR using synthetic wastewater, and GC-MS results revealed that the predominant DOMs were aromatics, long-chain alkanes, and esters. Kunacheva et al. (2017a) 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 showed that the main DOMs in the A-stage and B-stage included aromatics (27.9 and 21%), alcohols (25.6 and 15%), and acids (30.2 and 15%), respectively. 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 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 behavior 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.

Materials and methodologies

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) (Fig. S1). A hollow fiber 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 MBR fouling, 3 min of filtration was followed by 1 min of relaxation 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. Using a synthetic feed rather than sewage allows us to understand which molecules are produced by bacteria and end up as DOMs 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. An average sludge retention time (SRT) of 25 days was maintained by wasting approximately 280 mL of sludge daily. 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 influent feeding and production of membrane permeates. The MBR was fitted with a 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 acclimatization.

Analytical methods

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).

Determination of proteins and carbohydrate concentrations

The extraction of DOMs and bound EPS from the mixed liquor in the MBR anoxic and aerobic MBR stages 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 carbohydrate concentrations, respectively.

The BER and 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:

$$\mathrm{BER}\ (\%) = \frac{\mathrm{DOM}_{influent} - \mathrm{DOM}_{sludge}}{\mathrm{DOM}_{influent}} \times 100\% \quad (1)$$

$$\mathrm{MRR}\ (\%) = \frac{\mathrm{DOM}_{sludge} - \mathrm{DOM}_{permeate}}{\mathrm{DOM}_{permeate}} \times 100\% \quad (2)$$

where $\mathrm{DOM}_{\mathrm{influent}}$ is the DOM concentration in the influent (mg/L), $\mathrm{DOM}_{\mathrm{sludge}}$ is the DOM concentration in the aerobic supernatant (mg/L), and $\mathrm{DOM}_{\mathrm{permeate}}$ is the DOM concentration in membrane permeate (mg/L).

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):

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

Main components of DOMs

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 to 600 nm in 10-nm steps; for each excitation wavelength, the emission was detected from 300 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 lines are used to express the fluorescent 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.

Identification of low-MW DOMs (< 580 Da)

In this 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/mildly 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 soluble microbial products

(SMP) analysis using GC-MS (Wu and Zhou, 2010). All glassware was washed with acetone prior to the procedure. Mixing was for 3 min by manually inverting the extraction funnel and separation of the two phases occurred over 5 min. Traces of water were removed by mixing the solvent phase with two 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 analyzed 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 × 0.25 mm with a film thickness of 0.25 μm). Helium was the carrier gas at a flow rate of 1 mL/min. The injector temperature was set at 270 °C, and the MS was operated in the electron impact ionization mode (70 eV). The total runtime per sample was 60 min, and the GC-MS oven temperature program was 50 °C, hold for 7 min, rate 7 °C min^{-1} and then thereafter increased to 325 °C and hold for 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 analyzed 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 (deionized 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 (C10–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 minimize 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 understand which compounds were produced as SMPs and their approximate concentrations.

Results and discussion

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 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 very 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.

Proteins and carbohydrates concentrations in the MBR process

The variations in proteins and carbohydrate concentrations in the MBR treatment processes are shown in Fig. 1 a. 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, these values decreased to 2.9 ± 0.2 and 2.8 ± 0.2 mg/L, respectively. Our findings are consistent with Liang et al. (2007) who reported a protein concentration of 7.2 mg/L in the sludge supernatant and 3.6 mg/L in the MBR effluent, respectively, while the values for carbohydrates were 10.1 and 4.9 mg/L, respectively. In

Table 1 Treatment performance of general parameters in the MBR

	MLSS (g/L)	MLVSS (g/L)	COD (mg/L)	NH_4^+-N (mg/L)	PO_4^{3-} (mg/L)
MBR anoxic stage	4.3 ± 0.3	3.8 ± 0.3	35.9 ± 7.4 (94.4%)	8.1 ± 2.1 (76.7%)	2.9 ± 0.5 (58.9%)
MBR aerobic stage	4.7 ± 0.4	4.1 ± 0.3	21.8 ± 4.1 (96.6%)	4.2 ± 1.3 (88.9%)	2.7 ± 0.5 (61.7%)
MBR effluent	–	–	15.4 ± 2.2 (97.6%)	1.8 ± 0.2 (94.8%)	2.2 ± 0.3 (68.8%)

Note: the number in the parenthesis refers to the average removal efficiencies (n = 24); the number after the (±) symbol refers to standard deviation (SD)

