A COMPARATIVE STUDY OF BIOLOGICAL NUTRIENT REMOVAL PROCESSES WITH GRAVITY AND MEMBRANE SOLIDS-LIQUID SEPARATION

by

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Abstract

The replacement of the secondary clarifier with membrane filtration technology was explored as possible solution to accommodate increased loading rates in existing enhanced biological phosphorus removal (EBPR) plants. The present research study aimed to understand the impact of membrane solids-liquid separation on bioreactor performance, by (1) comparing a membrane and conventional EBPR process operated in parallel, and by (2) assessing the potential of membrane processes to perform EBPR satisfactorily under challenging operating conditions.

The utilization of membrane technology resulted in an overall reduced denitrification activity and a reduced observed sludge yield in the membrane process, due to the larger mass of aerobic sludge held in the system. The kinetics of phosphorus release and uptake, together with the stoichiometric coefficients, were intrinsically unaffected by the presence of membrane filtration. On the other hand, the maximum specific nitrification activity in the membrane sludge was significantly lower than that of the reference conventional sludge, possibly due to more extensive decay of nitrifiers originating from the high shear conditions that prevail in submerged membrane bioreactors. Using ribosomal intergenic spacer analysis, the bacterial community composition of the membrane process was found to be significantly different and less diverse than that of the corresponding conventional system, indicating that membrane solids-liquid separation *per se* is sufficient to shift the dynamics and composition of the microbial population. A last fundamental difference brought about by the employment of membrane filtration was the regular formation of a significant amount of foam on the surface of the anoxic bioreactor zone.

With sufficient volatile fatty acids (VFA) concentrations in the influent, the membrane-assisted process could maintain satisfactory nitrification and phosphorus removal performance under high rate conditions, with the lowest hydraulic retention time (HRT) tested being five

hours. A low phosphorus concentration in the effluent could be maintained when the solids retention time (SRT) of the process was extended from 12 to 20 days. However, this resulted in increased VFA utilization per unit mass of phosphorus removed, with the SRT having a significantly larger impact than the HRT. An innovative and sustainable membrane-assisted process was proposed with phosphorus removal and recovery merged in one single system.

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List of abbreviations

Aer	aerobic
Aer rec.	aerobic recycle
Ana	anaerobic
Anx	anoxic
Anx rec.	anoxic recycle
ATP	adenosine diphosphate
ASP	activated sludge processes
b_{AUT}	autotrophs decay rate
BC	British Columbia
bio-P	biological P removal
BNR	biological nutrient removal
CAS	conventional activated sludge
CEBPR	conventional enhanced biological phosphorus removal
COD	chemical oxygen demand
DGGE	denaturing gradient gel electrophoresis
DNA	deoxyribonucleic acid
DO	dissolved oxygen
EBPR	enhanced biological phosphorus removal
EDTA	ethylenediaminetetraacetic acid
FISH	fluorescence *in situ* hybridization
G+C	guanine and cytosine
GALO	*Gordonia amarae*-like organism
HAc	acetate
HRT	hydraulic retention time
MBR	membrane bioreactors
MEBPR	membrane enhanced biological phosphorus removal
MFA	microbial fatty acid
MLSS	mixed liquor suspended solids
MRPP	multi-response permutation procedures
N	nitrogen
NAD	nicotinamide adenine dinucleotide

NH_4-N	ammonium nitrogen
NO_X-N	nitrite plus nitrate nitrogen
OTUs	operational taxonomic units
OUR	oxygen utilization rate
P	phosphorus
PAOs	phosphorus-accumulating microorganisms
PCA	principal component analysis
PCR	polymerase chain reaction
PHA	polyhydroxyalkanoates
PO_4-P	orthophosphate phosphorus
poly-P	polyphosphate
RAS	return activated sludge
RIS	ribosomal intergenic spacer
RCF	Relative centrifuge force
RISA	ribosomal intergenic spacer analysis
RNA	ribonucleic acid
SOUR	specific oxygen utilization rate
rRNA	ribosomal ribonucleic acid
SRT	sludge retention time
SSU rDNA	small subunit ribosomal deoxyribonucleic acid
TCA	tricarboxylic acid
TKN	total Kjeldahl nitrogen
TN	total nitrogen
TP	total phosphorus
Tris-HCl	Tris (hydroxymethyl) aminomethane HCl
TSS	total suspended solids
UCT	University of Cape Town
UPGMA	unweighted pair group method with arithmetic mean
VFA	volatile fatty acids
VSS	volatile suspended solids
WWTPs	wastewater treatment plants
μ_{AUT}	maximum specific growth rate of autotrophic biomass

Preface

The present Ph.D. thesis has been prepared in manuscript-based format. A manuscript thesis, as described by the Faculty of Graduate Studies at The University of British Columbia, is a collection of published, in-press, accepted, submitted or draft manuscripts. The body of this thesis has been separated into eight main chapters. Chapter 1 is an introductory chapter presenting the background of the engineering problem and the main objectives of the thesis. The results of the research program are presented in Chapters 2 through 7. Chapter 8 relates the manuscript chapters to each others, outlines the engineering significance of the research work, and provides directions for future research.

The following is a list of the manuscripts submitted and in preparation that pertain to this thesis.

1. Monti, A., Hall E.R., Dawson, R.N, Husain, H., Kelly, H.G. (2005). A comparative study of biological nutrient removal (BNR) processes with sedimentation and membrane-based separation. Biotechnology and Bioengineering. In press.
2. Monti, A., Hall, E.R., Koch, F.A., Dawson, R.N., Husain, H., Kelly, H.G. (2005). Toward a high rate EBPR process in a membrane-assisted bioreactor. Submitted to Water Environment Research (October 30, 2005).
3. Monti, A., Hall, E.R. (2005). Impact of membrane separation on nitrification kinetics in a biological nutrient removal sludge. Submitted to Water Research (November 17, 2005).
4. Monti, A., Hall, E.R., van Loosdrecht, M.C.M. (2005). Kinetics of phosphorous release and uptake in a membrane-assisted biological nutrient removal process. Submitted to J. Environmental Engineering – ASCE (November 30, 2005).

