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Deciphering the core fouling-causing microbiota in a membrane bioreactor: Low abundance but important roles

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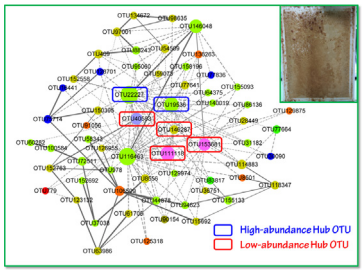
HIGHLIGHTS

- The microbiota structure related to membrane biofouling was investigated.
- 76 taxonomic clades were responsible for driving the divergences among communities.
- The fierce competition among species in communities shaped the bio-cake microbiota.
- The low-abundance species play an unrecognized role in bio-cake formation.

GRAPHICAL ABSTRACT



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ABSTRACT

Currently, membrane biofouling in membrane bioreactors (MBRs) is normally attributed to the occurrence of abundant bacterial species on membranes, whereas the roles of low-abundance bacteria have not been paid sufficient attention. In this study, the linear discriminant analysis (LDA) effect size (LEfSe) algorithm was used to identify active biomarkers, determining 67 different phylotypes among Bulk sludge, low-fouling Bio-cake (10 kPa), high-fouling Bio-cake (25 kPa) and Membrane pore in a membrane bioreactor with NaOCl backwash. Interestingly, a large proportion of the active biomarkers in bio-cake samples, such as *Methylophilaceae, Burkholderiaceae, Paucibacter* and *Pseudoxanthomonas,* did not fall within the abundant taxa (i.e., <0.05% relative abundance), indicating the preferential growth of these low-abundance bacteria on the membrane surface. Furthermore, the characterization of microbial interactions using a random matrix theory (RMT)-based network approach obtained a network consisting of 120 nodes and 228 edges. Specifically, network analysis showed the presence of an intense competition among bacterial species in the fouling-related communities, suggesting that negative interactions have an important effect on determining the microbial community structure. More importantly, the LEfSe algorithm and network analysis showed that most of the core species of the bio-cake, such as *Burkholderiaceae, Bacillus and Rhodothermaceae*, merely amounted to a very low relative abundance (<1%), suggesting their unrecognized and over-proportional ecological role in triggering the initial biofilm formation and subsequent biofilm maturation during MBR operation. Overall, this work should improve our understanding of the bacterial community structure on the fouled membranes in MBRs.

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1. Introduction

Membrane bioreactors (MBRs) are an efficient wastewater treatment technology that provide superior effluent quality compared with the conventional activated sludge processes. However, membrane biofouling, which can result in high operating costs, is still a primary obstacle in MBRs operation. The microbes and their metabolic products have been proven to play a crucial role in the biofouling process (Meng et al., 2017). Typical strategies developed for mitigating membrane biofouling were mainly based on physical and chemical principles (Wang et al., 2014), membrane modification (Yang et al., 2016) and biological-based strategies (Lee et al., 2016). Despite the advances in membrane biofouling control, complete prevention of its occurrence is not possible, leaving it the main challenge in the MBRs operation. With the development and application of molecular microbial techniques, progress has recently been made to characterize the microbiota compositions of the biofouling layer and bulk sludge. Such studies of the biofouling layer (i.e., bio-cake) and bulk sludge microbiota from a microbial ecology view are expected to provide more details about the development of biofouling and, meanwhile, provide new insight into biofouling control strategies.

