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Potential of fluorescence excitation emission matrix (FEEM) analysis for foulant characterisation in membrane bioreactors (MBRs)

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ABSTRACT

The relative impact of soluble microbial products (SMP) on membrane bioreactor (MBR) fouling is widely recognized. However, no direct relationship between fouling propensity and the SMP concentration in the bioreactor has been clearly established yet. This is due to (1) the lack of standard methods for SMP characterisation, (2) the limitations of the methods used so far, and (3) the characterisation of the SMP in supernatant rather than those deposited on the membrane surface. The aim of this paper is therefore to identify the feasibility of a novel technology, the fluorescence excitation-emission matrix (FEEM) spectrophotometry to characterise the supernatants and the foulant fractions. Foulant fractions are obtained by rinsing, backwashing (BW) and chemical cleaning (CC) the membrane modules, from two MBRs operated under different solid retention times (SRTs). FEEM was able to provide qualitative and quantitative information about the compounds present in MBR. In this study, FEEM results demonstrated that tryptophan-like proteins dominated in most samples rather than humic/fulvic-like substances. As expected, FEEM of the permeate collected from lab-scale ultrafiltration (UF) demonstrated that, these larger molecular weight proteins rather than humics were retained by membranes. These proteins were also in higher ratio in the chemical cleaning solutions of both MBRs, revealing preferential attachment to the membrane surface. Thus, proteins and humic compounds present in supernatants and their preferential deposition on or into membrane surface could be efficiently characterised in a simple, robust, non-destructive method like the FEEM analysis.

Keywords: Membrane bioreactor (MBR); Foulants; Cleaning; FEEM; Fouling layers; LC-OCD

1. Introduction

Soluble microbial products (SMP) have been extensively studied for their impact to fouling in membrane bioreactors (MBRs) [1]. SMP adsorb on the membrane surface, block pores and/or form gel layer on the membrane surface, and thus provide hydraulic resistance to permeate flow [2]. SMP consist of soluble and colloidal biopolymers, mostly polysaccharides and proteins. Studies regarding fouling of SMP usually report proteins and carbohydrate concentrations by conventional methods and the findings on their exact impact on MBR fouling are often contradictory [3–6]. Three dimensional fluorescence excitation-emission matrix (FEEM) spectroscopy is a rapid, selective and sensitive technique [7]. To date, few studies have applied FEEM to characterise SMP in MBR supernatants and foulant fractions [8,9]. Solid retention time (SRT) has been identified as the main parameter influencing SMP concentration [10]. Similarities between SMP and the extracted

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foulants from MBRs operating at different SRTs were observed by FEEM for synthetic wastewater [8]. The effect of SRT on the biodegradability of fluorescent substances in MBR was also estimated by using FEEM [9]. However sample preparation, foulant extraction, nature of wastewaters, operating conditions were different in all studies [8,9,11], making comparison difficult. In two different studies, FEEM of foulants extracted by chemical [8] and physical cleaning [11] were obtained. Therefore, the preferential deposition of foulants on the membrane surface could not be assessed properly from these previous studies. Some aspects to consider in the application of FEEM include: inner filtering effect (IFE), fluorescence quenching, temperature variations, pH change, presence of metal ions and oxidation process [12]. Furthermore polysaccharides, which are considered as a major foulant in MBRs [13], cannot be detected by FEEM.

The objective of this study is to characterise the SMP fluorescence nature for MBR supernatants and foulant fractions obtained by a three step cleaning protocol. The SMP samples and membrane foulants collected from pilot-scale MBRs operating under different SRTs conditions using real wastewaters were analysed by FEEM and liquid chromatography with organic carbon detector (LC-OCD).

2. Materials and methods

2.1. Origin and handling of samples

The pilot scale MBRs comprised two cylindrical stainless steel tanks (capacity of about 250 l) with total membrane surface area of 2 m^2 submerged hollow fibre module (Siemens, Memcor Australia). The hollow fibre modules were constructed from polyvinylidene fluoride (PVDF), with a pore size of 0.04 μm. Primary effluent from the Malabar Sewage Treatment Plant (STP) was screened through fine mesh (0.75 mm) and fed to the MBR rigs.

