



Characterizing fluorescent dissolved organic matter in a membrane bioreactor via excitation–emission matrix combined with parallel factor analysis



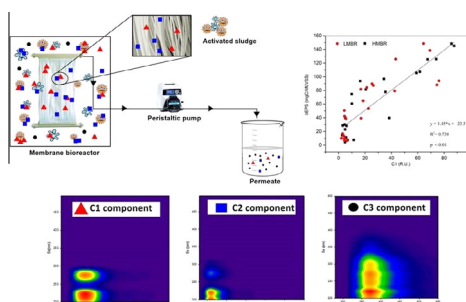
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HIGHLIGHTS

- EEM–PARAFAC was successfully applied to track fluorescent DOM in MBRs.
- Tryptophan-like component can be used as the surrogate of EPS production in sludge.
- Tyrosine-like component is closely related to the stability of sludge.
- Tryptophan-like component is transformed to microbial humic-like component.
- Protein-like components are mostly responsible for membrane fouling in MBRs.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, we successfully tracked the dynamic changes in different constitutes of bound extracellular polymeric substances (bEPS), soluble microbial products (SMP), and permeate during the operation of bench scale membrane bioreactors (MBRs) via fluorescence excitation–emission matrix (EEM) combined with parallel factor analysis (PARAFAC). Three fluorescent groups were identified, including two protein-like (tryptophan-like C1 and tyrosine-like C2) and one microbial humic-like components (C3). In bEPS, protein-like components were consistently more dominant than C3 during the MBR operation, while their relative abundance in SMP depended on aeration intensities. C1 of bEPS exhibited a linear correlation ($R^2 = 0.738$; $p < 0.01$) with bEPS amounts in sludge, and C2 was closely related to the stability of sludge. The protein-like components were more greatly responsible for membrane fouling. Our study suggests that EEM–PARAFAC can be a promising monitoring tool to provide further insight into process evaluation and membrane fouling during MBR operation.

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1. Introduction

The concentration and the quality of wastewater dissolved organic matter (DOM), which comprises thousands of organic compounds from microbial by-products and cell lysis, are critical factors in the assessment of operation performance and designing

optimum conditions of many biological treatment systems. The heterogeneous and complex structures of DOM often pose challenges to the improvement of system performance (Ishii and Boyer, 2012). Extracellular polymeric substances (EPS), excreted as a result of microbial activities (e.g., metabolism, biomass growth, and decay), are considered to be a major fraction of DOM in activated sludge processes and/or membrane bioreactors (MBR) (Meng et al., 2009). They mainly consist of proteins, polysaccharides, DNA, and humic substances. In particular, glue-like EPS

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operate as an important player on the surface of activated sludge, affecting the agglomeration of sludge. It was previously reported that soluble microbial products (SMP) and EPS were mostly responsible for biofouling in MBR (Guo et al., 2012).

Much of the previous literature revealed that the chemical composition of DOM played a critical role in MBR operation and performance (Liang et al., 2008; Yue et al., 2015). For example, large sized DOM fractions are typically rejected by the membrane, as they are accumulated inside bioreactors as a part of the sludge cake layer and the supernatant, which ultimately leads to a significant resistance to membrane filtration. The membrane fouling propensity of MBR strongly depends upon the composition, concentration, size, and hydrophobicity of DOM present in a given system. Previous studies have shown that hydrophobic fractions (e.g. aromatic proteins) in DOM had a higher membrane fouling propensity than hydrophilic fractions, while humic substances operated as the main constituents of sludge supernatant and permeate (Liang et al., 2008). DOM was also reported to be associated with a long term irreversible membrane fouling (Kimura et al., 2015). Shear stress driven by aeration in bioreactors could promote the release of microbial by-products from sludge (Wu and He, 2012). Meng et al. (2009) reported that bound EPS (bEPS) was a main source of sludge supernatant during the operation of MBRs.

It is widely recognized that DOM composition varies dynamically during biological treatment processes. However, the conventional quantity indices of DOM, such as dissolved organic carbon (DOC), chemical oxygen demand (COD), and the contents of proteins and/or carbohydrates, are very limited when attempting to attain detailed information of the compositional changes of DOM and their linkage with the system operation. Recently, optical measurements have gained a great deal of attention as an efficient tool for characterizing the structure and chemical composition of DOM, owing to the advantages of rapid detection and minimum requirement of sample pretreatment. Fluorescence excitation emission matrix (EEM) spectroscopy is a highly sensitive, potent, and useful tool for characterizing DOM in water/wastewater, and it has proven promising for probing the DOM composition involved in membrane fouling (Wang et al., 2009; Hur and Cho, 2012; Yue et al., 2015; Zhou et al., 2012). However, wastewater DOM is composed of various fluorophores, overlapping in EEM spectra, which makes the identification of dissimilar fluorescent groups difficult. Over the decades, different mathematical models have been utilized to decompose the EEM spectra in an effort to overcome the limitations, which include fluorescence regional integration (FRI), fluorescence ratio and parallel factor analysis (PARAFAC), and self-organizing maps (SOM) (Chen et al., 2003; Stedmon and Bro, 2008). Among those, PARAFAC, a mathematical separation tool, has proven capable in unraveling potential overlapped fluorophores with dissimilar chemical structures by successfully describing an EEM spectrum into a combination of several independent fluorescent groups. EEM-PARAFAC has emerged as an excellent technique to explore the dynamic changes of DOM in wastewater (Yang et al., 2015).

