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Mechanisms of microbial community structure and biofouling shifts under multivalent cations stress in membrane bioreactors

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HIGHLIGHTS

- Trivalent metal induced longer recovery on removal performance than bivalent metal.
- Trivalent metal cations reduced trans-membrane pressure by binding EPS and SMP.
- Fe(III)-tolerance genera were positively correlated and interacted with SMP.
- Inhibited genera and binding bridge reduced membrane fouling under Ca(II) stress.
- Microorganisms and EPS/SMP interacted and affected with each other.

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ABSTRACT

Five lab-scale membrane bioreactors (MBRs) were continuously operated to investigate the mechanisms and linkages of the microbial community and membrane fouling with trivalent metal cations (Fe(III) and Al(III)) and bivalent metal cations (Ca(II) and Mg(II)) shock loads. COD and NH_4^+-N removals showed recovery trends along with treatment process in the presence of metals. Trivalent metal cations reduced trans-membrane pressure (TMP) as well as fouling rate (dTMP/dt) and extended membrane module replacement period by binding activated sludge extracellular polymeric substance (EPS) and effluent soluble microbial product (SMP) productions. Illunima sequencing of 16S rRNA gene showed that metal stress stimulated specific metal-tolerance bacteria in the MBRs. Canonical correspondence analysis indicated that EPS and SMP made different contributions to the distribution of microbial community structure in Fe(III) and Al (III) systems, respectively. Under bivalent metal conditions, microbial community shifts and Ca(II) binding bridge worked together to inhibit EPS and SMP, while filamentous bacteria stimulated by Mg(II) that mainly controlled membrane fouling. This study has shown that the comparison of tri- and bivalent metals for membrane fouling control with binding bridge and functional microorganisms can provide a strategy for practical membrane bioreactor applications.

1. Introduction

Transition and alkali metal cations are ubiquitous in wastewater due to their diverse range of sources [1], including industrial activities, traffic-related emissions, small business activities, domestic effluents and other chemical treatment processes [2]. The effects of metal cations on conventional activated sludge processes have been extensively studied. However, contradictory results were reported that the low-level metals can stimulate microbial growth [3], while high concentrations of metals could actually inhibit growth [1] by altering enzyme conformation and blocking essential functional groups [4]. Membrane bioreactors (MBRs) could combine activated sludge process and membrane separation with advantages of small space requirements, better effluent quality, increased volumetric loadings, and less sludge production [5]. Thus, MBRs have been successfully applied as secondary treatment in wastewater treatment plants (WWTPs) for both industrial and municipal wastewater [6]. Meanwhile, the sludge mixture in MBRs could be affected by the uncertain effects of hardness (Ca(II) and Mg(II)) and hydrolysis (Fe(III) and Al(III)) cations in such wastewater [7], which is practical but rare to evaluate the performance when MBRs are subjected to metal cation loads [8].

Membrane fouling, an indicator of MBR biological performance [9], is also the concern when MBRs are employed to treat metal con-

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tained wastewater, and represents a major limitation in full-scale applications for WWTPs [10]. Metal cations could induce flocculent products and inorganic fouling in MBRs [11], which contribute more foulants than biopolymers [12]. Hardness metal cation Ca(II) was reported to enrich membrane surface microorganism as well as extracellular polymeric substance (EPS) and promote biological flocculation [13], while hydrolysis metal cations Fe(III) and Al(III) could reduce the concentration of soluble microbial product (SMP) in effluent [14]. Thus, it is necessary to identify the mechanisms of different multivalent metal cations on MBR sludge filterability and membrane separation processes for understanding pollution processes and controlling MBR mixture properties.

Additionally, microbial community structures and functions could also be influenced by metal cations, which could further impact ESP and SMP as well as MBR performance [15]. However, few studies have been conducted to explores the efficiency of cations on membrane fouling and removal efficiency by characterizing the evolution of microbial community. Recently, metagenomic analysis based on Illumina Miseq high-throughput sequencing has been widely applied for microbial community analysis due to its characteristics of low cost, adequate sequencing depth, high accuracy, and great coverage [4]. In addition, high-throughput technologies are increasingly used to explore microbial community evolution and physicochemical characteristics in membrane bioreactors [16], wastewater treatment plants [17], and metal-containing industrial wastewater [4].

