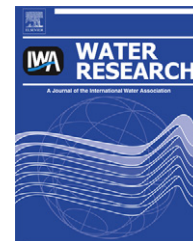


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# Correlating microbial community structure and composition with aeration intensity in submerged membrane bioreactors by 454 high-throughput pyrosequencing

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## ABSTRACT

For understanding of the microbial community structure and composition under different aeration intensities, 454 high-throughput pyrosequencing was applied to analyze the 16S rRNA gene of bacteria in two submerged membrane bioreactors (MBRs) under low ( $R_L$ ) and high aeration ( $R_H$ ) conditions. In total, 7818 ( $R_L$ ) and 9353 ( $R_H$ ) high-quality reads were obtained, and 1230 ( $R_L$ ) and 924 ( $R_H$ ) operational taxonomic units (OTUs) were generated at 3% cutoff level, respectively. 454 pyrosequencing could also reveal the minority bacteria that were hardly detected by the conventional molecular methods. Although the core populations were shared with highly functional organization (>80%), clear differences between the samples in the two MBRs were revealed by richness–diversity indicators and Venn analyses. Notably, microbial diversity was decreased under high aeration condition, and the evolution of the populations was observed mainly in the shared OTUs. Moreover, specific comparison down to the class and genus level showed that the relative abundances of  $\beta$ -Proteobacteria and  $\gamma$ -Proteobacteria in the  $R_H$  community were respectively decreased by 41.5% and 66.6%, consistent with the observed membrane fouling mitigation during the reactor operation. It was also found that *Nitrospira* and *Nitrosomonas*, being nitrite oxidizing bacteria (NOB) and ammonium oxidizing bacteria (AOB), were the dominant phylogenetic groups at the genus level of both reactors, and that the high ratio of NOB to AOB populations well supported the complete ammonium oxidation performance in the two reactors. Although some populations of NOB and AOB decreased with the increase of aeration intensity, the functional stability of the nitrification process was less affected, probably due to the low influent substrate concentration and the high level of functional organization.

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## 1. Introduction

In recent decades, submerged membrane bioreactor (MBR) technology has gained attention and popularity worldwide in water and wastewater treatment (Judd, 2008; Wang et al.,

2008). The submerged MBR process is considered as an innovative integration of conventional activated sludge (CAS) system and membrane separation process, thus enabling the independent control of sludge retention time (SRT) and hydraulic retention time (HRT) and retaining a high biomass

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concentration in the compact reactors. Compared to the CAS system, submerged MBRs have the distinctive advantages including a reduced footprint, a decreased sludge yield coefficient and a high-quality effluent (Calderon et al., 2012; Meng et al., 2009, 2010; Wang et al., 2009a).

Since the origin of the submerged MBR, aeration has been widely used to provide oxygen transfer into the liquid phase, but also to mitigate fouling by inducing a cross-flow velocity along the membrane surfaces (Delgado et al., 2008). An appropriate level of turbulent shear was conducive to the filtration process by preventing cake layer formation and membrane pore clogging (Verrecht et al., 2008). In the meantime, high shear conditions decreased the floc size, resulting in the increase of dispersed growth (Cicek et al., 1999) and the increase of dissolved organic matter (DOM) concentration through erosion of bound extracellular polymeric substances (EPS) (Menniti et al., 2009; Menniti and Morgenroth, 2010; Wang et al., 2009b), which consequently deteriorated the MBR performance (Meng et al., 2009; Wang et al., 2009a). In addition, aeration is reported to be the most expensive factor in terms of energy consumption and approximately 50% of the energy demand in an MBR arises from aeration (Gil et al., 2010). Therefore, a comprehensive investigation to optimize the aeration intensity is helpful for controlling membrane fouling, improving MBR performance and reducing energy demand.