5. Monti, A., Hall, E.R., Mohn, W.W. (2005). Characteristics and production of anoxic foam in membrane-assisted biological nutrient removal processes. In preparation.
6. Monti, A., Mohn, W.W., Hall, E.R. (2005). A comparative study of bacterial community dynamics in an enhanced biological phosphorus removal process with membrane and gravity solids-liquid separation. In preparation.

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Working in the Environmental Engineering Group at UBC has been a tremendous experience and I would like to thank past and present members for collaboration and encouragement. First of all, Fred Koch, research associate and manager of the UBC Pilot Plant, is thanked for his generous time and extensive discussions in all the stages of this project. Thank

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Co-Authorship Statement

Collaborations with several parties have provided valuable support for this thesis research. The submitted papers and manuscripts in preparation of this thesis have been strengthened by inputs given by my supervisor Dr. Eric Hall, coauthors, scientific colleagues and anonymous reviewers. Below is a summary of the contributions of coauthors to each chapter.

Chapter 2

The present research work was part of a broader study on membrane enhanced biological phosphorus removal supported by the Natural Sciences and Engineering Council of Canada (NSERC) in collaboration with Stantec Consulting, Zenon Environmental Inc., and Dayton and Knight Ltd. These three industrial partners were represented by Robert Dawson, Hadi Husain, and Harlan Kelly, respectively. The three individuals participated in several meetings and provided useful insights on the research activities and on the manuscript content.

Chapter 3

As a part of thesis proposal preparation, I visited the Environmental Biotechnology Group at the Technical University of Delft, The Netherlands, led by Dr. Mark van Loosdrecht. While there, I mastered batch test techniques that were subsequently implemented in this research work. Dr. Mark van Loosdrecht provided supervision during the visiting period and ongoing discussions in the following years. He also contributed with a critical review of the manuscript.

Chapter 5 and 7

The analysis of the bacterial community composition was conducted in the Microbial Ecology Group, headed by Dr. William Mohn, within the UBC Department of Microbiology. Dr. Mohn suggested the appropriate molecular technique to apply in this study, provided the required laboratory facilities and he provided valuable comments on the results and on the two resulting manuscripts..

Chapter 6

The representative individuals of the industrial partners contributed to this chapter for the same reasons presented earlier for Chapter 2. Fred Koch, research associate and manager of the UBC pilot plant, contributed with regular discussions on the research activities conducted at the pilot plant. He also provided helpful suggestions on the revision of the manuscript.

Chapter 1 Introduction

1.1 Preface

The activated sludge process still represents a widely used technology for biological wastewater treatment. The success of such technology over the decades originates from the ability of microorganisms to transform pollutants into less harmful byproducts at significantly reduced costs compared to other treatment processes (e.g. physical-chemical methods). The application of modern activated sludge processes in the industrialized world has expanded from the simple degradation of organic matter to the removal of nutrients such as nitrogen and phosphorus. These nutrients are notorious for stimulating excessive algal growth in stagnant water bodies, when they are present above a threshold concentration. In this regard, enhanced biological phosphorus removal has become one of the most sustainable solutions that is gradually replacing costly chemical treatments (Barnard, 1974).

Western Canada, and in particular the province of British Columbia (BC), has played a central role in the development and application of the biological nutrient removal (BNR) process as a sustainable alternative for the protection of sensitive aquatic environments. Since the construction of the first Canadian BNR process in Kelowna, BC (Oldham and Stevens, 1984), Western Canada witnessed a proliferation of BNR wastewater treatment plants (WWTPs), with flow rates ranging between 2.0 and 500 ML/d (Oldham and Rabinowitz, 2001). Recently, the increasing demand to expand the capacity of existing WWTPs, designed as BNR, and the implementation of more stringent limits on effluent quality have raised new challenges for various municipalities. In this regard, membrane-assisted processes are considered as an attractive solution to the engineering problem.

With the recent substantial decrease in the capital and operating costs of membrane technology, the application of membrane bioreactors (MBR) is also becoming a cost-effective

solution for large scale municipal WWTPs, especially when only the upgrading of an existing plant is needed (Trussel *et al.*, 2005). It is, in fact, well known among practitioners that MBR technology can operate at higher hydraulic loading rates compared to conventional activated sludge (CAS) processes, resulting in a more compact treatment. Other advantages of MBRs include a complete separation of the hydraulic retention time (HRT) from the sludge retention time (SRT) of the process and a superior quality of the effluent that could be recycled for downstream uses.

The combination of the BNR process with membrane filtration as the solids-liquid separator has been scarcely investigated, representing a clear knowledge gap in wastewater engineering field. Therefore, the ultimate question to be addressed is whether larger capacities of existing BNR plants can be accomplished by simply implementing membrane filtration in the existing treatment system or by building additional treatment trains to accommodate the increasing influent load. To this end, the Environmental Engineering group at The University of British Columbia (UBC) was funded with a Strategic Project from the Natural Sciences and Engineering Research Council of Canada (NSERC) to assess the technical feasibility of membrane technology coupled to the BNR process, as an innovative alternative to CAS treatment (Hall, 2001).

1.2 Literature review

1.2.1 Biological nutrient removal technology

The removal of nutrients such as nitrogen and phosphorus from wastewater by biological means is becoming a common practice owing to a more sustainable approach to solve environmental pollution problems. A new opportunity to combine the biological removal of both nitrogen and phosphorus in a single process came with the advent of the enhanced biological phosphorus removal (EBPR) process (Barnard, 1974). Special bacteria, collectively referred to as phosphorus-accumulating microorganisms (PAOs), have the ability to accumulate greater amounts of phosphorus from the wastewater than that which they require for basic metabolic purposes (Comeau *et al.*, 1986). In the following paragraphs, an overview on the current understanding of the mechanisms involved in the BNR process, as well as the state-of-the-art of process engineering, is presented.