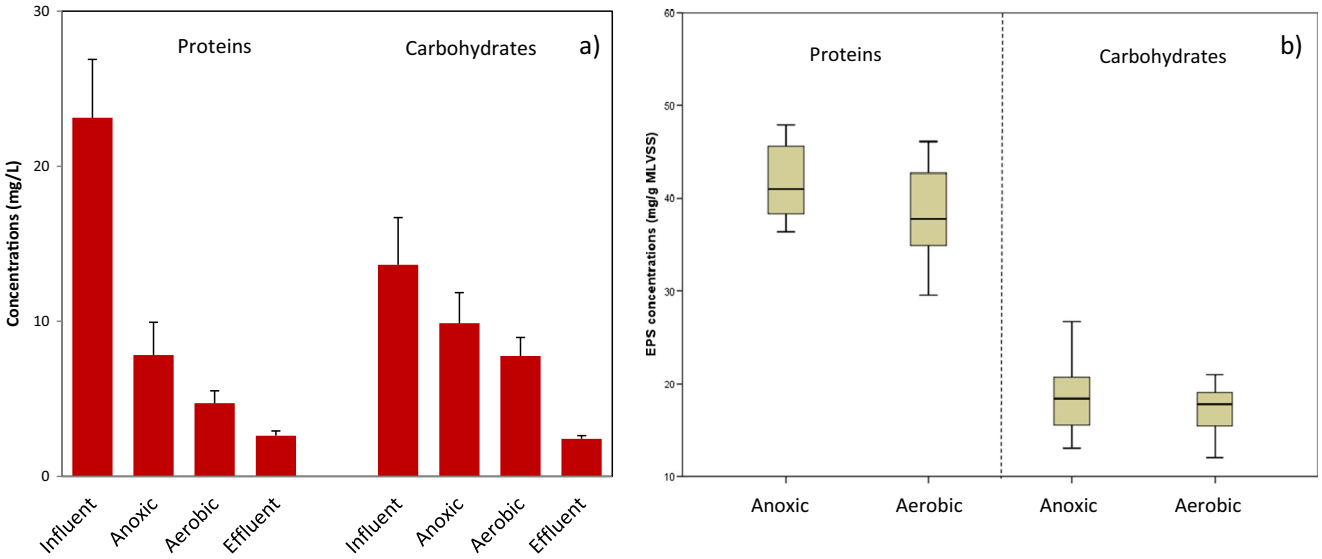

Fig. 1 **a** SMP concentration (mg/L). **b** EPS concentration (mg/g MLVSS) in the submerged anoxic-aerobic MBR

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 anoxic and aerobic MBR stage, while the values for carbohydrates were 18.7 ± 3.9 mg/g VSS and 17.1 ± 2.7 mg/g VSS, respectively (Fig. 1 b).

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 the proteins and 42% of carbohydrates in the influent could be removed through biodegradation. Moreover, the rejection rate of DOMs on average 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.

MW distribution of DOMs

The HP-SEC chromatograms representing different treatment stages are shown in Fig. 2. Five representative peaks in the chromatograms were identified. Peak 1 and peak 2 demonstrate high-MW SMPs (313.4 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 (14.1 and 10.3 kDa) fractions, in which their intensities reduced in the anoxic stage but increased in the MBR aerobic stage, implying that 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.

FEEM contours

The composition of DOMs was monitored by three-dimensional EEM spectroscopy, and representative fluorescence spectra are shown in Fig. 3. Five fluorescence peaks could be identified from the fluorescence excitation emission matrix (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)

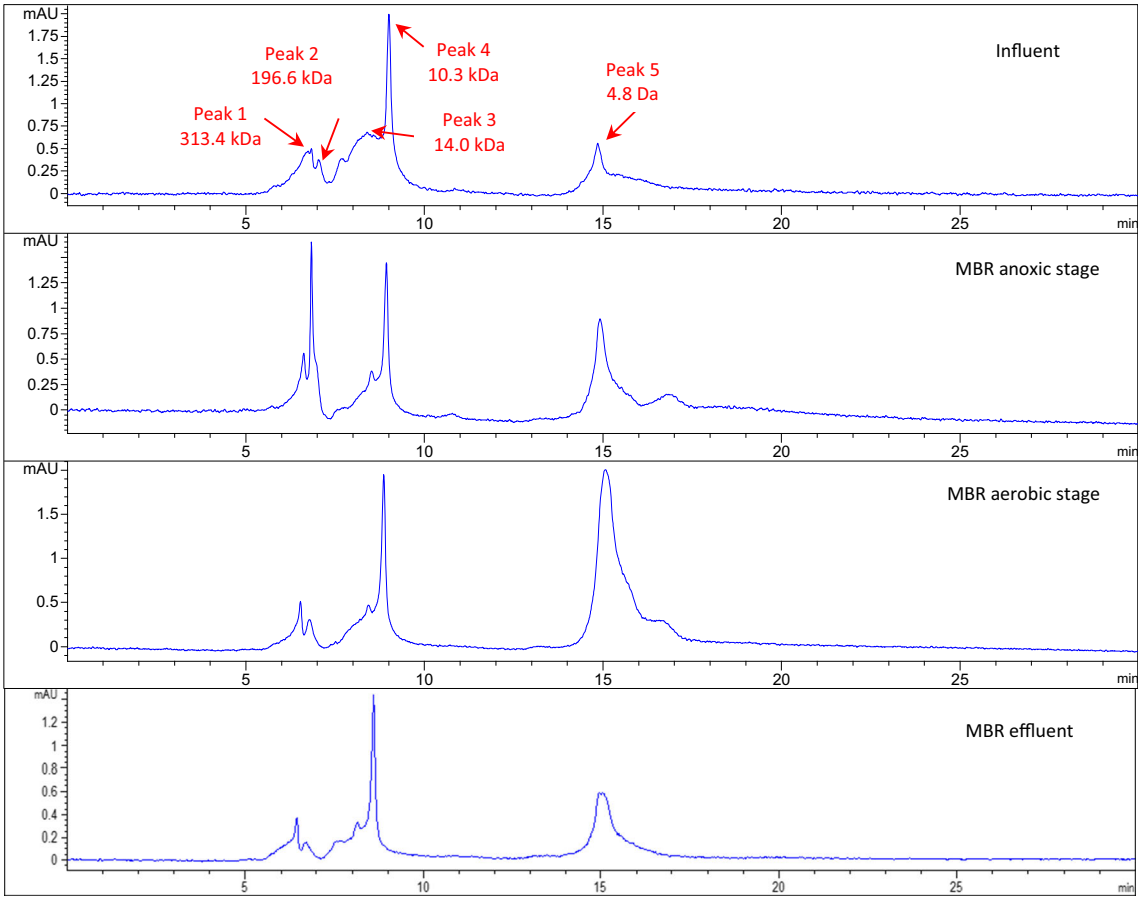

Fig. 2 The MW distribution of DOMs in the anoxic-aerobic MBR

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 analyze 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 analyze the excitation-emission regions, as described by previous researchers (Chen et al., 2003, Wang et al., 2009) (Fig. 4). Regions I, II, and III 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.