To date, most related studies have focused on addressing the influence of aeration intensity on flow regime and mass transfer (Delgado et al., 2008; Zhang et al., 2009), membrane permeability and biomass characteristics (e.g., floc size and DOM production) (Menniti et al., 2009; Menniti and Morgenroth, 2010; Wang et al., 2009b), and energy balance as well in submerged MBRs (Gil et al., 2010; Verrecht et al., 2008). Further attempts were conducted to evaluate the impacts of these factors on submerged MBR performance. For example, Meng et al. (2008) reported that aeration had a positive effect on cake layer removal while the heterogeneity of sludge supernatant was increased under high aeration conditions, which caused severe pore blocking. Recently, it has been observed that aeration intensity had a significant influence on predatory organisms (Menniti and Morgenroth, 2010; Wang et al., 2011). Menniti and Morgenroth (2010) found that *Aeolosoma hemprichi* emerged under lower shear conditions, and that their predation resulted in the increase of soluble microbial products (SMP) concentrations. In submerged MBR process, we hypothesize that the shear stress and dissolved oxygen (DO) concentration induced by different aeration intensity will influence the microbial community composition rather than merely the metazoan organisms (e.g., *A. hemprichi*). However, so far, the investigations performed to specify how the aeration intensity affects the microbial community structures have been based on the conventional molecular biology methods (e.g., PCR-DGGE) in submerged MBR systems. These methods enable the detection of abundant microbial species in function, but lack sufficient sequences to capture comprehensive and systematic information for analyzing complete community structures (Calderon et al., 2012; Hu et al., 2012; Zhang et al., 2012), which likely accounted for the contradiction of the results of previous studies. For instance, Ma et al. (2006) correlated the

microbial dynamics with the variation of DO in an anoxic/oxic MBR and a membrane-coupled sequencing batch reactor and found a significant difference in microbial communities between the two reactors, while Gao et al. (2011) reported the microbial community under high DO (4.0 mg/L) exhibited a great similarity (67%) with that under moderate DO condition (2.0 mg/L), including *Deltaproteobacteria*, *Betaproteobacteria*, *Dechloromonas*, *Rhodocyclus*, *Comamonas*, *Nitrospira* and some unidentified microorganisms. No more than 40 clones were sampled in these microbial community surveys (Gao et al., 2011; Ma et al., 2006), and the species of low abundance could not be detected efficiently. Besides, one band may contain more than one species in these PCR-DGGE tests. In view of these drawbacks of the conventional molecular biology methods, more sensitive detection is needed to further investigate the impacts of the aeration intensity on microbial community structure and composition in submerged MBRs.

Pyrosequencing developed by Roche 454 Life Science (Branford, CT, USA) is a high-throughput analytical method that can generate a large amount of DNA data through a massively parallel sequencing-by-synthesis approach (Margulies et al., 2005), and more than thousands of operational taxonomic units (OTUs) could be identified to investigate the microbial diversity in various environmental samples (Hu et al., 2012; Lu et al., 2012; Ye et al., 2011; Zhang et al., 2012). Pyrosequencing can provide significant insights into the evolution of microbial community resulting from the variation of aeration intensities in submerged MBR systems.

The overarching goal of this study is, therefore, to characterize and compare the bacterial communities under different aeration conditions in submerged MBRs. 454 high-throughput pyrosequencing was used to analyze the 16S rRNA gene of bacteria and to investigate their community structure and population composition under different aeration intensities.

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## 2. Materials and methods

### 2.1. Experimental setup and operating conditions

Two identical lab-scale submerged MBRs (working volume of 26 L each) under high ( $R_H$ ) and low ( $R_L$ ) aeration conditions were constructed to treat low-strength wastewater, and the characteristics of the influent wastewater are listed in Table 1A.

Each MBR tank was divided into a riser zone and two down-comer zones by two baffle plates, which could enhance the recirculation of mixed liquor and thus increase the cross-flow velocity (CFV) according to the theory of internal-loop-airlift reactor (Liu et al., 2003). Air diffuser was placed at the bottom of the riser zone to aerate the mixed liquors and induce a CFV along membrane surfaces. Due to the rapid recirculation of mixed liquors between the riser and down-comer zones, a relatively similar DO level was maintained in the whole reactor. Two 40 cm × 30 cm flat-sheet membrane modules (PVC, 0.40 μm, Kubota Corporation, Japan) were mounted vertically in the riser zone between the baffle plates. The membrane area of each module was 0.216 m<sup>2</sup>. The

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