Numerous researches have been carried out to investigate the effects of operational and environmental parameters on DOM composition in MBRs (Sabia et al., 2013; Zsirai et al., 2012). However, few studies have applied EEM-PARAFAC to track DOM changes to understand MBR processes (Hur et al., 2014; Zhou et al., 2012). In this study, we first used EEM-PARAFAC to monitor the changes of different fluorescent groups present in supernatant, membrane foulants, bEPS, and permeate over full operation of bench scale MBRs. The whole operation period was divided here into a before-acclimation and after-acclimation periods based on DOM concentrations of permeate, which were low during the before-acclimation period due to the domestication of microorganisms to a new environment (Fig. 1). The main objectives of this

study were (1) to examine temporal variations in bEPS, SMP, and permeate bench scale MBRs via EEM-PARAFAC, (2) to test the applicability of fluorescent DOM as signature for MBR operational parameters, and (3) to explore membrane capability to retain different DOM constituents. These investigations are beneficial in understanding the fate and role of fluorescent DOM in MBR, which helps to evaluate MBR performance via simple spectroscopic monitoring and enables the estimation of bEPS and SMP in both qualitative and quantitative manners, ultimately paving the way for the creation of a continuous on-line MBR monitoring system.

2. Methods

2.1. Experimental setup

Two parallel bench scale MBRs, having identical submerged PVDF hollow fiber membrane modules (Mitsubishi rayon, Japan) and an effective membrane surface area of 0.072 m², were operated with a working volume of 7 L each (Fig. S2). Both MBRs were run at the same filtration and relaxation modes of 8 min and 2 min, respectively. A constant filtration flux of 10 LMH (L/m²/h) was maintained for both systems. Air bubbles were supplied from the bottom of the reactors for membrane scouring and the proper growth and maintenance of microorganisms. In this study, two different aeration intensities were applied by air diffusers (i.e., air stones) at 6 L/min for a high aeration MBR (HMBR) and at 2 L/min for a low aeration MBR (LMBR) to induce different shear conditions associated with sludge characteristics (Lebegue et al., 2010). The low aeration condition was strong enough to keep activated sludge well mixed (Meng et al., 2008). Seed activated sludge was collected from a return line of a municipal wastewater treatment plant located in Seoul, South Korea. The hydraulic retention times (HRT) and sludge retention times (SRT) were adjusted to 10 h and 80 days, respectively, for both MBRs.

Synthetic wastewater with chemical oxygen demand (COD) ranging between 700 and 750 mg/L was used as feed, which corresponds to medium strength municipal wastewater. The composition of the feed wastewater is described in Table S1. The membranes of the two MBRs had a maximum trans-membrane pressure (TMP) of 30 kPa, after which physical and chemical (basic and acidic) cleaning treatments were required to recover the original flux. In brief, cake deposited on membrane surface was gently scraped off with a plastic sheet, and the membrane was washed with distilled water. The membrane was then soaked in NaOCl solution (3 g/L). For acidic cleaning, the membrane again was soaked in 2% solution of citric acid for 8 h.

2.2. EPS extraction protocol

EPS was extracted using the cation exchange resin (CER) (Dowex[®] Marathon[®] C, strongly acidic Na⁺ form, Sigma-Aldrich) method, which usually provides a high yield (Frølund et al., 1996). Briefly, CER was initially soaked in a phosphate-buffered saline (PBS) for 12 h and dried at room temperature for 24 h. 50 mL of activated sludge was taken in a conical tube from reactors and centrifuged at 4000 rpm under 4 °C to collect the supernatant containing soluble microbial products (SMP). The sludge pellets were suspended in a phosphate pH 7 buffer solution (50 mL), consisting of NaCl, KCl, Na₃PO₄, and NaH₂PO₄, to wash and completely remove loosely bound EPS before CER (70 g/g MLVSS), and a buffer solution was added (up to 50 mL). The suspension solutions containing biomass and CER were stirred for 18 h and centrifuged again at 4000 rpm under 4 °C to obtain bEPS samples. After filtering through a 0.4 μm cellulose tri acetate (CTA) membrane, portions of SMP and bEPS were lyophilized at -50 °C for further

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