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Effects of salinity build-up on the performance and bacterial community structure of a membrane bioreactor

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HIGHLIGHTS

- Salinity increase in the bioreactor affected MBR biological performance.
- Elevated salinity did not reduce microbial diversity in the bioreactor.
- Bacterial community in MBR could adapt to the elevated salinity condition.
- Bacterial succession could facilitate the recovery of MBR biological performance.

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ABSTRACT

This study investigated the effects of salinity increase on bacterial community structure in a membrane bioreactor (MBR) for wastewater treatment. The influent salt loading was increased gradually to simulate salinity build-up in the bioreactor during the operation of a high retention-membrane bioreactor (HR-MBR). Bacterial community diversity and structure were analyzed using 454 pyrosequencing of 16S rRNA genes of MBR mixed liquor samples. Results show that salinity increase reduced biological performance but did not affect microbial diversity in the bioreactor. Unweighted UniFrac and taxonomic analyses were conducted to relate the reduced biological performance to the change of bacterial community structure. In response to the elevated salinity condition, the succession of halophobic bacteria by halotolerant/halophilic microbes occurred and thereby the biological performance of MBR was recovered. These results suggest that salinity build-up during HR-MBR operation could be managed by allowing for the proliferation of halotolerant/halophilic bacteria.

1. Introduction

Water scarcity, exacerbated by climate change, population growth, and industrialization, has accelerated the use of alternative water sources, including reclaimed water (Shannon et al., 2008). Wastewater reclamation also effectively addresses environmental pollution. Thus, many dedicated attempts have been made to develop robust and highly efficient technologies, such as membrane bioreactor (MBR), for wastewater treatment and reuse (Melin et al., 2006). MBR integrates activated sludge treatment with membrane separation processes, such as microfiltration (MF) or ultrafiltration (UF). Compared with conventional activated sludge treatment, MBR has several advantages, including higher effluent quality, lower sludge production, and smaller physical footprint (Hai et al., 2014).

Further development of MBR has recently led to the concept of high retention-membrane bioreactor (HR-MBR) (Lay et al., 2010; Luo et al., 2014). Currently, there are three HR-MBR variations, namely osmotic membrane bioreactor (OMBR) (Achilli et al., 2009; Nawaz et al., 2013; Nguyen et al., 2015), membrane distillation bioreactor (Phattaranawik et al., 2008; Wijekoon et al., 2014), and nanofiltration membrane bioreactor (NF-MBR) (Choi et al., 2002, 2006). In these systems, the forward osmosis, membrane distillation, and nanofiltration membranes are utilized to extract treated water from the bioreactor mixed liquor. By employing these high retention membrane processes, the HR-MBR systems can potentially produce high quality water, particularly for regions

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facing severe freshwater scarcity and with stringent environmental regulations.

A major challenge to the development of HR-MBR is to manage salinity build-up in the bioreactor. High retention membranes can effectively reject inorganic salts, resulting in their accumulation or build-up in the bioreactor during HR-MBR operation (Lay et al., 2010). High salinity can also occur in the case of a conventional MBR due to seawater intrusion or during the treatment of highly saline wastewaters from seafood processing or the dairy industry (Reid et al., 2006).

It is well established that an elevated salinity condition can adversely affect MBR performance. Reid et al. (2006) observed that an increase in bioreactor salinity to 5 g/L sodium chloride (NaCl) increased the concentrations of soluble microbial products and extracellular polymeric substances in the mixed liquor and thus severely reduced the membrane permeability. Yogalakshmi and Joseph (2010) reported a reduction in biological performance as the bioreactor salinity increased. Jang et al. (2013) and Hong et al. (2013) subsequently attributed the reduced biological performance to the change of bacterial community structure in the highly saline environment of the bioreactor. Evidence of bacterial changes in response to the elevated salinity has also been reported by Qiu and Ting (2013) who investigated microbial community dynamics during OMBR operation. In these studies, denaturing gradient gel electrophoresis (DGGE) was applied to elucidate microbial response to the increase in bioreactor salinity. It is noteworthy that DGGE is a fingerprinting method and can only provide information of abundant microbial species (Boon et al., 2002). Moreover, crowding of DGGE bands due to identical positions of some bacteria in the gel may underestimate microbial diversity (Nübel et al., 1999; Choi et al., 2007).

In this study, high-throughput 454 pyrosequencing was used to systematically investigate impacts of salinity increase on the bacterial community structure of a conventional MBR equipped with an MF membrane. Basic performance of the MBR with salinity increase was also evaluated in terms of contaminant removal. The increase in bioreactor salinity simulated here was relevant to the range often encountered in HR-MBR operation. Thus, the results provide unique insight to the management of salinity build-up in the bioreactor during HR-MBR operation.

