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Characterization of the archaeal community fouling a membrane bioreactor

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ABSTRACT

Biofilm formation, one of the primary causes of biofouling, results in reduced membrane flux or increased transmembrane pressure and thus represents a major impediment to the wider implementation of membrane bioreactor (MBR) technologies for water purification. Most studies have focused on the role of bacteria in membrane fouling as they are the most dominant and best studied organisms present in the MBR. In contrast, there is limited information on the role of the archaeal community in biofilm formation in MBRs. This study investigated the composition of the archaeal community during the process of biofouling in an MBR. The archaeal community was observed to have lower richness and diversity in the biofilm than the sludge during the establishment of biofilms at low transmembrane pressure (TMP). Clustering of the communities based on the Bray–Curtis similarity matrix indicated that a subset of the sludge archaeal community formed the initial biofilms. The archaeal community in the biofilm was mainly composed of Thermoprotei, Thermoplasmata, Thermococci, Methanopyri, Methanomicrobia and Halobacteria. Among them, the Thermoprotei and Thermoplasmata were present at higher relative proportions in the biofilms than they were in the sludge. Additionally, the Thermoprotei, Thermoplasmata and Thermococci were the dominant organisms detected in the initial biofilms at low TMP, while as the TMP increased, the Methanopyri, Methanomicrobia, Aciduliprofundum and Halobacteria were present at higher abundances in the biofilms at high TMP.

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Introduction

Membrane bioreactors (MBRs) represent a class of technology integrating the activated sludge mediated nutrient removal and membrane mediated sludge–water separation (Kraume and Drews, 2010), and have many advantages in the wastewater treatment process (WWTP) in comparison with the conventional gravity dependent WWTP. These advantages include a smaller treatment space required, less hydraulic retention time, less activated sludge biomass production and a higher quality of effluent (Melin et al., 2006; Li et al., 2013). However, all MBR systems suffer from biofouling which negatively impacts on MBR performance, resulting in higher operating costs (Le-Clech et al., 2006). Biofouling is the result of deposition of the microorganisms and microbial extracellular polymeric substances (EPS), *e.g.*, polysaccharides and proteins, on the membrane surface. The microbes and their secreted EPS attach onto the membrane and proliferate to form a cake-layer on the membrane, leading to clogging of the membrane (Nguyen et al., 2012). Membrane clogging by the biofilm reduces the water permeability through the membrane, resulting in a lower permeate production (flux) or a higher transmembrane pressure (TMP) to maintain the constant flux.

Biofilm formation is a ubiquitous phenomenon for microorganisms in nature (Simões et al., 2010). The microbial community involved in the biofilm formation in MBR has been studied intensively. For example, bacterial communities including the Proteobacteria, Bacteroidetes and Actinobacteria, had been reported to compose the majorities of biofouling community in MBR (Miura et al., 2007; Ivnitsky et al., 2007). The Bacteroidetes and Firmicutes displayed a higher competence to live in the biofilm layers relative to the activated sludge (Lim et al., 2012). Additionally, fungal organisms, such as *Saccharomyces* sp. and *Cryptococcus* sp., are also present in both the anoxic and aerobic sludge and form biofilms on the MBR membranes (Ravi et al., 2009; Bojsen et al., 2012).