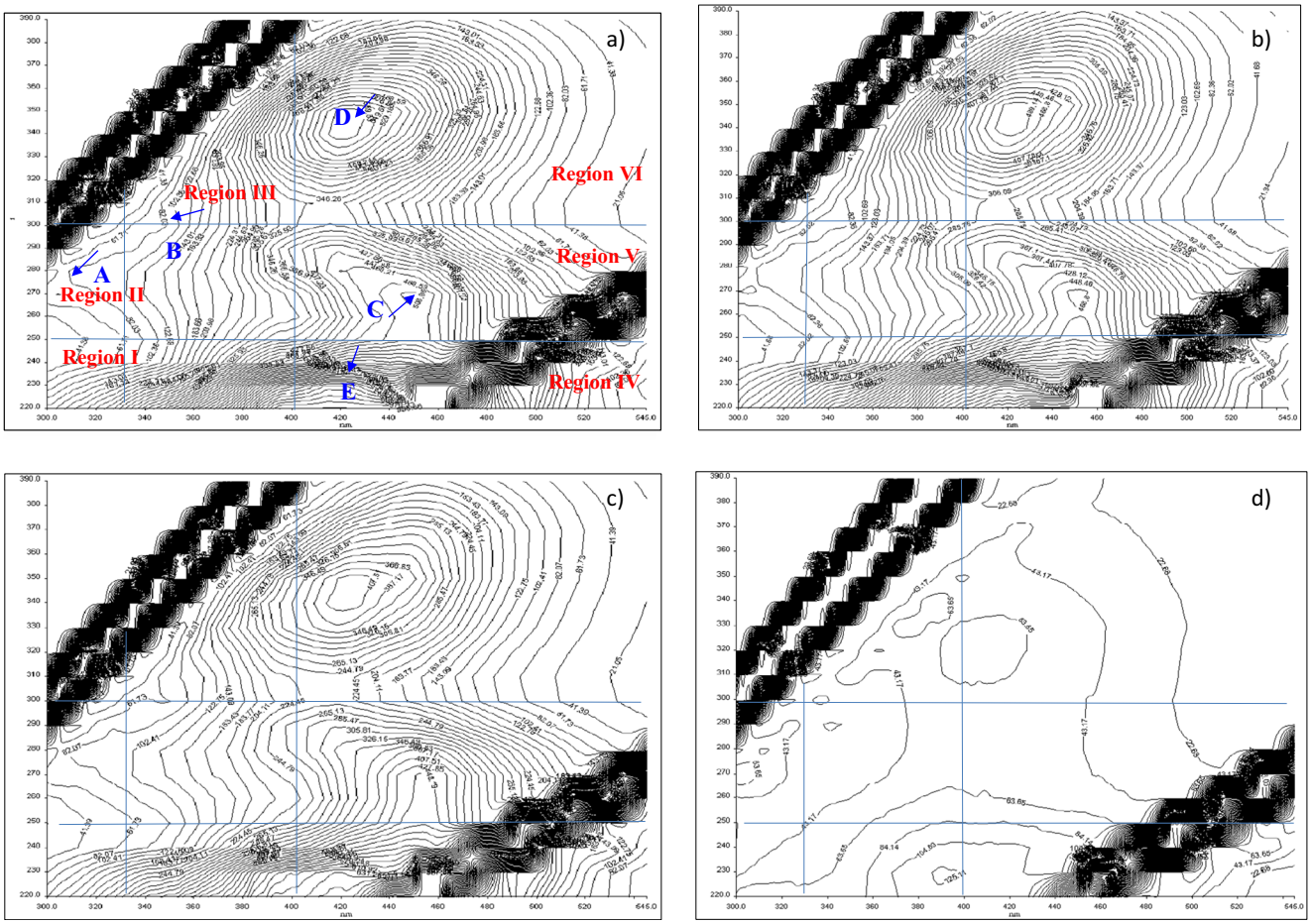

Fig. 3 FEEM florescence contours in **a** influent, **b** anoxic stage, **c** aerobic stage, and **d** MBR effluent. Abscissa represents "emission (nm)"; ordinate represents "excitation (nm)"

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.

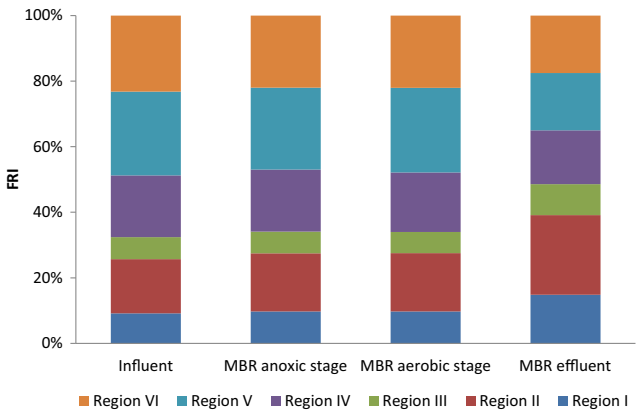

Fig. 4 Fluorescence regional integrating (FRI) distribution in the influent, MBR anoxic stage, MBR aerobic stage, and MBR effluent

Characterization of low-MW DOMs using GC-MS

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 can be seen in Fig. 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 was an obvious decrease in the response abundance between the raw influent and MBR effluent, with the removal of DOMs. The identification of DOM fractions in the raw