2. Methods

2.1. Experimental system and operational protocol

Two identical lab-scale MBR systems were used in this study. Detailed description of the MBR systems is available elsewhere (Luo et al., 2015). Briefly, each MBR system comprised a feed reservoir, an aerobic bioreactor and a submerged hollow fiber MF membrane module made of polyvinylidene fluoride (Mitsubishi Rayon Engineering, Tokyo, Japan). The MF membrane module had an effective surface area and a nominal pore size of 740 cm^2 and 0.4 μm, respectively. A Masterflex peristaltic pump (Cole-Parmer, Vernon Hills, IL) controlled by a computer was used to extract treated water through the MF membrane in a cycle of 14 min suction and 1 min off.

Activated sludge collected from the Wollongong Wastewater Treatment Plant (Wollongong, New South Wales, Australia) was acclimatized in the two MBR systems under the same conditions. A synthetic wastewater (Supplementary Information, Table S1), simulating medium strength municipal sewage, was used as the MBR influent. The mixed liquor suspended solids (MLSS) concentration in the two bioreactors was maintained at approximately 5 g/L by regular sludge wastage, which corresponded to a sludge retention time (SRT) of 50 days. The hydraulic retention time (HRT) was maintained at 24 h. The bioreactors were continuously aerated to maintain a mixed liquor dissolved oxygen (DO) concentration of approximately 5 mg/L. The bioreactor temperature was maintained at 26 ± 1 °C using a temperature-controlled water bath.

Once acclimatized in terms of bulk organic removal (i.e. over 97% total organic carbon (TOC) removal), the salinity of the influent to one of the MBRs (denoted "saline-MBR") was increased by raising the NaCl loading from 0 to 16.5 g/L with a gradient of 0.5 g/L day (Supplementary Information, Fig. S1). The range of salinity build-up simulated here was similar to that would occur during normal OMBR operation (Supplementary Information, Appendix A). To allow microbial adaptation to the highly saline condition, the influent NaCl loading was maintained at 10 and 16.5 g/L for 14 and 25 days, respectively. Therefore, the saline-MBR was continuously operated for 70 days (excluding the acclimatization period). Another MBR system (denoted "control-MBR") was operated concurrently under identical conditions, but without any increase in the influent salinity.

Mixed liquor samples were collected from the two MBR systems for microbial analysis on days 0, 33, 43, and 70 of the experiment, corresponding to 0, 10, 15 and 16.5 g/L NaCl loading in the saline-MBR.

2.2. Microbial community analysis

2.2.1. DNA extraction and 454 sequencing

Genomic DNA was extracted from all mixed liquor samples using the FastDNA® SPIN Kit for soil (MP Biomedicals, Santa Ana, CA). The integrity, purity and concentration of the extracted DNA were evaluated by electrophoresis in a 1% (w/v) agarose gel and the NanoDrop® ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE).

DNA samples were stored at −20 °C and then shipped to the Australian Genome Research Facility (Brisbane, Queensland, Australia) for amplicon pyrosequencing using a standard Roche 454/GS-FLX platform. Bacterial domain was targeted by selecting V1–V3 regions of the 16S rRNA genes with primers 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 519R (5′-GWATTACCGCGGCKGCTG-3′).

2.2.2. Sequence analysis

Raw pyrosequencing data were analyzed using the Quantitative Insights into Microbial Ecology software (QIIME 1.9.1) (Caporaso et al., 2010a). By using the "split_libararies.py" script, we removed defective sequences that contained ambiguous bases, had errors in the barcode or primer, a length outside the range 200–1000 nt, homopolymers greater than 6 nt, or an average quality score less than 25. The remaining sequences were denoised using the "den oise_wrapper.py" script and then clustered into operational taxonomic units (OTUs) using the GreenGenes 16S rDNA database with *uclust* based on the similarity of 97% (Edgar, 2010). OTUs containing less than two sequences (i.e. singletons) were excluded from the downstream analysis. The representative sequence of each OTU was aligned to the GreenGenes 16S rDNA database using *PyNAST* (Caporaso et al., 2010b). Chimeric sequences were identified by *ChimeraSlayer* (Haas et al., 2011) and subsequently removed from the OTUs using a python script. A Netwick formatted phylogenetic tree was constructed by employing *FastTree* (Price et al., 2010).

Both α- and β-diversity metrics were determined using a default setting in QIIME based on the even sequencing depth of 13,000 (i.e. the lowest sequences of each sample) to avoid the heterogeneity related to different sequencing depths. Specifically, α-diversity metrics included Chao1, Shannon index, and phylogenetic diversity, and β-diversity were indicated by the UniFrac distance metrics. Principal coordinate analysis (PCoA) and unweighted pair group method with arithmetic mean were used