1.2.1.1 Biochemical principles of EBPR

The first report of organisms capable of accumulating large amounts of polyphosphate from wastewater dates back to 1959 (Shrinath *et al.*, 1959). These authors provided evidence that an organism capable of using the energy stored in polyphosphate was responsible for enhanced phosphorus removal from wastewater. An anaerobic zone in the first stage of the process was the cause of the appearance of PAOs. It is striking to note that phosphorus removal was an undesirable process in this case, since nutrient-rich effluent was the main objective of the treatment for irrigation purposes of nearby rice fields. In later years, similar observations of biological phosphorus removal in activated sludge plants in the USA and South Africa were documented (Levin and Shapiro 1965, Barnard, 1976). During this time, some progress was made in understanding the mechanisms of this luxury phosphorus uptake. It became clear that the responsible microorganisms could profit from the absence of an electron acceptor in the

initial phase of the treatment plant. The polyphosphate (poly-P) can be used as an energy source for the uptake of fatty acids and their storage as polyhydroxybutyrate inside the cell. When organisms are exposed to aerobic conditions later in the process, they can grow on the internally-stored substrate and accumulate the anaerobically-released phosphorus again. Based on this early theory, full-scale installations to achieve low concentrations of phosphorus in the effluent were implemented in South Africa and Canada (Barnard, 1974, 1976; Oldham and Stevens, 1984).

It was during the mid-late 1980s that academic research started to focus on the biochemistry and metabolic principles, as well as the microbiology of the EBPR process. Tremendous advances have been made toward the scientific understanding of the mechanisms responsible for this process. Comeau *et al.* (1986) and Wentzel *et al.* (1986) were the first ones to propose a biochemical model that would explain the observed release and uptake of phosphate from the cell. The engineering outcome of those studies confirmed that predominance of PAOs in the anaerobic-aerobic system can be achieved as follows: if an anaerobic phase is introduced in which activated sludge is mixed with the influent wastewater, microorganisms capable of anaerobically taking up carbon sources from the influent are favored. PAOs can establish themselved in such conditions, because they are able to hydrolyze stored poly-P in order to supply energy for the anaerobic uptake of the carbon sources. Thus, in the anaerobic phase, PAOs take up the carbon substrate and store it in the form of polyhydroxyalkanoates (PHA) accompanied by the cleavage of poly-P and, consequently, release of orthophosphate. In the subsequent aerobic phase, PAOs can grow aerobically and take up orthophosphate to replenish the poly-P content by using the stored PHA as the carbon and energy source. The conversion of acetate, a favorable substrate for EBPR, to PHA, requires reducing power, because PHA is a more reduced compound than acetate. The idea that the tricarboxylic acid (TCA) cycle functions under anaerobic conditions to oxidize a part of the acetate to CO_2 and to generate reducing power

in the form of nicotinamide adenine dinucleotide (NADH) was the key-point of the *Comeau-Wentzel* model.

An alternative approach was proposed by Mino *et al.* (1987) in which anaerobic degradation of intracellularly stored glycogen to acetyl-CoA, as well as its partial oxidation to CO_2, was hypothesized to generate reducing power for PHA formation. This mechanism formed the basis for the *Mino* model, as an alternative biochemical model to explain the EBPR process (Figure 1.1). There are several experimental observations which strongly support the *Mino Model* (Mino *et al.,* 1998). Variations in the glycogen and PHB content of cells during the anaerobic uptake of acetate have been measured (Arun *et al.*, 1988; Smolders *et al.*, 1994). In all cases, the stoichiometry observed was close to that predicted by the *Mino* model, and it deviated significantly from the *Comeau-Wentzel* one. Other authors (Pereira *et al.*, 1996; Maurer *et al.*, 1997) demonstrated using ^{13}C nuclear magnetic resonance techniques that glycogen is involved in the anaerobic metabolism of EBPR sludges. This was a major step in the identification of the correct metabolic model for the anaerobic phase. The hypothesis of the TCA cycle, however, was not abandoned by Pereira *et al.* (1996), who measured a small fraction of the labeled carbon in acetate in the CO_2. Also based on a redox balance considerations, they concluded that the reducing power generated in the observed degradation of glycogen was insufficient to account for the PHA production. These were strong indications that a small fraction of acetate is metabolized through the TCA cycle under anaerobic conditions supplying a minor part (30%) of the reducing power for PHA formation. Therefore, Pereira *et al.* (1996) proposed a combination of the two models to explain the results obtained by in-vivo nuclear magnetic resonance (or NMR). In recent years, other metabolic pathways have been studied by looking at the utilization of glucose and propionate (other carbon source in real wastewater) from PAOs in the sludge (Wang *et al.*, 2002; Lemos *et al.*, 2003).

1.2.1.2 Microbiology of EBPR

Fuhs and Chen (1975) were the first to identify a bacterium that could accumulate excess phosphate internally when exposed to acetate-agar in the isolation procedure. They concluded that *Acinetobacter* sp. were the organisms responsible for the EBPR process. The abundance of *Acinetobacter* in EBPR systems and their chemical staining profile led to them being called PAOs. In parallel with efforts to understand the biochemical pathways involved in the EBPR process, microbiologists around the world devoted much attention to the investigation of species contributing to biological uptake of phosphorus. The tremendous progress in the techniques used in microbiology allowed researchers to conduct more sophisticated experiments on microbial populations acting in EBPR systems. As a result, it has been demonstrated that *Acinetobacter* is NOT primarily responsible for EBPR. An excellent review on these findings is reported by Mino *et al.* (1998). In short, by applying specific techniques such as fluorescent antibody staining, quinone profiles, biomarkers (e.g. polyamine, diaminopropane) and 16S-rRNA oligonucleotide probes, all targeting *Acinetobacter* presence in EBPR systems, it was possible to conclude that *Acinetobacter* was not detected or not detected sufficient to account for the EBPR observed. The observations made by Fuhs and Chen (1975) and other authors later (e.g. Wentzel *et al.*, 1988) were biased by the culture-dependent methods (Wagner *et al.*, 1993). In these methods, only those bacteria which are culturable on the artificial media used under the defined conditions can be isolated and identified. It is, therefore, likely that only a minor portion of bacteria in activated sludges can grow under such conditions and, thus, be detected. The aforementioned evidence against *Acinetobacter* was all obtained through culture independent methods.