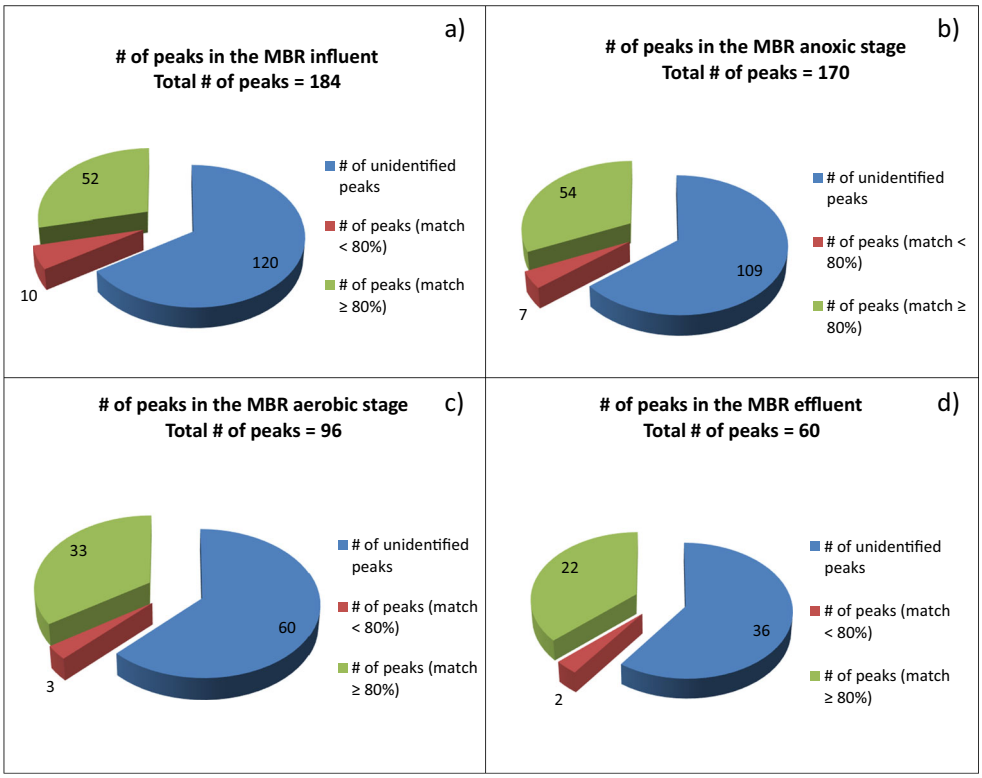

Fig. 5 GC-MS analysis for the DOM peak numbers in **a** influent, **b** MBR anoxic stage, **c** MBR aerobic stage, and **d** MBR effluent

influent is shown in Table S2. The total peaks detected were 184, but only 52 (29%) compounds were identified with a similarity greater than 80% (Fig. 5 a). Among the compounds identified, alkanes accounted for 37% of the total DOM, followed by alcohols (19%), aromatics (19%), and esters (19%), respectively (Fig. 6a).

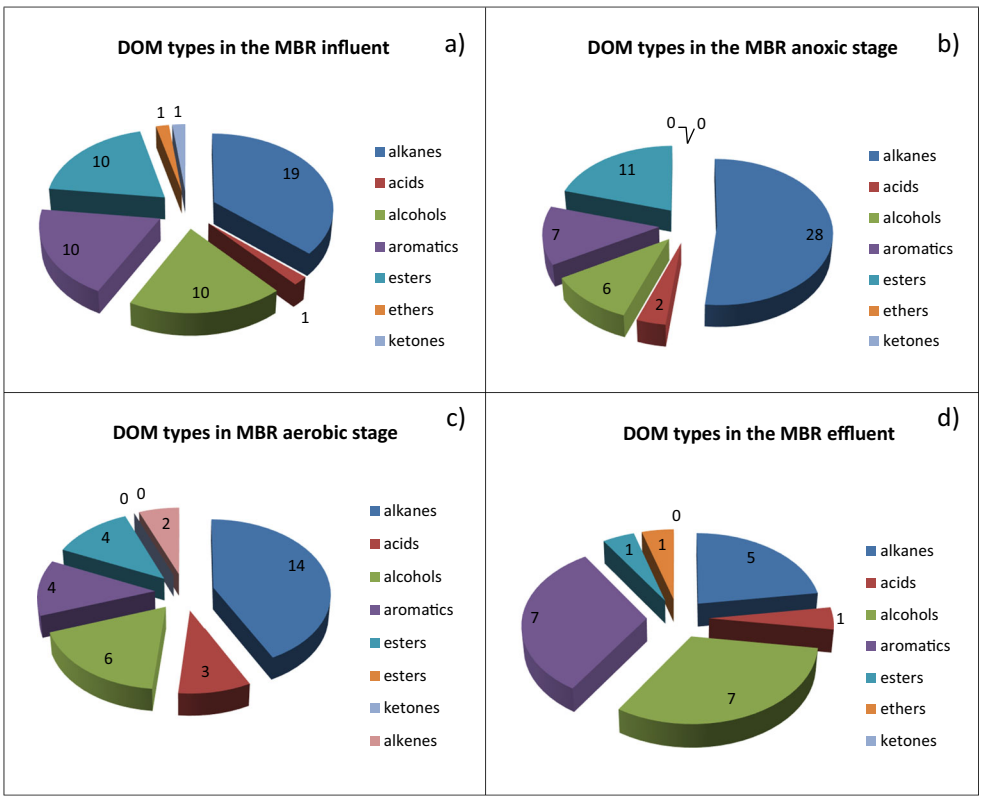

Fig. 6 GC-MS analysis for the typical DOM types in **a** influent, **b** MBR anoxic stage, **c** MBR aerobic stage, and **d** MBR effluent

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% (Fig. 5b). Among the compounds identified, alkanes accounted for 52% of the total DOMs, followed by esters (20%) and aromatics (13%) (Fig. 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, 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 finding indicated that slow biodegradable matter and inert matter in the feed could result in the generation and accumulation of DOM in the subsequent biological processes.

