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Comparison of microbial communities of activated sludge and membrane biofilm in 10 full-scale membrane bioreactors

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ABSTRACT

Operation of membrane bioreactors (MBRs) for wastewater treatment is hampered by the membrane biofouling resulting from microbial activities. However, the knowledge of the microbial ecology of both biofilm and activated sludge in MBRs has not been sufficient. In this study, we scrutinized microbial communities of biofilm and activated sludge from 10 full-scale MBR plants. Overall, *Flavobacterium*, *Dechloromonas* and *Nitrospira* were abundant in order of abundance in biofilm, whereas *Dechloromonas*, *Flavobacterium* and *Haliscomenobacter* in activated sludge. Community structure was analyzed in either biofilm or activated sludge. Among MBRs, as expected, not only diversity of microbial community but also its composition was different from one another ($p < 0.05$). Between the biofilm and activated sludge, community composition made significant difference, but its diversity measures (i.e., alpha diversity, e.g., richness, diversity and evenness) did not ($p > 0.05$). Effects of ten environmental factors on community change were investigated using Spearman correlation. MLSS, HRT, F/M ratio and SAD_m explained the variation of microbial composition in the biofilm, whereas only MLSS did in the activated sludge. Microbial networks were constructed with the 10 environmental factors. The network results revealed that there were different topological characteristics between the biofilm and activated sludge networks, in which each of the 4 factors had different associations with microbial nodes. These results indicated that the different microbial associations were responsible for the variation of community composition between the biofilm and activated sludge.

1. Introduction

Membrane bioreactors (MBRs), combining membrane separation with biochemical conversion, have led to a range of innovative environmental biotechnology applications for wastewater treatment and reuse (Hai et al., 2013). Biofouling is one of the main obstacles in MBR processes (Meng et al., 2009) because it results in decreased plant productivity/permeate yield, membrane lifespan and energy efficiency (Drews, 2010). Microbial production of membrane foulants in the activated sludge, as well as microbial colonization on membrane surfaces, are the main causal agents for biofouling (Malaeb et al., 2013). However, the microbial ecology has not been fully elucidated to date. Specifically, there are few published survey reports about microbial systems of both biofilm and activated sludge in actual MBRs.

Because activated sludge is the sole inoculum for biofilm formation on the membrane in a MBR, the microbial community in the biofilm is likely to resemble that in the activated sludge. However, many studies have reported differences between the activated sludge and biofilm communities in lab- and pilot-scale MBRs. For instance, Piasecka et al. (2012) found that the bacterial community in activated sludge differed from that in the membrane biofilm at the initial phase in a lab-scale MBR (an 18.6-L reactor with a flat sheet membrane). Lim et al. (2012) reported that the microbial composition of a biocake that was loosely attached to the membrane differed from that of activated sludge in a lab-scale MBR (a 6-

Abbreviations: A_2O, anaerobic/Anoxic/2Oxic; EPS, extracellular polymeric substances; F/M ratio, food-to-microorganism ratio; FS, flat sheet; HF, hollow fiber; HRT, hydraulic retention time; I, industrial wastewater; M, municipal wastewater; MLSS, mixed liquor suspended solids; MBR, membrane bioreactor; OTU, operational taxonomic unit; PCoA, principal coordinate analysis; PE, polyethylene; PES, polyethersulfone, polytetrafluoroethylene; PVDF, polyvinylidene fluoride; PVC, polyvinyl chloride; QS, quorum sensing; RCBD, completely randomized block design; SAD_m, specific aeration demand on membrane; SRT, solid retention time.

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L reactor with a hollow fiber membrane). These differences were primarily observed from single-membrane bioreactors, implying a need for microbial observations from multiple MBRs to confirm whether the biofilm evolves differently from the activated sludge.

In general, environmental factors can affect the microbial community in wastewater treatment systems. Many studies have shown that various operational factors (e.g., aeration, nutrient removal processes) have important effects on the microbial community in activated sludge (Hu et al., 2012; Zhang et al., 2012; Zhao et al., 2014). However, only a few studies have reported the effects of environmental factors (e.g., different characteristics of membranes) on the microbial community in biofilm. For example, Lee et al. (2014) found a community difference in biofilm on polyethersulfone (PES), polytetrafluoroethylene (PTFE), and PVDF with the same pore size.

Recently, network analysis has been applied for a better understanding of the complex microbial community within the system (Fuhrman, 2009; Faust and Raes, 2012). Network analysis offers new insight into the structure of complex microbial communities (Barberán et al., 2012; Williams et al., 2014). For instance, Kim et al. (2015) reported a microbial network of bacteria and archaea with environmental factors in the biomethane production system and found a key interaction between the bacterial and archaeal members for the energy production. The main objectives of this study were to elucidate the microbial complexities of biofilm and activated sludge and to determine the effects of environmental factors on microbial communities in both biofilm and activated sludge in full-scale MBRs. We surveyed 10 full-scale MBRs with different membrane materials, nutrient removal processes and operational factors using high-throughput sequencing (an Illumina MiSeq platform).