Many other studies to isolate pure cultures from EBPR processes were reported in the literature, but none of them exhibited all the characteristics which EBPR should possess. In many cases, the key characteristic which was lacking in these isolates was the anaerobic acetate metabolism. A review of these studies has been documented by Mino *et al.* (1998). In the search

for the real PAOs, fluorescence *in situ* hybridization (FISH) was used to investigate the microbial community in a full scale plant showing some degree of EBPR behaviour (Wagner *et al.*, 1994). The bacterial population of the plant comprised 36% *Actinobacteria*, 36% *betaproteobacteria*, 10% *gammaproteobacteria*, but *Acinetobacter* accounted for only 3-6% of the total bacterial community. Bond *et al.* (1995) applied the clone library approach and indicated that a critical difference in the microbial structure between EBPR and non-EBPR processes existed in the beta subclass of proteobacteria, suggesting that this group may have a specific role in the EBPR process. The *Rhodocyclus* group within the *betaproteobacteria*, was represented more in the reactor with greater phosphate removal. Bond *et al.* (1999) used FISH to determine that within the *betaproteobacteria*, the *betaproteobacteria*-2-subgroup comprised 55% of all bacteria in a efficiently operating laboratory scale EBPR reactor. Hesselmann *et al.* (1999) were the first to report the definitive phylogenetic placement of the *betaproteobacteria*-2-subgroup PAO as a close relative of and called the organism "*Candidatus* Accumulibacter phosphatis" (*Accumulibacter*, the short version). Crocetti *et al.* (2000) supported this finding and extended the knowledge by using FISH and post-FISH chemical staining to demonstrate that *Accumulibacter* cells cycled poly-P according to EBPR. Other authors confirmed that *Accumulibacter* was a PAO in laboratory scale (Onuki *et al.*, 2002) and full scale (Lee *et al.*, 2002 and Zilles *et al.*, 2002). Additional PAO candidates include *Actinobacteria* (Wagner *et al.*, 1994; Kawaharasaki *et al.*, 1999; Liu *et al.*, 2001), *alphaproteobacteria* (Kawaharasaki *et al.*, 1999), and *gammaproteobacteria* (Liu *et al.*, 2001). The denaturing gel gradient electrophoresis (DGGE) technique (Muyzer *et al.*, 1993) has revealed that the 16S-rDNAs extracted from EBPR sludges contain several different DNA fragments, implying that the sludges are not dominated by a single bacterium but composed of a few dominant bacterial strains (Brdjanovic *et al.*, 1997). Based on these recent results, it would appear that PAOs do not consist of one single dominant bacterium but, rather, several different bacterial groups. A dependency on interspecies

relationship's between different groups of microorganisms involved in EBPR prevent PAOs from growing as a single pure culture.

Due to the failure to isolate a true PAO organism, most experiments to study EBPR processes are still carried out with activated sludge. Isolation of PAOs remains essential, because experiments with pure culture will provide substantial information about the microbiology and biochemical aspects of the EBPR process (Mino *et al.*, 1998). This, in turn, will provide essential knowledge in the engineering applications to better design and optimization of biological phosphorus removal systems.

1.2.1.3 Process engineering aspects

The traditional method for phosphorous removal from wastewater is the addition of chemicals, such as iron or aluminum salts, to the process. In addition to the fact that in wastewater treatment, usage of chemicals should be minimized, there are several negative aspects of this practice. The counterion of the salts remains in the water, resulting in an increased salinity of treated waters. The chemicals precipitate in the sludge, leading to extra costs for treatment of the excess sludge. Moreover, since the sludge inventory in a treatment system is limited to a maximum amount, large treatment reactors are required in order to maintain the same amount of biological sludge (Van Loosdrecht *et al.*, 1997). Chemical phosphorus precipitation has, however, dominated as a standard process step due to the ease and stability of the method. In the recent years, a much better understanding of the EBPR fundamentals, together with the high costs of chemicals to achieve very low phosphorus concentrations (typically in sensitive areas) have favored the implementation of more environmentally friendly approaches. A number of different design solutions have been proposed to achieve biological phosphorus removal, often coupled with nitrogen elimination from wastewaters (Tchobanoglous et al., 2003). They can be grouped into two basic types: full biological processes and combined

biological/chemical processes. A basic scheme for a biological phosphate removal process is shown in Figure 1.2. All of the alternatives feature an anaerobic (absence of oxygen and nitrate) compartment as a common denominator in order to favor bacteria capable of accumulating large amounts of polyphosphate inside their cells. PAOs have a clear competitive advantage in that they can accumulate fatty acid substrate in the cells without the need of external electron acceptor. When the sludge becomes aerated (or nitrate is available) other heterotrophic organisms have no substrate to feed on, while PAOs can grow at the expense of their stored substrate. Therefore, an adequate design of the anaerobic phase is essential for a good EBPR process and this will heavily depend on the wastewater characteristics. The volatile fatty acids (VFA) content in the sewage is the most important variable that contributes to the sizing of the anaerobic zone of the process. The length of the aerobic phase in the treatment process is usually not limited by the phosphate uptake, but by nitrification, because of the slower growth rate of nitrifying bacteria.

To couple nitrogen removal and EBPR in a single process, a denitrification zone has to be added to the treatment line. It is traditionally assumed that the two processes compete for the same substrate, supported by the evidence that the presence of nitrate in the anaerobic zone gradually inhibits the biological phosphate removal process. In order to prevent the presence of nitrate in the anaerobic tank, researchers in South Africa pioneered a modified process layout to first introduce nitrate-containing return sludge to a denitrification reactor, after which the nitrate-free sludge/water mixture is partly recycled to the anaerobic tank (Figure 1.3). This design approach was named the University of Cape Town (UCT) process. Assuming a complete absence of nitrate in the anaerobic reactor, denitrification can be achieved through two processes: (1) heterotrophic denitrification in the anoxic zone using leftover carbon source as electron donor and nitrate (coming with the aerobic and returned sludge recycle) as the electron acceptor; (2) denitrifying dephosphatation, newly discovered process carried out by a sub-group of PAOs that

can use nitrate as the electron acceptor in the absence of oxygen. The latter process has been an active research area in the recent years due to its strong potential to reduce the use of chemical oxygen demand (COD) for nitrogen removal and save oxygen requirements in the subsequent aeration zone (Bortone *et al.*, 1996 and Kuba *et al.*, 1996).

