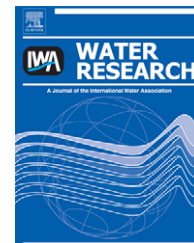


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A comparison of bacterial populations in enhanced biological phosphorus removal processes using membrane filtration or gravity sedimentation for solids–liquid separation

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ABSTRACT

In an earlier phase of this study, we compared the performances of pilot scale treatment systems operated in either a conventional enhanced biological phosphorus removal (CEBPR) mode, or a membrane enhanced biological phosphorus removal (MEBPR) mode. In the present investigation, we characterized the bacterial community populations in these processes during parallel operation with the same municipal wastewater feed. The objectives of the study were (1) to assess the similarity of the bacterial communities supported in the two systems over time, (2) to determine if distinct bacterial populations are associated with the MEBPR and CEBPR processes, and (3) to relate the dynamics of the community composition to changes in treatment process configuration and to treatment process performance. The characteristics of the bacterial populations were first investigated with ribosomal intergenic spacer analysis, or RISA. To further understand the bacterial population dynamics, important RISA phylotypes were isolated and identified through 16S RNA gene sequencing.

The parallel MEBPR and CEBPR systems developed bacterial communities that were distinct. The CEBPR community appeared to exhibit greater diversity, and this may have been the primary reason why the CEBPR treatment train demonstrated superior functional stability relative to the MEBPR counterpart. Moreover, the more diverse bacterial population apparent in the CEBPR system was observed to be more dynamic than that of the MEBPR process.

Several RISA bands were found to be characteristic of either the membrane or conventional biological system. In particular, the MEBPR configuration appeared to be selective for the slow-growing organism *Magnospira bakii* and for the foam-associated *Microthrix parvicella* and *Gordonia* sp., while gravity separation led to the washout of *M. parvicella*. In both pilot trains, sequence analysis confirmed the presence of EBPR-related organisms such as *Accumulibacter phosphatis*. The survey of the CEBPR system also revealed many uncultured organisms that have not been well characterized. The study demonstrated that a simple replacement of a secondary clarifier with membrane solids–liquid separation is sufficient to shift the composition of an activated sludge microbial community significantly.

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1. Introduction

The substitution of membrane bioreactor (MBR) technologies for conventional activated sludge processes can result in a number of important differences in the characteristics of the resulting treatment process. For example, when a traditional secondary clarifier is replaced with membrane filtration for solids–liquid separation, the following changes might be expected to occur: (1) all microbial species will be retained in the bioreactor irrespective of their settling properties, such that all microorganisms will be subjected to the same solids residence time that is governed by the sludge wasting rate; (2) colloids and macromolecules retained by the membrane will be subjected to a residence time that is equivalent to the solids retention time (SRT) rather than to the hydraulic retention time (HRT) (Ben Aim and Semmens, 2003); (3) the formation of microbial flocs may be hindered by the strong coarse-bubble aeration or the high shear environment that is often employed to reduce membrane fouling, resulting in a biomass particle size distribution that is finer than that in a conventional suspended growth system (Cicek et al., 1999). Significant process changes such as these have the potential to impact the microbial community composition in a biological treatment system, which may in turn, have important consequences for the performance of the overall system.

In an earlier phase of the present study, we compared the performances of pilot scale treatment systems operated in either a conventional enhanced biological phosphorus removal (CEBPR) mode, or a membrane enhanced biological phosphorus removal (MEBPR) mode. Among the observations reported, we noted that, for the MEBPR system, the absence of a secondary clarifier with an anoxic sludge blanket, caused a reduction of the denitrification capacity of the system and a lower overall biomass yield (Monti et al., 2006, 2007). Even more significantly, the maximum specific nitrification rate in the membrane process was observed to be significantly lower than that of a conventional, suspended growth EBPR process operated in parallel (Monti and Hall, 2008). These fundamental differences suggested to us that the presence of a membrane filtration module can indeed, favour the establishment of a microbial community with a distinct composition relative to that of a parallel, conventional system.

Whereas considerable research effort has been devoted to the behaviour of membrane filtration units in MBR systems (e.g. Fan and Zhou, 2007; Akram and Stuckey, 2008; Al-Halbouni et al., 2008), the microbiological aspects of membrane bioreactors remain largely unexplored. A few recent studies have attempted to compare the microbial community compositions in MBR and conventional systems, indicating that significant differences in the relative abundance and physiological state of the microorganisms do exist (Luxmy et al., 2000; Witzig et al., 2002; Gao et al., 2004). However, the operating conditions of the parallel systems used in these studies were far from identical. Although the previous results clearly contribute to our understanding of the microbiology of membrane bioreactors, only limited conclusions can be drawn regarding the direct impact of membrane filtration on the retained microbial population. In particular, it is not yet clear whether the observed differences in

performance of conventional and membrane systems (Monti et al., 2006) are actually accompanied by differences in microbial community composition. Further, the long-term community composition dynamics have not been compared in systems of these types under well-controlled experimental conditions.

In the present investigation, we characterized the bacterial community populations in parallel membrane and conventional EBPR processes that were operated under identical conditions and with the same municipal wastewater feed. The objectives of the study were (1) to assess the similarity of the bacterial communities supported in the two systems over time, (2) to determine if distinct bacterial populations are associated with the MEBPR and CEBPR processes, and (3) to relate the dynamics of the community composition to changes in treatment process configuration and to treatment process performance. The characteristics of the bacterial populations were first investigated with ribosomal intergenic spacer analysis, or RISA (Gürtler and Stanisich, 1996; Yu and Mohn, 2001), a molecular tool which differentiates microorganisms present in a population by analyzing the length heterogeneity of the ribosomal intergenic spacer region between the small (16S) subunit and large (23S) subunit rRNA genes. To further understand the bacterial population dynamics, important RISA phylotypes were identified through 16S RNA gene sequencing.

2. Materials and methods

2.1. Description of the pilot plant

The present study was conducted at The University of British Columbia (UBC) pilot plant, which operates with a municipal wastewater feed. The UBC pilot plant is a dual train facility that initially was operated for a comparative study of enhanced biological phosphorus removal processes operated in the conventional mode (CEBPR) using secondary clarification, and in the membrane bioreactor mode (MEBPR). The operating conditions of the MEBPR and CEBPR pilot scale systems in the present study were similar to those described in Monti and Hall (2008): a nine-month-long comparative period, followed by two dynamic or transition periods, during which the operating conditions of the pilot plant were first perturbed and then operated under steady state conditions.

Each EBPR pilot plant used a University of Cape Town (UCT) configuration (Tchobanoglous et al., 2003) with a total volume of 2500 L, that was divided into an anaerobic zone (11% v/v), an anoxic zone (28% v/v), and an aerobic zone (61% v/v). Zee-Weed®-140 membrane modules (Zenon Environmental Inc., now GE Water and Process Technologies, Oakville, Ontario, Canada) were installed in the aerobic zones for solids–liquid separation. The membrane filtration surface had a nominal pore size of 0.04 μm and each filtration module provided a total surface area of 12 m^2 (140 ft^2). The membranes were operated in a permeation mode for 9.5 min followed by a rapid backflush for 0.5 min using recently-stored permeate. Intermittent coarse-bubble aeration (10 s ON and 10 s OFF) was provided to the module at an air flow rate of 0.34 m^3/min

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