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 (Fig. 5c). Thirtt-three 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%) (Fig. 6c). In comparison, Zhang et al. (2016) investigated the behavior and characteristics of SMPs 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 aerobic MBR stage. This finding indicated that these simple compounds might have been completely biodegraded, especially in the aerobic stage, where biodegradability is enhanced by increased bacterial activity, and the aerobic degradation of organics might be more complete.

DOMs in the 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 units (chlorination, ozonation, 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, and 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 units and fine-tune them. From a scientific point of view, it is important to understand which type of compounds is present in 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% (Fig. 6d). The main compounds detected were aromatics (32%) and alcohols (32%), followed by alkanes (23%) (Fig. 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

Table 2 Compounds detected by GC-MS in the MBR anoxic stage (% match > 80%)

Similarity (> 80%)	Retention index (RI)	Compound name	Molecular weight (Da)	Formula	Concentration (μg/L)	Percentage (%)
91	1022	Octane, 3-ethyl-2,7-dimethyl-	170	$C_{12}H_{26}$	0.84	1.72
90	1131	Ethanol, 1-(2-butoxyethoxy)-	162	$C_8H_{18}O_3$	0.52	1.07
86	1304	1-tridecene	182	$C_{13}H_{26}$	0.46	0.94
83	1402	3,7-dimethyl-6-nonen-1-ol acetate	212	$C_{13}H_{24}O_2$	0.37	0.76
92	1331	Propanoic acid, 2-methyl-, 3-hydroxy-2,4,4-trimethylpentyl ester	216	$C_{12}H_{24}O_3$	0.94	1.93
95	1555	Phenol, 2,4-bis(1,1-dimethylethyl)-[a]	206	$C_{14}H_{22}O$	8.52	17.47
95	1514	Trifluoroacetic acid, n-tridecyl ester	296	$C_{15}H_{27}F_3O_2$	2.29	4.69
85	1701	1-heptadecene	238	$C_{17}H_{34}$	0.57	1.17
88	1753	Hexadecane, 2,6,10,14-tetramethyl-	282	$C_{20}H_{42}$	0.46	0.94
81	1627	5,5-diethyltridecane	240	$C_{17}H_{36}$	0.20	0.41
90	1746	Heptadecane, 3-methyl-	254	$C_{18}H_{38}$	0.28	0.57
94	1755	n-Pentadecanol	228	$C_{15}H_{32}O$	0.45	0.92
95	1711	Heptadecane[a]	240	$C_{17}H_{36}$	3.71	7.61
84	1852	Heptadecane, 2,6,10,15-tetramethyl-[a]	296	$C_{21}H_{44}$	0.32	0.66
90	1954	n-Heptadecanol-1	256	$C_{17}H_{36}O$	0.43	0.88
81	1814	Isopropyl myristate[a]	270	$C_{17}H_{34}O_2$	0.27	0.55
86	1805	m-Toluic acid, 2-ethylhexyl ester[a]	248	$C_{16}H_{24}O_2$	0.20	0.41
91	1971	Pentafluoropropionic acid, octadecyl ester	416	$C_{21}H_{37}F_5O_2$	0.23	0.47
96	1908	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester[a]	278	$C_{16}H_{22}O_4$	0.67	1.37
90	1910	Nonadecane	268	$C_{19}H_{40}$	0.72	1.48
93	1878	Hexadecanoic acid, methyl ester[a]	270	$C_{17}H_{34}O_2$	0.30	0.62
87	1945	10-methylnonadecane[a]	282	$C_{20}H_{42}$	0.34	0.70
91	1968	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	0.20	0.41
93	1973	1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester[a]	278	$C_{16}H_{22}O_4$	0.50	1.03
94	1900	1-nonadecene[a]	266	$C_{19}H_{38}$	0.41	0.84
93	1810	Octadecane	254	$C_{18}H_{38}$	2.98	6.11
90	2024	5,5-diethylheptadecane[a]	296	$C_{21}H_{44}$	0.55	1.13
89	2013	Isopropyl palmitate[a]	298	$C_{19}H_{38}O_2$	1.43	2.93
91	2024	3,3-diethylheptadecane[a]	296	$C_{21}H_{44}$	0.38	0.78
91	2071	Nonadecyl pentafluoropropionate[a]	430	$C_{22}H_{39}F_5O_2$	0.63	1.29
82	2073	Cyclohexane, tetradecyl-	280	$C_{20}H_{40}$	2.12	4.35
81	2045	Phytol	296	$C_{20}H_{40}O$	0.58	1.19
80	2085	9-octadecenoic acid (Z)-, methyl ester	296	$C_{19}H_{36}O_2$	0.29	0.59
83	2167	Octadecanoic acid[a]	284	$C_{18}H_{36}O_2$	0.68	1.39
81	2017	9-eicosene, (E)-	280	$C_{20}H_{40}$	2.13	4.37
85	2109	Heneicosane	296	$C_{21}H_{44}$	0.91	1.87
88	2124	5-ethyl-5-methylnonadecane[a]	310	$C_{22}H_{46}$	0.45	0.92
93	2009	Eicosane[a]	282	$C_{20}H_{42}$	2.07	4.24
86	2223	5-butyl-5-ethylheptadecane[a]	324	$C_{23}H_{48}$	0.26	0.53
90	2351	1-heneicosanol[a]	312	$C_{21}H_{44}O$	1.20	2.46
87	2370	Sulfurous acid, pentadecyl 2-propyl ester	334	$C_{18}H_{38}O_3S$	0.67	1.37
87	2506	10-cyclohexylnonadecane	350	$C_{25}H_{50}$	2.16	4.43
85	2407	Tetracosane[a]	338	$C_{24}H_{50}$	0.37	0.76
89	2210	Eicosyl trifluoroacetate[a]	394	$C_{22}H_{41}F_3O_2$	0.85	1.74
83	2477	Heneicosane, 11-(1-ethylpropyl)-[a]	366	$C_{26}H_{54}$	0.16	0.33
86	2442	2-methyltetracosane[a]	352	$C_{25}H_{52}$	0.71	1.46
93	2575	Phthalic acid, di(6-methylhept-2-yl) ester[a]	390	$C_{24}H_{38}O_4$	0.45	0.92
86	2619	Squalane[a]	422	$C_{30}H_{62}$	0.85	1.74
83	2740	Docosane, 7-hexyl-	394	$C_{28}H_{58}$	0.33	0.68
88	2641	2-methylhexacosane	380	$C_{27}H_{56}$	0.31	0.64
81	2632	Sulfurous acid, hexyl tetradecyl ester[a]	362	$C_{20}H_{42}O_3S$	0.11	0.23
83	2840	2-methyloctacosane[a]	408	$C_{29}H_{60}$	0.17	0.35
86	2804	Octacosane	394	$C_{28}H_{58}$	0.53	1.09
83	3401	Tetratriacontane[a]	478	$C_{34}H_{70}$	0.25	0.51