The archaea represent the third domain of life and exhibit considerable diversity. Indeed, this group of microorganisms is best known for their ability to adapt to extreme environments, such as the hot springs (Song et al., 2013), acidic environment (Edwards et al., 2000) and hypersaline lakes (Oren, 2001). They have even been identified in activated sludge from wastewater treatment plants (Gómez-Silván et al., 2010; Zhang et al., 2011). Some archaeal groups, *e.g.*, Haloarchaea and Crenarchaea, were demonstrated to be capable of adhering to surfaces to form biofilms (Fröls et al., 2012; Koerdt et al., 2010). The quorum sensing (QS) signals, which regulate the biofilm formation processes of bacterial community, were also reported to coordinate the development of archaeal biofilm (Orell et al., 2013). The carboxylated acyl homoserine lactones (C-AHLs) were used in the QS system of the methanogenic euryarchaea (Zhang et al., 2012). This was different from the bacterial QS system, where the N-acyl-homoserine lactones were used as the signals in QS phenomenon (Krysciak et al., 2011). The biofilm archaeal community was diverged in different wastewater treatment processes. In an anoxic/aerobic submerged biofilter system, the archaea Methanobacteria and Methanomicrobia were observed to compose the majority of the biofilm community (Gómez-Silván et al., 2010). In a reverse osmosis membrane system, the Methanomicrobia were also the dominant archaea of the biofilms on membrane surface (Al Ashhab et al., 2014). Besides, the Thermoprotei, Methanopyri, and Thermoplasmata were prevailing in the membrane biofilms in the reverse osmosis membrane system (Al Ashhab et al., 2014). In a pilot-scale membrane-coupled upflow anaerobic sludge blanket bioreactor, the methanogenic archaea, *e.g.*, Methanosarcinales and Methanospirillaceae, persistently composed of the biofouling microbial community, even the membranes were cleaned by the chemical reagents (Calderón et al., 2011). Despite these reports, there is still very little data on the association of archaea with biofilm formation on MBR membranes and their impact on MBR performance (Calderón et al., 2013). Since some archaeal groups, such as methanogenic archaea and ammonia oxidizing archaea, are very important in nitrogen and carbon removal processes and prevailing in wastewater treatment activated sludge (Fredriksson et al., 2012; Tabatabaei et al., 2010), it is necessary to study the community diversity of archaeal biofilm on membranes in order to control the biofouling phenomenon in MBR systems.

In this study, the archaeal communities on hollow fiber membranes and in activated sludge were characterized in a MBR through high-throughput sequencing of the 16S rRNA gene. The phylotypes of archaea were determined correlating with the increase in TMP.

1. Materials and methods

1.1. MBR set-up and operation

A laboratory scale anoxic/oxic membrane bioreactor (A/O MBR) was conducted to treat the synthetic wastewater, which was composed of glucose (320 mg/L), beef extract (60 mg/L), peptone (80 mg/L), KH_2PO_4 (7 mg/L), $MgSO_4{\cdot}7H_2O$ (14 mg/L), $FeSO_4{\cdot}7H_2O$ (7.3 mg/L) and sodium acetate (90 mg/L). The total organic carbon (TOC) of synthetic wastewater was 200 mg/L. The A/O MBR system was composed of an anoxic sludge tank, an aerobic sludge tank and a filtration tank in which a membrane module was installed (Fig. 1a). The membrane module was made with the hollow fiber PVDF membranes (ZeeWeed, GE, Singapore) and designed to be a "curtain" mode, where one end of the membrane piece was sealed and hung down into the sludge tank (free end), while the other end of the membrane was open and sealed into a collection chamber that was linked to the suction pump (Fig. 1a). The activated sludge was collected from the Ulu Pandan wastewater treatment plant in Singapore and acclimated in synthetic wastewater for 60 days before the start of the experiment. The concentrations of dissolved oxygen (DO) in the anoxic tank and aerobic tank were 0.1–0.2 mg/L and 5–6 mg/L, respectively. A peristaltic pump was used to re-circulate the liquor sludge from the filtration tank to anoxic tank. The mixed liquor suspended sludge (MLSS) was maintained at 6–8 g/L and 3–5 g/L in the anoxic tank and aerobic tank respectively. The MBR was operated with the constant flux, 15 ± 1 L/(m^2·hr) (LMH), at the room temperature of 25–26°C. The hydraulic retention time (HRT) and sludge retention time (SRT) for the MBR were approximately 10 hr and 25 days respectively. The parameters, such as membrane flux, TMP, pH, DO and temperature, were monitored and automatically recorded using a data logger and a computer. The TOC of the influent and permeate was measured using a multi N/C® 2100s (Analytik Jena AG, Thuringia, Germany).

1.2. DNA extraction

DNA of the biofilms and activated sludge was separately extracted by a modified CTAB-PEG protocol (Paithankar and Prasad, 1991; Griffiths et al., 2000). The biofilm and sludge samples were collected at two time points of low TMP stage (7 and 10 kPa) and two time points of high TMP stage (30 and 60 kPa). The mixed liquor suspended sludge was collected in microfuge tubes containing lysing matrix (FastPrep Kit, MP