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Biodiversity and population dynamics of microorganisms in a full-scale membrane bioreactor for municipal wastewater treatment

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ABSTRACT

The total, ammonia-oxidizing, and denitrifying Bacteria in a full-scale membrane bioreactor (MBR) were evaluated monthly for over one year. Microbial communities were analyzed by denaturing gradient gel electrophoresis (DGGE) and clone library analysis of the 16S rRNA and ammonia monooxygenase (*amoA*) and nitrous oxide reductase (*nosZ*) genes. The community fingerprints obtained were compared to those from a conventional activated sludge (CAS) process running in parallel treating the same domestic wastewater. Distinct DGGE profiles for all three molecular markers were observed between the two treatment systems, indicating the selection of specific bacterial populations by the contrasting environmental and operational conditions. Comparative 16S rRNA sequencing indicated a diverse bacterial community in the MBR, with phylotypes from the α- and β-Proteobacteria and Bacteroidetes dominating the gene library. The vast majority of sequences retrieved were not closely related to classified organisms or displayed relatively low levels of similarity with any known 16S rRNA gene sequences and thus represent organisms that constitute new taxa. Similarly, the majority of the recovered *nosZ* sequences were novel and only moderately related to known denitrifiers from the α- and β-Proteobacteria. In contrast, analysis of the *amoA* gene showed a remarkably simple ammonia-oxidizing community with the detected members almost exclusively affiliated with the *Nitrosomonas oligotropha* lineage. Major shifts in total bacteria and denitrifying community were detected and these were associated with change in the external carbon added for denitrification enhancement. In spite of this, the MBR was able to maintain a stable process performance during that period. These results significantly expand our knowledge of the biodiversity and population dynamics of microorganisms in MBRs for wastewater treatment.

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1. Introduction

Submerged membrane bioreactors (MBR) combine efficient biological treatment with membrane separation and are now widely accepted as an advanced technology for obtaining high-quality effluent. This process offers several important advantages over conventional wastewater treatment systems, including high biodegradation capacity and efficiency, excellent permeate quality and low sludge production. As more stringent effluent standards are expected and the costs of membrane and membrane process continue to fall, the applications of MBR in municipal and industrial wastewater treatment are becoming increasingly widespread around the globe (Judd, 2007).

The bacterial communities present in active biomass in activated sludge mixed liquor represent the core component of every MBR for biological carbon and nutrient removal. While bacteria in wastewater treatment plants (WWTP) have been intensively studied by culture-dependent methods (Ueda and Earle, 1972) and nucleic acid-based molecular approaches (Bond et al., 1995; Nogueira et al., 2002; Wagner and Loy, 2002), surprisingly little research has been conducted to explore the influence of membrane separation and operating conditions on the overall microbial community structure and diversity of the MBR. Consequently, much of what is known or assumed concerning biological processes in MBRs has primarily come from investigations of conventional activated sludge (CAS) systems, regardless of the fact that significant differences in operating conditions exist between the two treatment processes. Importantly, MBRs are typically operated at high sludge concentrations and low food-to-microorganism (F/M) ratios. Consequently, since energy supply is limited, the microorganisms would preferentially use the carbon sources to satisfy their maintenance energy demands as opposed to biomass growth (Muller et al., 1995; Low and Chase, 1999). In addition, other contrasting operational aspects of MBRs, including longer sludge retention time (SRT), shorter hydraulic retention time (HRT) and shear forces, would also have an impact on the activated sludge communities. To date, denaturing gradient gel electrophoresis (DGGE)-based community structure analysis has indicated that the bacterial communities in pilot-scale MBRs fed with raw sewage were distinct from that in the CAS process (Luxmy et al., 2000), while another investigation (through fluorescent *in situ* hybridization, FISH) has revealed minor differences in the nitrifying community composition between parallel-running MBR and CAS pilot systems (Manser et al., 2005). In addition, pilot studies monitoring long-term community structure changes have demonstrated diverse and dynamic bacterial populations in MBRs for graywater (Stamper et al., 2003) and municipal wastewater (Miura et al., 2007) treatment even during periods of stable operation. More recently, Huang et al. (2008) have revealed by 16S rRNA clone library analysis that novel members of the Bacteria domain are ecologically significant in laboratory-scale municipal wastewater treatment MBRs operated under different conditions. These pioneering works highlight the need for exploring the microbial community composition and diversity in these relatively new biological wastewater treatment systems. To our knowledge, there have been no molecular microbial diversity surveys of full-scale MBR systems for municipal wastewater treatment, and seasonal variations of these diverse communities in relation to process performance remain little known.

We hypothesized that the contrasting operational and environmental conditions of full-scale MBRs as opposed to CAS systems will have great impact on the physiological state and bacterial community structure and population dynamics of mixed liquor. Our study site (in a European country) represents a unique and ideal WWTP at which to examine and compare the microbial community composition, since there is an MBR and a CAS system running in parallel treating the same municipal wastewater and the MBR was originally inoculated with activated sludge from the CAS process. We used DGGE fingerprinting technique to examine how the structure of bacterial populations varied seasonally in both environments. Clone library analysis of phylogenetic and functional markers provided the first detailed molecular look at the composition and diversity of total community and ammonia- oxidizing and denitrifying Bacteria in a full-scale municipal wastewater treatment MBR. Additionally, we examined the impact of changes in MBR operating conditions on the key microbial groups when external carbon sources for the stimulation of denitrification were switched from one to another during the study period. Significant shifts in total bacteria and denitrifying community were observed with some of the major shifting bands in the 16S rRNA gene DGGE profiles corresponding to microorganisms capable of denitrification.

2. Materials and methods

2.1. *Full-scale MBR*

The submerged MBR (6520 m^3/d in capacity) was constructed in 2003 as an extension of the existing CAS system to comply with a more stringent effluent regulation and an increase in load. It consists of a denitrification compartment where pretreated municipal wastewater is introduced, a nitrification compartment, and a filtration compartment where activated sludge is retained by submerged hollow-fiber microfiltration membrane modules (Zenon, ZeeWeed, total membrane surface area 10,160 m^2 and nominal pore diameter 0.03 μm). The compartments are well-mixed either by continuous stirring (denitrification) or aeration (nitrification and filtration). The MBR is operated at a constant flow of 230 m^3/h, with the remaining flow (ranging between 0 and 1440 m^3/h, with an average value of 418 m^3/h) being treated in the parallel CAS system. Detailed set-up and operational information of the full-scale MBR was given elsewhere (Fenu et al., 2010). Initially, acetate was added to the denitrification compartment to enhance denitrifying activity. This carbon source was then changed to butyrate on December 5 2006. Subsequent switches of carbon source between butyrate and acetate occurred on April 4 and in the end of May 2007 (Fig. 1).

In contrast to the MBR, the CAS process has no denitrification zones. Pretreated wastewater is introduced directly to the aeration compartments. Separation of treated water from