[a] Compounds that were also detected in the influent

Table 3 Compounds detected by GC-MS in the MBR aerobic stage (% match > 80%)

Similarity (> 80%)	Retention index (RI)	Compound name	Molecular weight (Da)	Formula	Concentration (μg/L)	Percentage (%)
94	1555	Phenol, 2,4-bis(1,1-dimethylethyl)-[b]	206	$C_{14}H_{22}O$	4.12	22.49
94	1502	1-pentadecene	210	$C_{15}H_{30}$	0.28	1.53
91	1512	Pentadecane	212	$C_{15}H_{32}$	0.30	1.64
91	1731	Ethanol, 2-(dodecyloxy)-	230	$C_{14}H_{30}O_2$	1.13	6.17
95	1755	n-Pentadecanol[a]	228	$C_{15}H_{32}O$	0.23	1.26
95	1711	Heptadecane[a]	240	$C_{17}H_{36}$	0.98	5.35
87	1945	10-methylnonadecane[a]	282	$C_{20}H_{42}$	0.38	2.07
83	1999	Octadecanal	268	$C_{18}H_{36}O$	0.29	1.58
95	1908	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester[b]	278	$C_{16}H_{22}O_4$	0.68	3.71
88	1910	Nonadecane[a]	268	$C_{19}H_{40}$	1.06	5.79
94	1878	Hexadecanoic acid, methyl ester[b]	270	$C_{17}H_{34}O_2$	0.45	2.46
93	1968	n-Hexadecanoic acid[a]	256	$C_{16}H_{32}O_2$	0.66	3.60
93	1973	1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester[b]	278	$C_{16}H_{22}O_4$	0.20	1.09
95	1900	1-Nonadecene[b]	266	$C_{19}H_{38}$	0.58	3.17
80	1855	Methoxyacetic acid, tridecyl ester	272	$C_{16}H_{32}O_3$	1.07	5.84
85	2089	Methoxyacetic acid, 4-hexadecyl ester	314	$C_{19}H_{38}O_3$	0.64	3.49
91	2024	3,3-diethylheptadecane	296	$C_{21}H_{44}$	1.29	7.04
92	2153	n-Nonadecanol-1	284	$C_{19}H_{40}O$	0.15	0.82
86	2167	Octadecanoic acid[b]	284	$C_{18}H_{36}O_2$	0.11	0.60
85	2017	5-eicosene, (E)-[a]	280	$C_{20}H_{40}$	0.27	1.47
88	2009	Eicosane[b]	282	$C_{20}H_{42}$	0.12	0.66
91	2071	Nonadecyl pentafluoropropionate[b]	430	$C_{22}H_{39}F_5O_2$	0.21	1.15
89	2109	Heneicosane	296	$C_{21}H_{44}$	0.34	1.86
86	2223	5-butyl-5-ethylheptadecane[b]	324	$C_{23}H_{48}$	0.42	2.29
91	2351	1-heneicosanol[b]	312	$C_{21}H_{44}O$	0.23	1.26
85	2407	Tetracosane[b]	338	$C_{24}H_{50}$	0.48	2.62
87	2472	1-heneicosyl formate	340	$C_{22}H_{44}O_2$	0.11	0.60
87	2442	2-methyltetracosane[b]	352	$C_{25}H_{52}$	0.18	0.98
93	2575	Phthalic acid, di(6-methylhept-2-yl) ester[b]	390	$C_{24}H_{38}O_4$	0.17	0.93
87	2641	2-methylhexacosane[a]	380	$C_{27}H_{56}$	0.21	1.15
85	2840	2-methyloctacosane[b]	408	$C_{29}H_{60}$	0.19	1.04
85	2948	1-heptacosanol	396	$C_{27}H_{56}O$	0.35	1.91
80	3138	Docosane, 11-decyl-	450	$C_{32}H_{66}$	0.44	2.40

[a] Compounds that were also detected in the MBR anoxic stage

[b] Compounds that were also detected in the influent and MBR anoxic stage

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-benzenedicarboxylic acid, butyl 2-methylpropyl ester), which were detected in the influent, remained in the MBR effluent; these results indicate 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 to 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, mildly volatile, and thermos-stable DOMs (< 580 Da) which limits the interpretation of results to a very