Experienced gained in North America and also in various other parts of the world has provided the following general design philosophy for BNR processes (Oldham and Rabinowitz, 2001):

- As stated above, the selection of the process configuration depends on historical influent average and peak monthly characteristics. In addition, effluent quality requirements together with expected minimum and maximum monthly mixed liquor temperature play an important role in the overall design.
- The need for primary sludge fermentation (or provision of an alternative source of VFA) is dependent upon how much VFA is naturally present in the raw wastewater and the mass of phosphorus that must be reliably removed from the influent.
- For typical North American wastewaters, a main bioreactor SRT of about 10 days in conjunction with an HRT of about 8 hours is capable of good nitrogen and phosphorus removal, as long as careful attention is paid to the design of the secondary clarifiers.
- The overall size of the bioreactor to achieve a given level of total nitrogen removal can be reduced substantially by the use of primary sludge fermentation. Where fermenter supernatant is added to the anaerobic zone to provide VFA to the phosphorus removal process, the remainder of fermentation products spill over into the anoxic zone, thereby increasing the rate of denitrification that is realized in that zone.

Chemical precipitation to remove phosphorus from the wastewater is often applied in conjunction with biological means, to further improve the quality of the final effluent. Generally, chemicals are added to the main sludge line with the disadvantage of accumulating precipitates

in the bioprocess. As an alternative, a fraction of the return sludge is introduced to a *stripper* tank in which anaerobic conditions are maintained. After sludge/water separation an enriched phosphate-containing flow is obtained and treated downstream with chemical precipitation (PhoStip process) or, lately, with more sustainable methods such as crystallization, resulting in phosphorus recovery (Britton *et al.*, 2005). Reducing the process SRT is claimed to be another method to improve the efficiency of EBPR systems. This results in an increase of net biomass yield and, as a consequence, more phosphorus-rich sludge to be wasted.

1.2.2 Membrane bioreactor technology and its application

Membranes can be introduced into microbiological processes in two ways (Rittmann, 1998). The first is a solids-liquid separator that replaces a conventional settling tank in which biomass is captured and recycled. This application directly improves effluent quality and biomass retention and may lead to improved space utilization. The second is as a bubbleless gas-transfer device to supply gaseous substrates. In this second application, the membrane becomes colonized with a biofilm (Rittmann, 2002). The use of a membrane in the present research work belongs to the first type of application.

The first application of membrane technology in wastewater treatment dates back in the mid-60's (Smith *et al.*, 1969) with the intent to achieve a compact treatment system and a superior effluent quality compared to the conventional activated sludge treatment. The long list of advantages of this technology attracted some companies in North America and Japan to develop better membranes materials and system configurations to make the application of membrane in wastewater treatment more acceptable. After 30 years since its conception, MBR technology has become a serious alternative to conventional treatment (van der Roest *et al.*, 2002). The principle of this technology, its advantages and drawbacks, and the scientific research

background in the fields of MBR and biological nutrient removal are discussed in the following paragraphs.

1.2.2.1 Principle of MBR processes

Membrane bioreactors can be defined as the combination of two basic processes, biological degradation and membrane separation, into a single process in which suspended solids and microorganisms responsible for biodegradation are separated from the treated water by a membrane filtration unit (Manem and Sanderson, 1996). The MBR system configurations used wastewater treatment in the last 10 years can be divided in two groups.

- "Conventional MBR": the activated sludge is pumped to tubular or flat sheet modules where it flows at high velocities (greater than 2 m/s and often greater than 4 m/s) with consequently quite high pressure drop and thus quite high transmembrane pressure. This is a typical cross flow filtration.
- MBR with immersed membranes: hollow fibers or hollow panes are immersed in the aerated tank and treated water is generated by applying suction in the inner part of the fibers.

A schematic representation of the operation of these two types of MBR is shown in Figure 1.4. The cross-flow filtration is able to achieve higher flux but requires a high tangential velocity (3 to 5 m/s) and a significantly higher applied pressure because of the pressure drop along the membrane length. This results in a large recycle ratio (feed flow to a membrane module over permeate flow) of between 25 and 75 % and an energy consumption of between 4 and 12 kWh per m^3 of water treated. In contrast, the immersed membrane works at much smaller applied pressure, without recirculation of biomass, but with aeration for membrane scouring. The energy consumption for filtration (including pumping and aeration) is between 0.3 and 0.6 kWh per m^3 of water treated, 10 to 20 times smaller than for the cross-flow filtration system (Côté and

Thompson, 2000). This quantum gain, in terms of energy, is the primary reason for the success of immersed membranes in the recent MBR applications around the world, at full scale.

1.2.2.2 Advantages and disadvantages of MBRs

One of the major advantages of membranes to replace a conventional settler lies in the superior quality of the effluent. Basically, suspended solids-free treated water is generated from the bioreactor, with very high removal of pathogens (Manem and Sanderson, 1996). Such effluent quality opens the way for direct water recycle and re-use in a era where the Earth, with its diverse and abundant life forms, including over six billion humans, is facing a serious water crisis (United Nations, 2003). From the process standpoint, MBR offers the possibility to completely separate the HRT from the SRT, providing optimum control of the biological reactions and greater reliability and flexibility in process use. Therefore, the selection of bacteria and microorganisms present in the system is no longer dependent on their ability to form biological flocs and thus to settle: all the species present in the aerated tank have the same residence time that is defined the wasting of the sludge (Ben Aim and Semmens, 2003). The ability of the membrane to keep all the microorganisms in the bioreactor without worrying about sludge settling characteristic has permitted the operation of biological processes at substantially higher biomass concentrations, i.e. running processes at very high SRT, even infinitely high (Yamamoto *et al.*, 1989; Yasui *et al.*, 1996; Sakai *et al.*, 1997). The ability to accommodate such a large mass of sludge in the process provides the benefit to obtain satisfactory effluent quality with a small foot-print system. Thus, especially in high densely populated area, MBR technology offers a valuable alternative to the conventional activated sludge treatment.

Until recently, the advantages listed above were defeated by the capital and operating costs behind an MBR process. Therefore, the sporadic early MBR applications around the world were basically designed for small scale municipal WWTPs (Fan *et al.*, 1996) and special cases of

industrial wastewater, such as landfill leachate treatment. With the introduction of immersed membranes and the consequent decrease in the operating costs, together with the exponential reduction of the membrane production costs, the new MBR processes stand a better chance of becoming a cost-effective solution when compared with the standard conventional activated sludge. However, hollow fiber modules, as well as all the other membranes, are prone to fouling, a process that gradually reduces the permeate production rate. This requires a periodic cleaning of the membrane to restore its filterability. Fouling has been since a subject of active research in the field of MBR technology for some time. A clear understanding of the interactions between biological process behavior, membrane fouling rates, membrane flux and the operating conditions is required to confidently rely on the application of future MBR technology at large scale.