Imposed flux of 12 l/m^2h resulting in hydraulic retention time (HRT) of 10 h was applied to both reactors. MBR1 was operated under no sludge wastage condition. A flow rate of 12.6 l/d sludge was wasted for MBR2 to operate the system under 20 d SRT condition. Samples were collected from the two MBRs on the same day and transported to the lab for preparation and analysis. Biomass were centrifuged at 3000 rpm (approximately 2000 g) for 8 min and the supernatants were filtered through 0.45 μm filter papers for further analysis. After one and half year of continuous operation, the membrane modules were taken out from the MBRs and were cleaned by following a three-step-cleaning protocol, namely: rinsing and backwashing with MilliQ water (18.2 MΩ.cm) and chemical cleaning with NaOH (pH 11). Three foulant fractions were obtained namely: the upper (rinsed), intermediate (backwashed) and lower (chemical cleaned) layers. The cleaning solutions were filtered through 0.45 μm filter papers for analysis.

2.2. Experimental setup

In order to assess their fouling potential, supernatant and cleaning solutions were also filtered in a dead-end filtration cell (17.35 cm^2 surface area, 100 kDa polyethersulfone ultrafiltration (UF) membrane, 1 bar transmembrane pressure). The solution, filled into the feed reservoir (2 l), was pressurized into the filtration cell using nitrogen gas. The permeates from the filtration tests were collected and analysed to identify foulants.

2.3. FEEM and LC-OCD analysis

FEEM were obtained using Cary Eclipse fluorescence spectrophotometer (Varian, Australia) with a 4 ml, 10 mm path length quartz cuvette. Emission (Em) spectra were scanned from 280 to 500 nm at 2 nm increments and excitation (Ex) spectra were scanned from 200 to 400 nm at 5 nm increments. The slit widths for excitation and emission were 5 nm. The photomultiplier tube voltage was set to 800 V, providing Raman intensity at 348 nm of 20±2 arbitrary units (a.u.). MilliQ water was run as blank to monitor the stability of the instrument. According to literatures [7,11,14], fluorophores in FEEM can be divided into five regions (Table 1). In addition to FEEM, LC-OCD (DOC-Labor, Germany) was performed with a TSK 50S column, according to method described by Huber [15].

3. Results and discussion

3.1. FEEM analysis of MBR supernatants

FEEM spectra (Fig. 1) illustrate spectral information about the chemical compositions of SMP present in MBR supernatant. Tryptophan-like and humic-like

Table 1
Major fluorescent components identification, peak locations (Excitation; Ex, Emission; Em) observed in different studies [7,11,14]

Identification	Ex (nm)	Em (nm)	Compounds
B	225–237 275	309–321 310	Tyrosine-like, protein-like
T1	275–285	320–350	Tryptophan-like
T2	225–240	340–381	
A	237–260	400–500	Humic acid-like
C	300–340	405–430	
D	220–240	410–450	Fulvic acid-like