It is generally agreed that multivalent metal cations are associated with the biofouling process and sludge characteristics as well as microbial community structures. However, the specific mechanisms of how microbial community and biofouling change and correlate with each other over time in the presence of hardness and hydrolysis metal cations are still unknown. This study aims to link microbial community, biofouling process and multivalent metal cations stress by using metagenomics and biostatistics analyses. Additionally, the results could shed lights onto regulation and optimization of membrane treatment processes of wastewater containing hardness and hydrolysis metal cations, as well as advance our understanding and engineering of molecular mechanisms during ecological evolution in MBRs.

2. Materials and methods

2.1. MBRs operation and sample collection

Five pilot-scale MBRs with a working volume of 13.5 L were used to treat synthetic simulating municipal wastewater [18]. Polyvinylidene fluoride (PVDF) membrane module (Tengxiang Corp., Hangzhou, China) were employed with an effective filtration area of 0.2 m^2. The seeding activated sludge used in MBRs was collected from Jiangxinzhou Wastewater Treatment Plant (Nanjing, China). The MBRs were controlled to acclimatize activated sludge with increasing influent COD from 0 to 300 mg/L during start-up period. The optimal parameters were finally set at pH of 7.0–7.3, hydraulic retention time (HRT) of 5.4 h and solid retention time (SRT) of 25 d.

After start-up period, the five MBRs were continuously operated for 60 days, divided into 5 phases with 12 days per phase. The actual wastewater is more complex than synthetic wastewater, where a lot of other components influence sludge growth, characteristics and behaviors. But in this study, we focus on effect of multivalent metals on MBRs, thus the synthetic wastewater was used to highlight the important roles of metal cations. The influent of each MBR contained different types of metal salts, $CaCl_2$, $MgCl_2$, $FeCl_3$ and $Al_2(SO_4)_3$ with the exposure concentration at 200, 200, 750 and 216 mg/L, respectively. During the operation, chemical membrane cleaning was conducted when the trans-membrane pressure (TMP) was over 40 kPa [19] by immersing the modules into a NaOCl solution (1000 mg/L Cl_2) for 6 h and then in 3% sulfuric acid solution for 8 h [2,6]. After the cleaning process, the membrane modules were continuously applied into the next phase.

The activated sludge samples of five MBRs were collected at the end of each phase, and divided into two parts, one for EPS analysis, and the other for genomic DNA extraction. The second part of activated sludge were mixed with 100% ethanol immediately at a ratio of 1:1 (v/v) for DNA protection. The effluents were collected in every other days.

2.2. Chemical analyses

Laboratory analyses were conducted to determine the characteristics of the effluent wastewater, activated sludge and permeate. Among them, COD and NH_4^+-N were measured by using microwave digestion method and Nesslerizaion spectrophotometry method, respectively [4]. EPS of activated sludge and SMP of effluent were extracted using a heat extraction method [11]. Specifically, activated sludge was washed in a buffer solution, then transferred to an extraction vessel with rubber cap. Polysaccharide (Ps) and protein (Pn) were selected as indicators for EPS and SMP, and were measured by using phenol-sulfuric acid method with glucose as standard and Folin method with bovine serum albumin (BSA) as a standard, respectively [20]. The concentrations of Ps and Pn in EPS of activated sludge were normalized to VSS as mg/g VSS [18], respectively. In each run, the membrane fouling rate was indicated by the value of TMP, which was measured by a Chartless Recorder (ZYW130-RG, Zhongyi Electronics Co., LTD.).

2.3. DNA extraction and Illumina Miseq sequencing

The mixture of activated sludge was centrifuged at 4000 rpm for 10 min at 4 °C to collect the pellets (~20 mg) 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 was 5′-AGAGTTTGATYMTGGCTCAG-3′ and the reverse primer was 5′-TGCTGCCTCCCGTAGGAGT-3′. Barcodes and adapters 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). About 500 ng of purified PCR product for each sample was mixed and sent to Jiangsu Zhongyijinda Analytical & Testing Co., Ltd (Jiangsu, China) for Illumina Miseq sequencing. The raw data has been submitted to NCBI Sequence Read Archive (SRA) with accession number of SRR2086960.

2.4. Data and bioinformatics analysis

After sequencing, we used Mothur (http://www.mothur.org/) to sort sequences exactly matching the specific barcodes into different samples following commands ‘fastq.info’, ‘trim.seqs’ and ‘make.fastq’. Then, the separated forward and reverse reads were merged by using the commands ‘make.contigs’. Initial denoising step was to reduce sequencing and PCR errors with command