1.2.2.3 MBR technology coupled to EBPR process

MBR technology for wastewater treatment has been typically coupled to carbon and nitrogen removal biological processes (Côté *et al.*, 1998; de Silva *et al.*, 1998; Côté and Thompson, 2000; van der Roest *et al.* 2002; Rosenberger *et al.*, 2002). Phosphorus removal in MBR systems has been typically achieved with chemical precipitation. As already discussed earlier, the EBPR process seems to be not compatible with long sludge ages, according to the experience gained in conventional activated sludge systems. The promising aspect of implementing membranes in EBPR processes consists of producing an effluent containing only soluble phosphorus (mainly ortho-P), generally at very low concentrations in efficient EBPR systems. In addition, MBR technology offers the possibility to design ultra-high rate processes, resulting in an important saving on the treatment plant foot-print.

Recently, the first attempts to couple membrane solids-liquid separation with EBPR have been documented in the technical literature. Adam *et al.* (2002) first, and Patel *et al.* (2005) later,

indicated that biological P removal (bio-P) can be successfully accomplished in MBR systems operated at relatively long SRTs of about 20 days and with a high ratio of short chain VFA to total P in the feed. On the other hand, satisfactory P removal in the study of Fleisher *et al.* (2005) could only be achieved with addition of alum, using a combination of biological and chemical treatment methods. Even though these investigations have offered an important preliminary assessment on the feasibility of bio-P in MBR systems, little is still known about the impact of membrane technology on the EBPR process.

The MBR approach seems to represent one of the most intriguing process technologies available to cope with future stringent quality criteria in wastewater treatment. It appears evident from the information available in the literature that a more comprehensive and thorough study is needed to better understand the kinetics and process stability of the membrane EBPR process over different operating conditions.

1.2.3 Composition and dynamics of microbial populations

The methods that have been recently developed to study structure and function of microbial aggregates including activated sludge flocs and biofilms have greatly increased our knowledge about the microbial systems upon which the performance of wastewater treatment plants depend (Wilderer *et al.*, 2002). The microbial community, which is responsible for the removal of pollutants, consists of a wide variety of often non-culturable bacterial species, protozoa and metazoa, possibly also of fungi and yeast cells. Molecular techniques have been developed in the last decade as a tool to directly study microorganisms in the bioreactor, i.e. not dependent on culture methods. The invaluable contribution of molecular techniques in wastewater engineering is the identification of bacteria and the characterization of their physiological properties. This will eventually lead to the development of specific control strategies to favor or suppress the growth of individual functional group of microorganism for

the benefit of the intended treatment (Wagner *et al.*, 2002). In the last decade, the application of these techniques has identified different groups of non-culturable bacteria responsible for sludge bulking, EBPR, nitrification and denitrification. Surprisingly, the model organisms originally thought to be responsible for these processes and ammonia oxidation have been shown to be of little actual importance in wastewater treatment (Wagner *et al.*, 1994; Wagner *et al.*, 1996; Crocetti *et al.*, 2000). In order for the observations gained at the microscale to be helpful on the macroscale (i.e. to the engineering of the process), the microscale microbiological results have to be correlated with functional process parameter values characteristic for the system as a whole. It is in fact, irrelevant to know which types of bacteria are present in certain activated sludge flocs, unless the appearance and abundance of certain species or groups of species correlates with the process conditions under which the microbial community has formed, and with the performance data obtained for the reactor in question (Wilderer *et al.*, 2002).

In the following sections, a description of the principles behind commonly applied techniques is provided. In addition, the main results gained from the application of molecular tools with regards to MBR processes are briefly reported.

1.2.3.1 Oligonucleotide probing

When we want to understand community structure, we need to identify and enumerate the different microorganisms according to their inheritable genetic content. The common approach is to target the small subunit (SSU) (i.e. 16S or 18S) rRNA, which is a powerful phylogenetic marker. An oligonucleotide probe directed toward the SSU rRNA is the most direct approach. An oligonucleotide probe is single-stranded DNA fragment comprised of 15 to 25 bases whose sequence is complementary to a region in the target-cell's SSU rRNA. Under strictly controlled assay conditions, the probe DNA hybridizes to the complementary region of the target-cell's RNA, but it does not hybridize to the RNA from any other cells, due to mismatches in the

sequence. If the RNA is fixed in place, un-hybridized probe can be washed away, leaving only probe hybridized to the target RNA. As long as the hybridized probe can be detected, the presence and quantity of target rRNA can be detected (Rittmann and McCarty, 2001).

The first basic format to carry out oligonucleotide hybridization is called *slot blotting*, and it requires that RNA be extracted from the sample. The probe's radioactivity, labeled with ^{32}P, is later detected and quantified. Slot blotting was one of the first molecular techniques applied to bioreactors to study the community structure of methanogens carrying out anaerobic treatment (Raskin *et al.*, 1994). A second and more recent approach for oligonucleotide probing is *fluorescent in situ hybridization*, or FISH (Manz *et al.*, 1994). Probes in FISH applications are labeled with a molecule that fluoresces when excited by light of a given wavelength. Therefore, detection is through fluorescence microscopy. The RNA is not extracted with FISH, but remains inside the cells (*in situ*), which are fixed and made porous to the probe. Because the cells are not destroyed, as in slot blotting, FISH is able to provide information on the spatial relationships among different species. Countless are the examples in the literature in which FISH was used as an invaluable tool to investigate the microbial community carrying out different metabolic function.