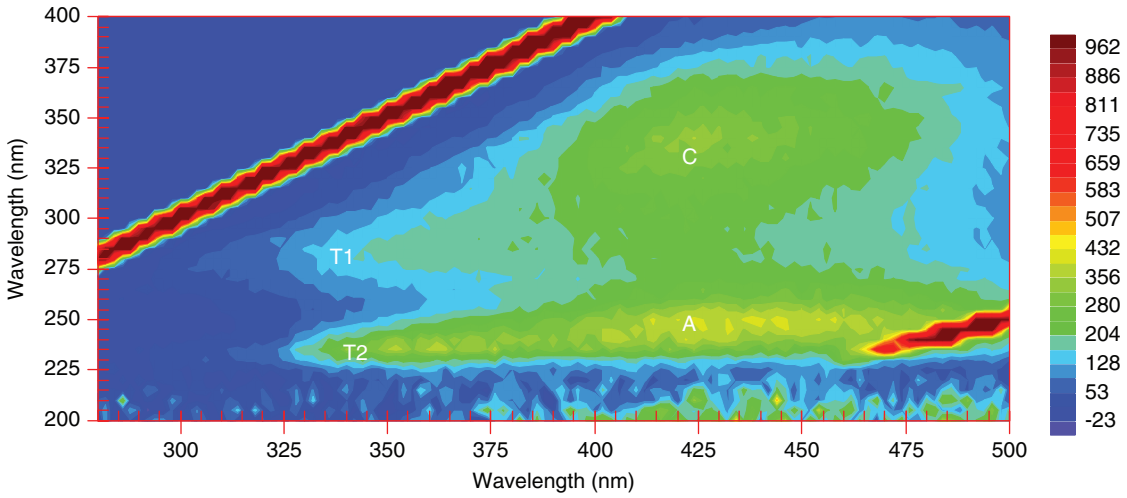

Fig. 1. FEEM spectra of MBR1 supernatant.

fluorescence dominated in all FEEMs for MBR1 (Fig. 1), which was also observed in a previous study [16]. The main peaks were located at the excitation wavelengths of 235–240 nm (peak T2) and 285–290 nm (peak T1) for emission wavelength of 350 nm, for MBR1 supernatant. Compared to the fluorescence peak location of proteins observed for some sewage-impacted rivers (Ex/Em of 276–281/340–370 nm) described by Baker [17], the location of peak T1 of MBR1 supernatants and permeates showed a red shift to longer wavelengths in term of excitation wavelengths. Humic/fulvic acid-like fluorescence was also detected at excitation wavelengths of 250 nm (peak A) and 335 nm (peak C) for emission wavelength of 420 nm. Compared to the fluorescence peak locations for peaks A and C observed for algogenic organic matter [18], samples in this study showed a red shift. The changes in peak shifts among different studies illustrate the structural variability of fluorescent dissolved organics. This is anticipated that these structural differences could play a role in the biodegradability of the compounds present in MBR systems [9]. The effect of peak shifts on biodegradability has not been assessed yet [7,9] and this prediction still requires further assessment. Nevertheless, the peak intensity reported in this study are the maximum values recorded for each peak. Since, peak intensity depends on the relative concentrations of the different compounds [7], the comparison of peak intensities and the intensity ratios between the supernatant and the UF filtrate allows the assessment of the level of removal of the compounds by UF filtration (Fig. 2). The peak intensities for T1 and T2 reduced from 181 to 77, and from 352 to 84 a.u. respectively. There was no significant removal of humic/fulvic-like compounds for MBR1 supernatants, as observed by peak intensities for peaks A and C.

As supernatant was filtered through 0.45 µm before FEEM and LC-OCD analysis, the further characterisation of the 100 kDa UF filtrate also allowed the assessment of the nature of the compounds based on their size. As a 100 kDa membrane is generally recognised to feature pore size of around 0.01 µm, the characterisation of the 0.01–0.45 µm compounds is possible. The significant decreased intensity of fluorescence associated with T1 and T2 indicates that supernatant contains a substantial amount of tryptophan-like fluorescence materials in this size range. LC-OCD also illustrated significant removal of biopolymers eluting between 25–35 min. However humics, building block and low molecular weight organics eluting after 40 min were not significantly retained by the 100 kDa UF membrane.