Table 4 Compounds detected by GC-MS in the MBR effluent (% match > 80%)

Similarity (> 80%)	Retention index (RI)	Compound name	Molecular weight (Da)	Formula	Concentration (μg/L)	Percentage (%)
88	1228	Phenol, 2-(1,1-dimethylethyl)-	150	$C_{10}H_{14}O$	0.30	0.82
92	1457	1-dodecanol	186	$C_{12}H_{26}O$	0.58	1.58
94	1555	Phenol, 2,4-bis(1,1-dimethylethyl)-[c]	206	$C_{14}H_{22}O$	6.37	17.32
93	1656	1-tetradecanol	214	$C_{14}H_{30}O$	0.27	0.73
93	1731	Ethanol, 2-(dodecyloxy)- [a]	230	$C_{14}H_{30}O_2$	2.26	6.15
95	1755	n-Pentadecanol[b]	228	$C_{15}H_{32}O$	1.41	3.83
92	1908	1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester[c]	278	$C_{16}H_{22}O_4$	0.38	1.03
82	1936	1,4-diphenyl-1-pentanone	238	$C_{17}H_{18}O$	0.28	0.76
91	1968	n-Hexadecanoic[b]	256	$C_{16}H_{32}O_2$	2.32	6.31
83	1877	2-methyl-1-octadecene[a]	266	$C_{19}H_{38}$	0.53	1.44
95	1973	1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester[c]	278	$C_{16}H_{22}O_4$	2.46	6.69
96	1900	1-nonadecene[c]	266	$C_{19}H_{38}$	1.38	3.75
81	1890	Methoxyacetic acid, 2-tetradecyl ester	286	$C_{17}H_{34}O_3$	1.69	4.60
84	2167	Octadecanoic acid[c]	284	$C_{18}H_{36}O_2$	0.18	0.49
94	2153	n-Nonadecanol-1[a]	284	$C_{19}H_{40}O$	0.52	1.41
82	2328	Ethanol, 2-(octadecyloxy)-	314	$C_{20}H_{42}O_2$	0.79	2.15
90	2281	Triethylene glycol monododecyl ether	318	$C_{18}H_{38}O_4$	0.36	0.98
85	2351	1-heneicosanol[c]	312	$C_{21}H_{44}O$	0.25	0.68
90	2407	Tetracosane[c]	338	$C_{24}H_{50}$	0.15	0.41
87	2584	Phthalic acid, 4,4-dimethylpent-2-yl octyl ester	376	$C_{23}H_{36}O_4$	13.75	37.39
92	2641	2-methylhexacosane[b]	380	$C_{27}H_{56}$	0.41	1.12
87	2840	2-methyloctacosane[c]	408	$C_{29}H_{60}$	0.13	0.35

[a] Compounds that were also detected in the MBR aerobic stage

[b] Compounds that were also detected in the MBR anoxic and aerobic stage

[c] Compounds that were also detected in the influent and MBR anoxic and aerobic stage

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), in contrast to the COD concentration of 15.4 mg/L (Table 1), clearly indicating that high MW compounds make up the bulk of the 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 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, although it represents a substantial improvement on past methods. 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 non-polar (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 stages (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 consistent recalcitrance through biological wastewater treatment process, while phthalic acid, 4,4-dimethylpent-2-yl octyl ester (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 DOM rejection by membrane filtration (37.5%) could be due to the cake gel layer formed by the physical deposition of large DOM aggregates, which were mainly composed of soluble and colloidal materials (e.g., proteins, 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-min filtration followed by 1-min relaxation. The TMP gradually increased over time but remained lower than 100 mbar throughout the study, indicating the presence of a biofilm/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 clearly explain the fate of DOM in the MBR, since DOM production and accumulation (e.g., non-biodegradable fractions in the influent, cell lysis, and bound EPS released during the biological processes), and DOM elimination (e.g., biodegradation, adsorption, membrane filtration etc.), always occur simultaneously.

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 the anoxic and aerobic MBR stages 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 dominant fractions of DOMs. That is, fulvic and humic acid-like substances and the changes in their location and peak intensities indicated the alteration in these DOM properties.
3. The GC-MS analysis indicated that the highest peak numbers (170) were found in the anoxic stage, and 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, resulting in a 37.5% rejection of the DOM. 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.

References

APHA (2012). APHA, AWWA, WEF 2012. Standard Methods for the Examination of Water and Wastewater 22nd edition, American Public Health Association, Washington D.C

Aquino SF, Hu AY, Akram A, Stuckey DC (2006) Characterization of dissolved compounds in submerged anaerobic membrane bioreactors (SAMBRs). J Chem Technol Biotechnol 81(12):1894–1904

Aquino SF, Stuckey DC (2004) Soluble microbial products formation in anaerobic chemostats in the presence of toxic compounds. Water Res 38(2):255–266

Barker DJ, Stuckey DC (1999) A review of soluble microbial products (SMP) in wastewater treatment systems. Water Res 33(14):3063–3082

Chen L, Gu Y, Cao C, Zhang J, Ng JW, Tang C (2014) Performance of a submerged anaerobic membrane bioreactor with forward osmosis membrane for low-strength wastewater treatment. Water Res 50: 114–123

Chen W, Westerhoff P, Leenheer JA, Booksh K (2003) Fluorescence excitation-emission matrix regional integration to quantify spectra for dissolved organic matter. Environ Sci Technol 37(24):5701–5710

Drews A, Mante J, Iversen V, Vocks M, Lesjean B, Kraume M (2007) Impact of ambient conditions on SMP elimination and rejection in MBRs. Water Res 41(17):3850–3858

