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Correlation between microbial community structure and biofouling as determined by analysis of microbial community dynamics

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HIGHLIGHTS

- Research investigates the operation of continuous MBRs to treat saline wastewater.
- COD and NH_4^+–N removal could recover along with operation under saline stress.
- Polysaccharide in EPS and SMP played an important role in the membrane fouling.
- Strength or duration of salinity stimulated microorganisms with similar functions.
- The change of microbial community could alter the level of membrane fouling.

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ABSTRACT

Three lab-scale membrane bioreactors (MBRs) were continuously operated to treat saline wastewater under 0%, 0.75% and 1.5% NaCl stress. 0.75% and 1.5% NaCl reduced the COD and NH_4^+–N removal at the beginning, while the removal efficiencies could be recovered along with the operation of MBRs. Also, the polysaccharide in extracellular polymeric substances (EPS) and soluble microbial products (SMP) played an important role in the membrane fouling. Illumina sequencing of 16S rRNA gene showed that the increasing level of salinity reduced the diversity of the microbial community, and a higher salinity stimulated the growth of *Bacteroidetes*. At genus level, *Flavobacterium*, *Aequorivita*, *Gelidibacter*, *Microbacterium*, and *Algoriphagus* increased with the increase of salinity, which are shown to be highly salt tolerant. The strength of salinity or the duration of salinity could stimulate the microorganisms with similar functions, and the changes of polysaccharide in EPS and SMP were closely related to the membrane fouling rate as well as correlated with some saline-resistance genera.

1. Introduction

Saline wastewater can be produced by many industries such as food processing, canning, petroleum, and petrochemical industries (Sudarno et al., 2011), as well as by seawater infiltration and seawater sewage flushing (Lefebvre and Moletta, 2006). Physical and chemical methods can treat saline wastewater efficiently, but they are extremely expensive. A membrane bioreactor (MBR), combining the activated sludge process and membrane separation, is widely used in wastewater treatment with recognized advantages (Judd, 2010), including small space and reactor requirements, better effluent quality, increased volumetric loadings, and less sludge production (Trouve et al., 2014). The application of MBR treating saline wastewater has been previous studied (Guan et al., 2014; Pendashteh et al., 2011).

However, membrane foulants such as soluble microbial products (SMP) and extracellular polymeric substances (EPS) (Gao et al., 2010; Neyens et al., 2004) often hamper the MBR application. The compositions of SMP and EPS are bacterially polymeric, lysis and hydrolysis products (Huang et al., 2011), as well as polysaccharides, nucleic acids, and proteins (Pan et al., 2010). High salinity or fluctuations in saline wastewater can cause cell plasmolysis and death of microbes, and significantly affect physical and biochemical properties of the biomass, leading to a change of EPS and SMP contents (Vyrides et al., 2010). Jang et al. (2013) investigated the effects of high salinity on the performance and membrane fouling of MBR with saline wastewater, found that high salinity increases the EPS contents, which can impact membrane filtration and fouling properties. Although current literatures show that salinity could change the generation of EPS and SMP and result

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in the changes of membrane fouling, the underlying mechanisms are still unclear.

The EPS and SMP are generated by microbiota in MBR, and salinity could directly change the microbial ecology. Therefore, characterization of microbial ecology might provide insights into how salinity influences membrane fouling. Previous researches just showed that salt could change the MBR fouling (Jang et al., 2013), without reporting relationship among EPS and SMP, as well as microbial ecology. Therefore, the structural changes of microbial communities should be meaningfully characterized. High-throughput sequencing technologies have widened the scope of data generation and allowed us to view this picture in greater detail, which has been widely used to characterize the microbial community and function in wastewater treatment (Yadav et al., 2014; Zhu et al., 2015) and activated sludge (Miao et al., 2015a).

In this study, three MBRs were operated at different salinity levels (0%, 0.75% and 1.5% NaCl). Besides of measuring the basic effluent parameters, including COD, NH_4^+–N, EPS and SMP concentration, the fouling behavior of MBRs was also monitored by measuring the trans-membrane pressure (TMP). The microbial community was analyzed by high-throughput 16S rRNA gene amplicon sequencing on Illumina Mi-Seq platform. The interaction and correlation among fouling rate, SMP and EPS, and microbial community under different salinity levels was studied along with membrane fouling. This study aims to reveal the complete ecology process and the trends of membrane fouling in MBRs after salt shock.