Different values of peak intensity ratios were observed for MBR1 supernatants and permeate. The relative ratio between humic-like (C+A) and protein-like (T1+T2) substances increased significantly for MBR1 permeate, this ratio was 1.43 for supernatant and 4.33 for permeate. This increased ratio indicated that compared to humic-like substances, tryptophan-like proteins were more retained by UF membranes. For surface water samples, the molecular weight of biopolymers

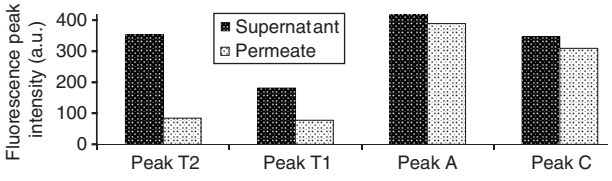

Fig. 2. Fluorescence peak intensity of four different peaks for MBR1 supernatant and permeate from dead-end UF (100 kDa).

ranged from 50–2000 kDa and for humics 0.1–10 kDa as measured by liquid chromatography [19]. Therefore, larger molecular weight tryptophan-like proteinaceous materials were retained by the 100 kDa membrane.

3.2. FEEM of SMP at different SRTs

The peak locations and corresponding peak intensities were obtained for maximum peak intensity values for MBR1 (infinite SRT) and MBR2 (20 d SRT) supernatants. The FEEM analysis illustrated that, all four peak locations were similar to those previously reported (Fig. 3), indicating that the fluorescent organics in the two MBRs had similar characteristics [14]. However, the peak intensities for T1 and T2 were different for the two supernatants. The T1/T2 ratio was 0.5 in MBR1 and 0.9 in MBR2. Humic-like fluorescence denoted by peaks A and C were also higher in MBR2 supernatants. Higher humic concentration at low SRT was also observed in another study, based on colorimetric assessment of SMP [20]. The effect of SRT on biodegradable fluorophore was assessed by the peak intensity ratio of humic-like (C+A) to protein-like (T1+T2) compounds. Humic to protein-like peak intensity ratio is considered as a reliable factor determining biodegradability of dissolved organics [21]. For MBR1, the ratio was 1.43, while the ratio of 1.77 was observed for MBR2. LC-OCD analysis of supernatants illustrated a higher amount of biopolymers (elution time 25–35 min) in MBR2 compared to MBR1. Therefore, it appears that long SRT is favourable to produce more biodegradable products, i.e., more biodegradable fluorophores were produced by microorganisms at long SRT. High SRT has been reported to produce sludge with better filterability, and containing a lower amount of floc-bound extracellular polymeric substances (EPS) [20]. In a previous study assessing the biodegradability by FEEM, contradictory results were observed for dissolved organics and SMP at different SRTs. While SRT value increased from 20 to 60 d, the humic to protein-like peak intensity ratio increased from 0.89 to 1.44 (for dissolved organic matter) and 0.22 to 0.25 for SMP indicating non-biodegradable fluorophore were produced in higher amount at long SRT [9]. Such discrepancies could be due to different reasons: in the present study two MBRs were operated in parallel instead of adopting different SRTs, consecutively in the same MBR system, leading to variation in wastewater characteristics during the experiments.

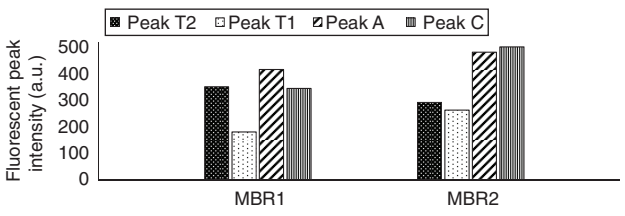

Fig. 3. Fluorescence peaks intensities of four peaks in MBR1 and MBR2 supernatants.

