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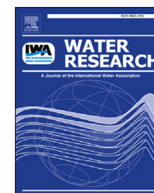

Membrane biofouling in a wastewater nitrification reactor: Microbial succession from autotrophic colonization to heterotrophic domination

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Membrane biofouling is a complex process that involves bacterial adhesion, extracellular polymeric substances (EPS) excretion and utilization, and species interactions. To obtain a better understanding of the microbial ecology of biofouling process, this study conducted rigorous, time-course analyses on the structure, EPS and microbial composition of the fouling layer developed on ultrafiltration membranes in a nitrification bioreactor. During a 14-day fouling event, three phases were determined according to the flux decline and microbial succession patterns. In Phase I (0–2 days), small sludge flocs in the bulk liquid were selectively attached on membrane surfaces, leading to the formation of similar EPS and microbial community composition as the early biofilms. Dominant populations in small flocs, e.g., *Nitrosomonas*, *Nitrobacter*, and *Acinetobacter* spp., were also the major initial colonizers on membranes. In Phase II (2–4 d), fouling layer structure, EPS composition, and bacterial community went through significant changes. Initial colonizers were replaced by fast-growing and metabolically versatile heterotrophs (e.g., unclassified *Sphingobacteria*). The declining EPS polysaccharide to protein (PS:PN) ratios could be correlated well with the increase in microbial community diversity. In Phase III (5–14 d), heterotrophs comprised over 90% of the community, whereas biofilm structure and EPS composition remained relatively stable. In all phases, AOB and NOB were constantly found within the top 40% of the fouling layer, with the maximum concentrations around 15% from the top. The overall microbial succession pattern from autotrophic colonization to heterotrophic domination implied that MBR biofouling could be alleviated by forming larger bacterial flocs in bioreactor suspension (reducing autotrophic colonization), and by designing more specific cleaning procedures targeting dominant heterotrophs during typical filtration cycles.

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1. Introduction

The submerged membrane bioreactor (MBR) is a well-developed process that combines membrane filtration with biological treatment, providing better effluent quality and lower sludge production compared to the conventional activated sludge processes (Judd, 2011). A significant portion of MBR operating cost is attributed to fouling control. Membrane fouling occurs as a result of the accumulation of solutes, colloids and bacterial flocs on membrane surfaces, and biofouling contributes to about 45% of the overall fouling (Komlenic, 2010). Traditional methods developed for mitigating membrane biofouling are mainly based on physicochemical principles, and factors modulating biofouling behaviors have been extensively studied, including feed characteristics, membrane properties, and reactor operation conditions (Ahmed et al., 2007; Park et al., 2006; Tsuyuhara et al., 2010). However, a comprehensive understanding of biofouling mechanisms from a microbial ecology perspective is still limited, hampering the applications of innovative, biological fouling control strategies, including enzymatic disruption of extracellular polymeric substances (EPS) excretion, quorum quenching, and biofilm disruption by adding bacteriophage (Xiong and Liu, 2010). In order to

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transform these novel solutions into more effective, long-term fouling control strategies, it remains important to study biofilm structure, EPS and microbial community succession in mixed-culture SMBR systems.

Compared with natural environments or media-based biofilm reactors, MBR provides a unique habitat for biofilm growth. The transmembrane pressure generates a drag force that dominates the transport of particulates, colloids, cells, and nutrients from the mixed liquor to the membrane surfaces. One of the typical membrane fouling mechanisms is standard blocking, where small particles pass the membrane pores and a finite number adhere to the walls causing pore blockage (Nguyen et al., 2010). Only a few studies have examined the relationship between small sludge flocs in a MBR and the initial microbial colonizers on membrane surfaces (Piasecka et al., 2012; Zhang et al., 2006a; Gao et al., 2011). These studies observed that the biofilm community succession could be influenced by the enhanced flux of nutrients and metabolites within the fouling layer, and bulk sludge and the fouling layer microbial communities could be different. However, how various physical and biological factors contribute to the fouling layer microbial community succession throughout the fouling process remains unknown.

This study aimed at examining the microbial community changes and the associated fouling behaviors of ultrafiltration membrane (UF) in a lab-scale wastewater nitrification reactor. It is hypothesized that 1) initial membrane filtration favors the attachment of small flocs, and initial colonizers are closely related to species present in small flocs; 2) the biofilm community succession is dominated by the pressure-driven deposition of bulk microbes at an early stage and species interactions among autotrophs and heterotrophs at later stages. Specific objectives were to 1) investigate the bacterial populations in size-fractioned bulk sludge and their correlations with the early colonizers on membranes; 2) monitor the changes of biofilm structural parameters and EPS composition during biofouling; and 3) evaluate the biofilm community succession and the spatial distribution of major bacterial groups within the fouling layer. By depicting a more comprehensive and detailed picture of biofouling processes, this study can potentially inspire novel anti-fouling strategies for membrane bioreactors in general, and for aerobic submerged MBRs in particular.