1.2.3.2 Community fingerprints

One drawback of oligonucleotide probing is that it can be used confidently only for strains that have been isolated and sequenced. Therefore, it is very useful to have a molecular technique that provides a fingerprint of the community's diversity, whether or not the key strains have been isolated and sequenced. New fingerprinting techniques are being developed. The basic principle underlying them is that the DNA coding for a specified and universal function of the microorganisms of interest is selectively amplified by polymerase chain reaction (PCR). The amplified DNA must be analyzed to create and interpret the fingerprint. A method whose use is

growing is denaturing gradient gel electrophoresis (DGGE) of 16S rRNA gene fragments (Muyzer *et al.*, 1993). A acrylamide gel is prepared in such a way that it has a continuous gradient of denaturing agent, typically urea plus formamide. As the DNA moves toward the positive electrode, it encounters increasingly denaturing conditions. Depending mainly on the DNA's G+C content (two nucleotide bases), it denatures, greatly expands in size, and stops at different locations between the electrodes. This creates a pattern of DNA bands that constitute the fingerprints. There are many examples documented in the literature in which the use of DGGE was able to reveal the diversity of the microbial population and its change with time. Despite their usefulness in characterizing microbial communities, 16S rRNA gene sequences are sometimes not divergent enough to distinguish species from the same genus (Normand *et al.*, 1996). In addition, bacterial strains with distinct physiologies have been reported to have identical 16S rRNA genes (Palys *et al.*, 1997). On the other hand, the region between the 16S and 23S rRNA gene, known as the ribosomal intergenic spacer (RIS), has a highly variable length (Gürtler and Stanisich, 1996). The RIS has been used as a biomarker to distinguish species and strains of a species (Garzía-Martínez *et al.*, 1999). The PCR amplicons of the variable region are separated in acrylamide gels, resulting in a fingerprint band pattern. The RIS analysis, or RISA, has also been applied in studying the bacterial community composition of activated sludge systems (Yu and Mohn, 2001; Smith *et al.*, 2003).

Even though molecular techniques have opened new grounds in the knowledge of environmental microbial communities, they are not exempt from biases. DNA extraction methods may select for certain types of microorganisms (Martin-Laurent *et al.*, 2001). In addition, the biases and limitations in the PCR include: the existence of multiple 16S rRNA gene operons, primer annealing specificity, differences in the ease of amplification of DNA from different organisms, and the need to design primers that will bind to as many target organisms as possible. As explained above, DGGE and RISA techniques generate a pattern of bands on a gel.

Although theoretically, each band represents a unique sequence and therefore a unique species, this is not always the case. Several sequences may co-migrate to form what appears to be a single band (Buchholz-Cleven *et al.*, 1997) and some organisms generate more than one band (Nubel *et al.*, 1996). Despite these limitations, a relative comparison between samples is still possible if all samples are treated in the same manner.

Another method to qualitatively assess the biomass community structure is through the chemotypic analysis of the microbial fatty acid (MFA) composition contained in the biosolids (Werker and Hall, 2000). Microbial fatty acids compose the lipid structures that form the cellular membranes of all microbes. There are many different chemical forms of fatty acids and each species of microorganism has a unique MFA contribution that is linked directly to its genetic makeup. The assortment of types of fatty acids presented in the biomass sample provides a pattern or fingerprint that reflects the community structure. In addition, since changes in the biomass MFA profile will be strongly influenced by shifts in the balance of constituent species, the rate of change of the profile should provide a means for quantifying population dynamics.

1.2.3.3 Microbial community composition in MBR systems

Luxmy *et al.* (2000) were among the first to investigate the bacterial community in an MBR process by applying FISH and DGGE techniques. Given the infinite sludge age, they claimed that the predator-prey relationship plays a very significant role, because a long SRT is suitable for the generation of the higher trophic-level organisms like protozoa and metazoa. It was found that the alpha- and beta- subclasses of proteobacteria were the dominant groups in the MBR process. Different shapes of clusters were observed when targeting nitrifiers with FISH, later detected with the confocal laser microscope. Based on DGGE profiles, it was concluded that bacterial communities in MBRs were different from those in CAS with less than 0.4 similarity index. Another relevant study on the microbiology of MBR was conducted by Witzig

et al. (2002), within a broader MBR project focusing on aerobic treatment of municipal wastewater (Rosenberger *et al*, 2002). The low food/microorganism ratio typical for MBRs resulted in significantly fewer bacteria cells detectable by FISH, compared to conventional activated sludge. Therefore, the majority of cells were assumed to reside in a physiological state not allowing for cell division. Contrary to Luxmy *et al.* (2000), the system was virtually free of grazing organisms, and therefore lacking one of the most important mechanisms responsible for cell elimination and ecological selection in conventional activated sludge.

More investigation and understanding of microbial composition in MBRs and how it differs when compared to CAS processes is definitely needed to generate information what will ultimately lead to a better design of these complex biological systems.

1.3 Research objectives

From the literature review presented in the previous chapter, it appears that the biological process aspects behind an MBR system have been scarcely investigated, mainly due to the limited application of MBR technology in the field of wastewater treatment. In particular, studies on the combination of membrane filtration with the EBPR process are just emerging in the last few years and they are only limited to assessment of the technical feasibility of the process. Therefore, a more comprehensive and thorough study is needed to better understand the kinetics and process stability of the membrane EBPR (MEBPR) process under different operating conditions.

A common approach taken by practitioners and researchers is to regard the membrane unit as a biomass separator that simply replaces the conventional clarifier normally employed in biological processes for wastewater treatment. Although this modification alone offers several advantages, it also changes the conditions that determine the presence of certain microorganisms in the treatment system. As a matter of fact, the selection of bacteria is no longer dependent on their ability to form biological flocs and thus to settle: all the species present in the process will have the same residence time given by the sludge wasting rate. This is also true for colloids and macromolecules retained by the membrane: their residence time is no longer equivalent to the HRT but rather to that of the SRT (Ben Aim and Semmens, 2003). This is one of the main differences between a conventional activated sludge process and a MBR operated at the same conditions. In addition, a membrane-assisted process with immersed membranes features strong course-bubble aeration as a means to scour the bundle of fibers and reduce the fouling. This intermittent vigorous aeration tends to hinder the formation of flocs, resulting in a biomass particle distribution that is finer than in the equivalent CAS (Zhang *et al.*, 1997). Last, the sludge blanket normally present at the bottom of secondary clarifiers is eliminated, as is the anoxic

environment associated with the sludge blanket. The aforementioned dissimilarities are expected to alter the microbiology and kinetics of the membrane process which the performance of wastewater treatment plants depend on. Thus, an MBR process not only differs from a CAS process for the long SRTs and relatively short HRTs operating conditions, but also for the intrinsically different environment created by the presence of a membrane instead of a gravity solids-liquid separator.