Horng RY, Huang C, Chang MC, Shao H, Shiau BL, Hu YJ (2009) Application of TiO2 photocatalytic oxidation and non-woven membrane filtration hybrid system for degradation of 4-chlorophenol. Desalination 245(1–3):169–182

Huang X, Liu R, Qian Y (2000) Behaviour of soluble microbial products in a membrane bioreactor. Process Biochem 36(5):401–406

Imai A, Matsushige K, Nagai T (2003) Trihalomethane formation potential of dissolved organic matter in a shallow eutrophic lake. Water Res 37(17):4284–4294

Ishii SKL, Boyer TH (2012) Behavior of reoccurring parafac components in fluorescent dissolved organic matter in natural and engineered systems: a critical review. Environ Sci Technol 46(4):2006–2017

Janga N, Ren X, Kim G, Ahn C, Cho J, Kim IS (2007) Characteristics of soluble microbial products and extracellular polymeric substances in the membrane bioreactor for water reuse. Desalination 202(1–3): 90–98

Jarusutthirak C, Amy G (2006) Role of soluble microbial products (SMP) in membrane fouling and flux decline. Environ Sci Technol 40(3): 969–974

Kunacheva C, Soh YNA, Trzcinski AP, Stuckey DC (2017a) Soluble microbial products (SMPs) in the effluent from a submerged anaerobic membrane bioreactor (SAMBR) under different HRTs and transient loading conditions. Chem Eng J 311:72–81

Kunacheva C, Le C, Soh YNA, Stuckey DC (2017b) Chemical characterization of low molecular weight soluble microbial products in an anaerobic membrane bioreactor. Environ Sci Technol 51(4):2254–2261

Kunacheva C, Stuckey DC (2014) Analytical methods for soluble microbial products (SMP) and extracellular polymers (ECP) in wastewater treatment systems: a review. Water Res 61:1–18

Liang S, Liu C, Song L (2007) Soluble microbial products in membrane bioreactor operation: behaviors, characteristics, and fouling potential. Water Res 41(1):95–101

Ma H, Allen HE, Yin Y (2001) Characterization of isolated fractions of dissolved organic matter from natural waters and a wastewater effluent. Water Res 35(4):985–996

Meng F, Chae SR, Drews A, Kraume M, Shin HS, Yang F (2009a) Recent advances in membrane bioreactors (MBRs): membrane fouling and membrane material. Water Res 43(6):1489–1512

Meng F, Drews A, Mehrez R, Iversen V, Ernst M, Yang F, Jekel M, Kraume M (2009b) Occurrence, source, and fate of dissolved organic matter (DOM) in a pilot-scale membrane bioreactor. Environ Sci Technol 43(23):8821–8826

Namour P, Müller MC (1998) Fractionation of organic matter from wastewater treatment plants before and after a 21-day biodegradability test: a physical-chemical method for measurement of the refractory part of effluents. Water Res 32(7):2224–2231

Ren X, Shon HK, Jang N, Lee YG, Bae M, Lee J, Cho K, Kim IS (2010) Novel membrane bioreactor (MBR) coupled with a nonwoven fabric filter for household wastewater treatment. Water Res 44(3): 751–760

Rojo F (2009) Degradation of alkanes by bacteria: minireview. Environ Microbiol 11(10):2477–2490

Rosenberger S, Kraume M (2002) Filterability of activated sludge in membrane bioreactors. Desalination 146(1–3):373–379

Seo DJ, Kim YJ, Ham SY, Lee DH (2007) Characterization of dissolved organic matter in leachate discharged from final disposal sites which contained municipal solid waste incineration residues. J Hazard Mater 148(3):679–692

Sheng GP, Yu HQ, Li XY (2010) Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review. Biotechnol Adv 28(6):882–894

Shin HS, Kang ST (2003) Characteristics and fates of soluble microbial products in ceramic membrane bioreactor at various sludge retention times. Water Res 37(1):121–127

Stuckey DC (2012) Recent developments in anaerobic membrane reactors. Bioresour Technol 122:137–148

Tang S, Wang Z, Wu Z, Zhou Q (2010) Role of dissolved organic matters (DOM) in membrane fouling of membrane bioreactors for municipal wastewater treatment. J Hazard Mater 178(1–3):377–384

Trzcinski AP, Ganda L, Yan Ni AS, Kunacheva C, Qing ZD, Lin LL, Tao G, Lee Y, Ng WJ (2016) Identification of recalcitrant compounds in a pilot-scale AB system: an adsorption (a) stage followed by a biological (b) stage to treat municipal wastewater. Bioresour Technol 206:121–127

Wang Z, Wu Z, Tang S (2009) Characterization of dissolved organic matter in a submerged membrane bioreactor by using three-dimensional excitation and emission matrix fluorescence spectroscopy. Water Res 43(6):1533–1540

Wang ZP, Zhang T (2010) Characterization of soluble microbial products (SMP) under stressful conditions. Water Res 44(18):5499–5509

Wu B, Zhou W (2010) Investigation of soluble microbial products in anaerobic wastewater treatment effluents. J Chem Technol Biotechnol 85(12):1597–1603

Zhang D, Trzcinski AP, Kunacheva C, Stuckey DC, Liu Y, Tan SK, Ng WJ (2016) Characterization of soluble microbial products (SMPs) in a membrane bioreactor (MBR) treating synthetic wastewater containing pharmaceutical compounds. Water Res 102:594–606

Zhang DQ, Eng CY, Stuckey DC, Zhou Y (2017) Effects of ZnO nanoparticle exposure on wastewater treatment and soluble microbial products (SMPs) in an anoxic-aerobic membrane bioreactor. Chemosphere 171:446–459