2. Methods

2.1. Membrane bioreactor

Three bench-scale MBRs were set up with a working volume of 13.5 L. The membrane module (PVDF, 0.03 μm, Tengxiang Corp., Hangzhou, China) had an effective filtration area of 0.2 m^2 and a nominal pore size of 0.25 μm. The sludge used in the MBRs was collected from Jiangxinzhou Wastewater Treatment Plant (Nanjing, China). Before usage, the sludge was acclimatized for 2 weeks. In order to better define the underlying mechanisms and phenomena in MBR fouling, a synthetic wastewater simulating municipal wastewater contained glucose (300 mg/L), peptone (50 mg/L), NH_4Cl (114.6 mg/L), and KH_2PO_4 (12 mg/L) as primary nutrients and required trace elements. The three MBRs were set as Control, 0.75% NaCl and 1.5% NaCl groups, in which the NaCl concentrations were 0, 7.5 g/L and 15 g/L, respectively. A balance box with a float-ball valve was used to control the water level in the reactor. Hydraulic retention time (HRT) and solid retention time (SRT) were set to be 5.4 h and 25 d, respectively. The operating flux was 12.5L/m · 2 h. TMP was recorded every 6 h to show the fouling trend. When the TMP reached about 40 kPa, the membrane was extracted and washed. Briefly, the membrane was firstly cleaned with high-pressure water, then soaked in 3% NaClO solution for about 6 h and finally in 3% H_2SO_4 solution for about 4 h.

2.2. Analytical methods

The volatile suspended solids (VSS), and COD were measured by Standard Methods, and NH_4^+–N was analyzed according to Nesslerizaion Method (Miao et al., 2015a). EPS and SMP were extracted using the heat extraction method (Wang et al., 2009). Protein (Pn) and polysaccharide (Ps) were analyzed using the Lowry method (Wang et al., 2014) and the modified Anthrone method (Zhu et al., 2014), respectively. Then the concentrations of Pn and Ps were normalized to VSS as mg/g VSS, respectively. During the operation time, the membrane fouling state was denoted by the value of TMP, which was determined by Chartless Recorder (ZYW130-RG, Zhongyi Electronics Co., Ltd.).

2.3. DNA extraction and PCR

The sludge samples from three MBRs were extracted in week 1, 3, 5, 7, 9. For DNA protection, the sludge was mixed with 100% ethanol immediately at a ratio of 1:1 (v/v). The mixture was centrifuged at 4000 rpm for 10 min under 4 °C to collect the pellets (~20 mg) and to extract DNA by using FastDNA® SPIN Kit for Soil (MP Biomedicals, USA). Concentrations and purity of extracted DNA were determined with microspectrophotometry (NanoDrop® ND-1000, USA).

The extracted DNA samples were amplified with a set of primers targeting the hypervariable V1–V2 region of 16S rRNA gene. The forward primer is 5′-AGAGTTTGATYMTGGCTCAG-3′ and the reverse primer is 5′-TGCTGCCTCCCGTAGGAGT-3′. Adapter and barcode were incorporated between the adapter and forward primers. The PCR amplification was conducted in a 50 μL reaction system containing 2 μL forward primer (10 μM) and 2 μL reverse primer (10 μM), 25 μL 2×EasyTaq® PCR SuperMix (Transgene, Beijing), 40 ng template DNA and 21 μL ddH_2O. The PCR was conducted under following protocol: 98 °C for 5 min; 20 cycles of 98 °C for 30 s, 50 °C for 30 s, 72 °C for 40 s, and a final extension at 72 °C for 10 min. PCR products were purified with TaKaRa MiniBEST DNA Fragment Purification Kit Ver. 4.0 (TaKaRa, Japan).

2.4. High-throughput sequencing and data analysis

High-throughput sequencing was performed with Illumina Miseq sequencer at Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China). After sequencing, Mothur (http://www.mothur.org/) was used to sort sequences exactly matching the specific barcodes into different samples. Then, Sickle tool (https://github.com/najoshi/sickle) was used to perform the quality filtering to remove the reads with averages quality score <20 or with any unknown bases. Then, the reads were assembled by Mothur command 'make.contigs' with the criteria of 'maxambig = 0', 'maxhomop = 8' and 'minoverlap = 10'. The quality-filtered reads were then processed by Mothur with commands 'trim.seq', 'pre.cluster' and 'chimera.uchime' to remove chimera and sequencing noise. In order to compare all the samples at the same sequencing depth, 10,000 reads were randomly extracted from each of the 15 samples. Taxonomic classification of each sample was individually conducted using Ribosomal Database Project (RDP) (http://rdp.cme.msu.edu/) Classifier (version 2.6) with confidence threshold of 50%. 'Aligner' and 'Complete Linkage Clustering' were applied to calculate richness and diversity indices including operational taxonomic units (OTUs), Shannon Index, and Chaos Index (Miao et al., 2015a).

2.5. Statistical analysis

Microbial communities in the 15 samples were clustered based on abundances of genera using the cluster analysis (CA). Correlation analysis between measured variables and sludge microbial community was performed using canonical correspondence analysis (CCA). The Pearson Indices (R) and Significant Correlation (P) of the correlations among different parameters were calculated by R (v.3.0.2). CCA identifies an environmental basis for community ordination by extracting the maximum relationship between community composition and measured environmental variables. Both CA and CCA were performed with R (v.3.0.2).