To date, the microbial community in MBRs has been investigated extensively, including the community compositions of the bulk sludge and bio-cake (in different fouling stages), and the impacts of various physicochemical conditions. For example, Jo et al. found that community compositions in the bio-cake were significantly different from those in the bulk sludge in 10 full-scale MBRs (Jo et al., 2016). Likewise, Lim et al. demonstrated that the bulk sludge mainly consisted of *Enterobacteriales* (43.65%), *Sinomonas* (11.22%) and *Xanthomonadales* (9.89%), while *Enterobacteriales* (68.41%), *Bacteroidales* (7.35%) and *Clostridiales* (6.11%) were dominant in the bio-cake (Lim et al., 2012). In addition, the bacterial richness and abundance of the bio-cake were consistently higher than that of the bulk sludge, as revealed by Shannon and Chao1 index (Gao et al., 2014). Nonetheless, the microbial community compositions in the bio-cake were not consistent throughout the biofouling process. According to the study of Ziegler et al., *Beta-proteobacteria*-affiliated bacteria, including *Limnohabitans*, *Hydcrogenophaga* and *Malikia,* and filamentous-affiliated bacteria, including *Chloroflexi* and *Gordonia*, were the dominant populations at the initial and last biofouling stages, respectively (Ziegler et al., 2016). Moreover, the microbial community in the chemically backwashed MBR could differ from that in the conventional MBR due to different microbial responses towards chemicals (Lee et al., 2013; Cai and Liu, 2016). For instance, Navarro et al. observed an increase in microbial evenness with no significant decrease in richness for the bio-cake (Navarro et al., 2016a), indicating that the dominant species rather than the rare species were significantly affected by NaOCl. Briefly, all the previous literature concerning microbial compositions in MBRs have reached an agreement that the biofouling microbial community showed a dynamic shift and differed from that of the bulk sludge (Choi et al.; Lim et al., 2012), which was commonly attributed to the different physicochemical factors such as dissolved oxygen, transmembrane pressure (TMP), fluxes and sparging rates (Gao et al., 2013; Ziegler et al., 2016). However, how the inter-species interactions in MBRs shape the biofouling community structure and thus contribute to the bio-cake formation remains unknown. More importantly, high-abundance microbes were always considered to play a critical role in membrane fouling development. Nonetheless, by evaluating the fouling potentials of 41 bacterial strains isolated from fouled membranes, Ishizaki et al. recently demonstrated that the high-abundance species may not necessarily be fouling-causing bacteria (Ishizaki et al., 2016). Meanwhile, recent studies highlighted the importance of low-abundance microbes for ecosystem function, such as biochemical processes (Vuono et al., 2016), community assemblies (Elsas and Salles, 2012) and microbiome functionalities (Hol et al., 2010). On the basis of this knowledge, it is plausible to speculate that low-abundance microbes can play an over-proportional role in bio-cake microbiota. Therefore, it is necessary for us to conduct in-depth analysis for the fouling-causing microbiota in MBRs.

Recently, network analysis approach has been developed to evaluate and characterize the complicated ecological networks of biological communities (Faust et al., 2012). This approach has been widely applied to discern taxon co-occurrence patterns among populations in microbial communities in various natural habitats, such as oceans (Chow et al., 2014), soils (Zhou et al., 2010; Barberán et al., 2012) and groundwater (Deng et al., 2016). These studies demonstrated that microorganism co-occurrence patterns are ubiquitous, which is of great importance for the ecological structure and function of the microbial community. In particular, this analysis method can give new insights into the complex microbiota topological structure, including community organization principles, keystone species and interactions between community members (Deng et al., 2012), rather than the simple species richness and abundance. As such, the in-depth analysis on fouling-related bacterial consortia with this method can definitely increase our understanding on bio-cake microbiota during the biofouling development. As an artificial microbial ecosystem with high biomass concentrations (Grady et al., 2011), activated sludge ecosystems definitely contain a multitude of interactions, such as mutualism and competition. However, currently, information regarding microbial ecological network association in activated sludge ecosystems, especially in the bio-cake of fouled membranes, is rarely reported (Ju and Zhang, 2014; Peng et al., 2014; Jeong et al., 2016).

The objective of this study was, therefore, to investigate the potential roles of individual species and species-species interactions in the microbiota organization of the activated sludge ecosystem in MBRs. The microbial community structure in both bulk sludge and bio-cake were analyzed by 16S rRNA gene-based sequencing. The bacterial consortia inhabiting membrane pores were also characterized. Moreover, linear discriminant analysis (LDA) effect size (LEfSe) was employed to distinguish specific differentially abundant species over represented in different communities and to explain the biologically differential bacteria among various habitats (i.e., bulk sludge, bio-cake and membrane pores). Additionally, a phylogenetic molecular ecological network was constructed to deduce the potential interactions among microbial populations, and the pivotal roles of the low-abundance species in bio-cake microbiota were illuminated. Overall, this study is expected to improve our comprehensive understanding of microbial community structures in MBRs and shed light on the development of biofouling control strategies.

2. Methods and materials

2.1. Operation of the MBR and sample collection

Three flat-sheet membrane modules (PVDF, 0.1 μm, SINAP, Shanghai, China), defined as M.1, M.2 and M.3 hereafter, were submerged in the aerobic tank of an anoxic/aerobic MBR with a total working volume of 24 L (12 L each for the anoxic and aerobic tanks, respectively) (see Fig. S1 in the supplementary materials). Each membrane module had an effective filtration area of 0.1 m^2. The hydraulic retention time (HRT) and solid retention time (SRT) were set at 5.33 h and 30 d, respectively. Transmembrane pressure (TMP) was monitored every 30 s using Labviews (National