2. Materials and methods

2.1. Nitrifying bioreactor

A 20-L nitrification bioreactor was operated in continuous flow mode at ambient temperature with a hydraulic retention time (HRT) of 4 d and solids retention time (SRT) of 15 d (Fig. 1). The seed sludge was obtained from a lab-scale nitrifying reactor operated under similar conditions (Ahn et al., 2011). The feed medium contained 500 mg-N/L ammonium and was devoid of organic carbon as described previously (Hockenbury and Grady, 1977; Ahn et al., 2008). Reactor pH was automatically controlled at 7.5 ± 0.1 by adding 1 mol/L $NaHCO_3$ solution. Aeration was continuously provided at a rate of 1 L/min through flexible rubber tubing placed at the bottom edges, resulting in a constant level of dissolved oxygen in the bioreactor (2.2 ± 0.5 mg O_2/L, specific aeration demand was about 0.6 $m^3/m^2/h$ during the operation of submerged membrane filtration). The bioreactor was monitored for its performance (ammonia, nitrite, nitrate, total and effluent chemical oxygen demand concentrations) using standard methods (Rice, 2012).

Submerged membrane filtration was operated for different time periods (3 h, 6 h, 12 h, 18 h, 1 d, 2 d, 3 d, 5 d, 7 d, and 14 d) in the nitrification bioreactor using flat-sheet polysulfone membranes (avg. pore diameter = 86 nm) placed in plexiglass membrane cartridges. Membrane fabrication procedures and more details about UF operation are described in Supplementary Information. Triplicate membranes were operated simultaneously in the reactor for each time period, and completely sacrificed for imaging and community analysis. Air scouring on membrane surfaces was eliminated by positioning the membrane cartridge away from air diffusers. Permeate was driven across the membrane by a vacuum pump at a constant transmembrane pressure (TMP, 25 kPa), weighed by a digital scale, and recorded by the LabView software (National Instruments, TX). The constant TMP instead of the constant flux mode was selected mainly to simplify the analysis of biofilm community variations caused by pressure-driven attachment. In addition, it has been suggested that UF performance could potentially get improved by allowing flux to decline naturally, i.e., a constant-TMP condition (Bourgeous et al., 2001). Freshly harvested membranes were cut into equal-width pieces (1.54 × 2.54 cm) and rinsed twice by gently dipping sequentially in 40 mL 1× PBS buffer. Two buffer washes were previously proven to be an optimal procedure for examining membrane fouling (Xue et al., 2014), i.e., 0–1 buffer wash was insufficient to remove planktonic cells, but >2 washes will remove loosely attached flocs. Rinsed membranes were analyzed freshly for biofilm structure, fixed for fluorescent *in situ* hybridization (FISH), or stored at −80 °C for less than a week prior to EPS and DNA extraction as detailed in the following sections.

2.2. Floc separation, size measurement, and EPS extraction

Activated sludge flocs collected from the nitrification reactor (prior to membrane operation) were size-fractioned by gravity settling. Briefly, 500 mL activated sludge was transferred into a 1 L separatory funnel. 20 mL from the bottom layer was collected after 30 min settling time to represent the large floc fraction, and another 60 mL was collected from the top after another 30 min settling to represent the small floc fraction. The floc size distribution in each fraction and the original bulk sludge was obtained by microscopic analysis. The micrographs were obtained with a Zeiss Axio Observer Z1 microscope (40× objective, phase contrast, Carl Zeiss AG, Germany) and analyzed for particle size using ImageJ software. For each sample, over 50 images were taken and 5000–30,000 particles were examined to set up a representative particle size distribution.

To quantify the bound EPS, 15 mL sludge liquor from each fraction was filtered through a 0.22-μm membrane filter and the collected sludge was processed for EPS extraction using the formaldehyde and NaOH method (Liu and Fang, 2002). Protein and polysaccharide concentrations in the extracted EPS were measured by the standard Lowry (Total Protein Kit, Sigma–Aldrich, MO) and the phenol-sulfuric acid methods, respectively (Eaton et al., 2005). The bound EPS content in each fraction was calculated as protein or polysaccharide concentrations per unit TSS of sludge, and the TSS was measured according to the standard method (Rice, 2012).

2.3. Imaging analysis for membrane biofilm structure

The rinsed membrane biofilm samples were mounted on glass slides with double-sided tape. An extra layer of the double-sided tape was placed outside each end of the membrane to create a space and to prevent the cover slide from disturbing the biofilm structure. The biofilm samples were examined by confocal laser scanning microscopy (CLSM) and subject to imaging and quantitative structural analysis. The total biofilm cells were fluorescently tagged by nucleic acid stain SYTO 9 (2.5 μmol/L, Sigma–Aldrich, MO). Three EPS markers were applied to target the polysaccharide (Alexa 633 conjugated Concanavalin A and Wheat Germ Agglutinin,

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