3.3. FEEM of foulant layers

In order to better define the effects of SMP on membrane fouling, the FEEM spectra of membrane foulants were analysed in Table 1. Four peaks could be observed in the FEEM spectra. Comparison of the peak locations of the supernatants (Fig. 3) and foulants (Table 2) illustrated that the fluorescence characteristics of membrane foulants were not the same as those of SMP samples from MBR1 and MBR2. The locations of peak T2 in MBR1 rinsed and desorbed fractions were slightly blue shifted compared to the supernatant. For the backwashed fraction the location of peak T1 was red shifted to longer wavelengths by 5 nm. The location of peak A was blue shifted by 15 and 40 nm for backwashed and desorbed fractions respectively, compared to the supernatant. The peak location of peak C for rinsed fraction was red shifted by 5 nm compared to the supernatant. These variations in peak locations of the foulant layers were also observed for MBR2 (data not presented). These changes in peak locations illustrated that the SMPs in the supernatants and that in the foulant layers were slightly in different compositions. A red shift is related to the presence of carbonyl containing substituents for example, hydroxyl, alkoxyl, amino groups and carboxyl constituents [22,23]. A blue shift is associated with the break-up of a large molecules into smaller fragments [14,24]. The desorbed fraction was obtained by extracting the foulants with NaOH, therefore pH alteration and break-up of some large molecules could be responsible for the changes in fluorescence characteristics between supernatant and desorbed fraction [7]. The blue shift of peak A in backwashed fraction could be due to presence of relatively higher amount of humics in this foulant layer, these humics were responsible for pore blocking.

In MBR systems, the fouling layer is partly formed by the rejected particulate materials of biological origin, which actively excrete slimy, glue-like materials (EPS and SMP). Irreversibly attached biofloc residues and planktonic bacteria act as the seed for biofilm growth. Furthermore, the structure of the fouling layer also differs along the fibre length of hollow fibre [25]. The constituents of membrane foulants are related to the interactions between organic materials and membrane surface, therefore the shifts in peak locations illustrated the structural differences in aromatic proteins, and humics in different foulant layers and supernatants.

Table 2
Excitation (Ex) peak locations and FEEM intensities (Int in a.u.) for MBR1 foulant fractions

MBR1	Peak T2		Peak T1		Peak A		Peak C	
	Ex	Int	Ex	Int	Ex	Int	Ex	Int
Rinsed	230	528	285	138	250	188	340	167
Backwashed	235	537	285	205	235	440	335	200
Desorbed	220	24920	290	5640	210	17520	325	1640

The structural differences could be due to poor oxygen and substrate transfer in the dense foulant layers as no maintenance cleaning was performed in the pilot plant during the operating period [25].

The peak intensities of protein-like substances were significantly higher compared to humic-like compounds in most of the foulant fractions, except for the rinsed fraction from MBR2 (data not presented). This suggests that the protein-like substances in membrane foulants, indicated by peaks T1 and T2, were dominant compared to the humic-like substances, represented by peaks C and A. In addition, the protein peak intensities in desorbed fractions for both MBRs were significantly higher (24920 and 14480 a.u. in MBR1 and MBR2, respectively) compared to the rinsed and backwashed fractions. Significantly higher amount of biopolymers, compared to humics was also observed by LC-OCD for these fractions. Proteins have been widely reported as one of the major foulants in MBR systems. The retention of proteins could severely affect membrane fouling in MBRs [26]. In two previous studies, it was observed that proteins have higher fouling potential, as these compounds adsorb directly on the membrane surface, which can be partly removed by backwashing and chemical cleaning [27,28].

4. Conclusions

Simultaneous characterisation of SMP present in supernatants and foulant fractions using FEEM technology was discussed in this paper. Based on this study, four fluorescence peaks were identified and it was observed that, protein-like fluorophores dominated in MBR supernatants and foulant layers. Humic to protein peak intensity ratio illustrated that, long SRT is favourable to produce more biodegradable products. It was also found that, the dominant fluorescent compounds in membrane foulants were protein-like substances, which were strongly attached to membrane surface. However, polysaccharides, which are also considered as major foulant in MBRs, cannot be detected by FEEM analysis. In this study, LC-OCD complemented the trends observed by FEEM. LC-OCD illustrated, that, higher amount of biopolymers (protein, carbohydrates and inorganic colloids) were tightly bound to membrane surface and were responsible for irreversible fouling in MBR systems. Limitation of fouling in MBRs still requires the selection/design of more appropriate membrane material featuring chemical stability and unfavourable environment for foulant adhesion.

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