2. Materials and methods

2.1. Sampling sites and methods

The characteristics and specific processes of 10 MBRs are shown in Table 1, Tables S1 and S2. The environmental factors (flux, flow rate, F/M ratio, size of membrane tank, aeration rate in the membrane tank, total membrane area, membrane materials, SRT, BOD, COD, TN, TP and operation mode) were provided by the operator in each MBR. The permanganate ($KMnO_4$) was used for measuring COD in this study. Because this method only measures part of the organic matter (Henze, 2008), most of the BOD values were greater than the COD values. SAD_m was calculated based on the aeration in the membrane tank and total membrane area. The HRT in the membrane tank was calculated based on the flow rate and size of the membrane tank. The concentration of MLSS in the membrane tank was measured in triplicate by standard methods (APHA et al., 2005). The temperature in the membrane tank was measured three times using a digital thermometer (Center technology corp., model CENTER 309, Taiwan).

The membrane module in the membrane tank was removed from the membrane tank with a crane, and the surface of the membrane with biofilm (transmembrane pressure > 20 kPa) was softly rinsed with tap water to remove debris and excess cells from the activated sludge. Sterilized gauze was used to detach the biofilm from the membrane surface, and it was kept in a sterilized conical tube (50 ml). Three sampling spots of biofilm (resulting in triplicate) in the membrane module were randomly selected within the middle part of the membrane module. Samples of activated sludge were collected from three sampling spots in the membrane tank. Activated sludge was sampled into 50 ml of a sterilized conical tube.

2.2. DNA extraction, PCR amplification and Miseq platform sequencing

The sample was prepared as follows for DNA extraction. A slice of gauze (1 cm × 1 cm) with biofilm was cut into small pieces. 1.5 ml of activated sludge was concentrated by centrifugation (10,000 g, 1 min, 25 °C), and the pellet was re-suspended in 0.5 ml of distilled water. These samples were placed into the bead tube for DNA extraction. DNA was extracted from the precipitates using the NucleoSpin Soil kit (Macherey-Nagel GmbH, Düren, Germany). The DNA was eluted in 100 μl of the elution buffer. The eluted DNA was quantified using the ND-1000 spectrophotometer (NanoDrop Inc., Wilmington, DE, USA).

To determine the microbial communities, the Illumina MiSeq platform sequencing technique of partial 16S ribosomal RNA gene was conducted. Each of the sequenced samples was prepared according to the protocols of Illumina 16S Metagenomic Sequencing Library Preparation (Illumina, San Diego, USA). The quantification of DNA and the DNA quality were measured by PicoGreen and NanoDrop. The 16S rRNA genes were amplified with primer 341F (5′-CCTACGGGNGGCWGCAG-3′) and 785R (5′-GACTACHVGGGTATCTAATCC-3′). These regions (V3–V4) were selected from Klindworth et al. (2012). Illumina adapter overhang nucleotide

Table 1
Characteristics of 10 different membrane bioreactors.

MBR	Flow rate (m^3/day)	Flux (LMH)	MLSS in the membrane tank (mg/L)	HRT in the membrane tank (h)	SAD_m (Nm^3/m^2 h)	Average temperature in the membrane tank (°C)	F/M ratio (kgBOD/kgMLSS day)	Average influent (mg/L)				Membrane type, materials, pore size
								BOD	COD	TN	TP	
A-M	15	10.5	5093 ± 114	44.8	0.72	14.1 ± 0.3	0.01*	124.1	125.3	32.9	3.2	HF, PE, <0.4 μm
B-M	9	10.8	3887 ± 442	51.7	0.62	13.7 ± 0.6	0.01*	119.6	127.0	28.7	2.9	HF, PE, <0.4 μm
C-M	1200	6.5	6643 ± 83	18.3	0.17	14.7 ± 0.4	0.02	160.0	150.0	40.0	4.0	HF, PTFE, 0.1 μm
D-M	850	12	5520 ± 480	13.1	0.23	13.1 ± 0.3	0.02*	116.2	95.8	27.2	2.8	HF, PE, 0.4 μm
E-M	2300	8	7860 ± 370	4.8	0.19	16.1 ± 1.2	0.06	154.4	89.9	40.3	4.3	HF, PVDF, <0.1 μm
F-M	7800	13.4	8720 ± 1186	3.4	0.22	15.8 ± 0.8	0.24	225.0	151.9	43.9	5.5	HF, PE, <0.4 μm
G-M	6000	12	8413 ± 46	5.5	0.31	18.1 ± 0.5	0.06	268.0	155.0	80.0	5.5	FS, c-PVC, 0.4 μm
H-M	5000	7.2	10,213 ± 1473	8.8	0.28	21.7 ± 0.6	0.06	190.0	173.0	52.4	5.2	FS, c-PVC, 0.4 μm
I-I	250	16.7	4447 ± 114	9.12	1.7	15.6 ± 0.4	0.06*	103.0	133.6	45.0	10.0	FS, c-PVC, 0.4 μm
J-I	25,000	12.5	7606 ± 404	2.7	0.15	22.0 ± 0.4	0.03	200.0	140.0	35.0	7.0	HF, PE, <0.4 μm

Abbreviations: F/M ratio; Food to microorganism ratio, FS; Flat sheet, HF; Hollow fiber, HRT; Hydraulic retention time, I; Industrial wastewater, M; Municipal wastewater, MLSS; Mixed liquor suspended solids, PE; Polyethylene, Polytetrafluoroethylene, PVDF; Polyvinylidene fluoride, c-PVC; Chlorinated Polyvinyl chloride, SAD_m; Specific aeration demand on membrane.
An asterisk indicates that the F/M ratio was calculated based on the aeration tank.