In light of the overall MEBPR project goals outlined in the preface and the above discussion on the MBR research needs, the objectives of the present research work were addressed in two interconnected experimental programs.

- A comparative study in which the fundamental differences between a membrane and conventional process were investigated in the context of biological phosphorous removal.
- A development study in which the MEBPR process was run under operating conditions which progressively moved toward those typically applied to MBRs, i.e. long SRTs and short HRTs.

The information gathered from the above two programs should lead to a solid and cost-effective process design that can guarantee satisfactorily treatment performance. A more detailed overview of the research questions within the two experimental phases is presented in the following sections.

1.3.1 Research questions for the comparative study

The fundamental research question guiding the first phase of the present research work was "Is the membrane module just a replacement of the secondary clarifier?".

To address the above enigma from the best possible angle and in the context of biological phosphorus removal, the following additional research questions were investigated.

- Is the EBPR process performance any different when a membrane solids-liquid separator replaces the conventional clarifier? (Chapter 2)
- Are the observed nitrification and EBPR kinetics and stoichiometries affected by the use of membrane solids-liquid separation? (Chapter 3 and 4)
- What is the impact of a membrane module on the composition of the microbial population carrying out the biological activity? (Chapter 5)

To properly tackle all the above points, it was proposed to run two parallel identical treatment systems fed with the same influent under the same operating conditions. The only dissimilarity between the two processes consisted of the means by which the biomass was separated from the treated water: membrane filtration on one side (MEBPR process) and gravity sedimentation on the other (CEBPR). Under these conditions, the process performance, nitrification and EBPR kinetics, and the microbial community composition of the two systems could be analyzed and compared.

1.3.2 Research questions for the development study

The utilization of a membrane solids-liquid separation in a bioreactor allows for a set of operating conditions which are not technically achievable in a conventional activated sludge process. Membrane processes have been shown to function well at significantly longer SRTs, due to their ability to perform the solids-liquid separation independently from the settleability of the biomass. In addition, the smaller footprint of the membrane separation unit, compared to the secondary clarifier, opens new avenues to operate the process at high rate conditions typical of short HRT systems.

In the context of biological nutrient removal processes, it was imperative to address the following research questions.

- Can an MEBPR process maintain satisfactory removal of COD, nitrogen and phosphorus at increasingly higher loading rates?
- If short SRTs are recommended for successful operation of conventional EBPR processes, what is the impact of extending the SRT on biological phosphorous removal?

To this end, once the comparative study was completed, the CEBPR treatment train was retrofitted to become a second MEBPR process to study different combinations of operational set points (i.e. HRT and SRT). The ultimate research objective for this second phase was to investigate the potential of the MEBPR process as a cost-effective solution to upgrade existing BNR plants (Chapter 6 and 7). In other words, experimental evidence should be given to the feasibility and reliability of membrane technology to support high rate and relatively long SRT process conditions.

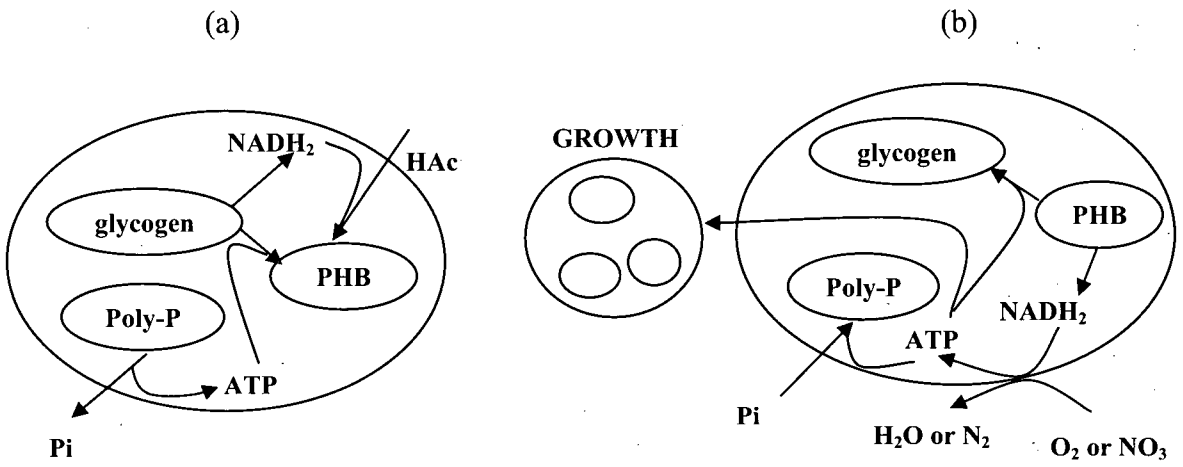

Figure 1.1 **Schematic representation of the metabolism of biological phosphorus removal organisms during (a) anaerobic conditions and (b) anoxic or aerobic conditions.**

(a) (b)

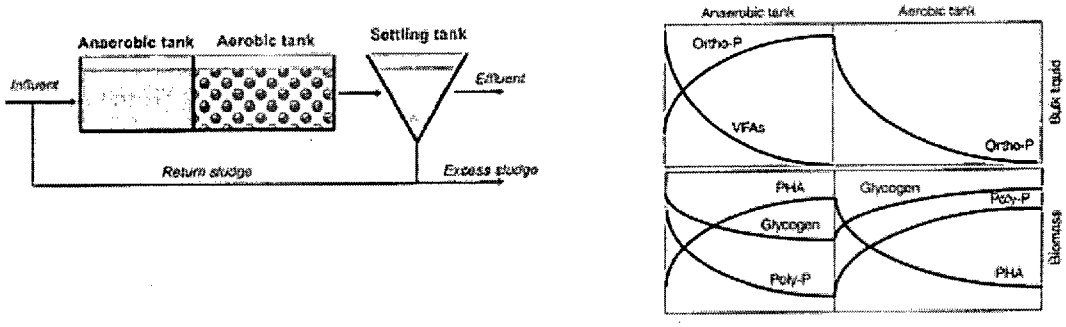

Figure 1.2 (a) Representation of an EBPR process, and (b) concentrations profiles of key compounds in a plug flow type of process. VFA: volatile fatty acids.

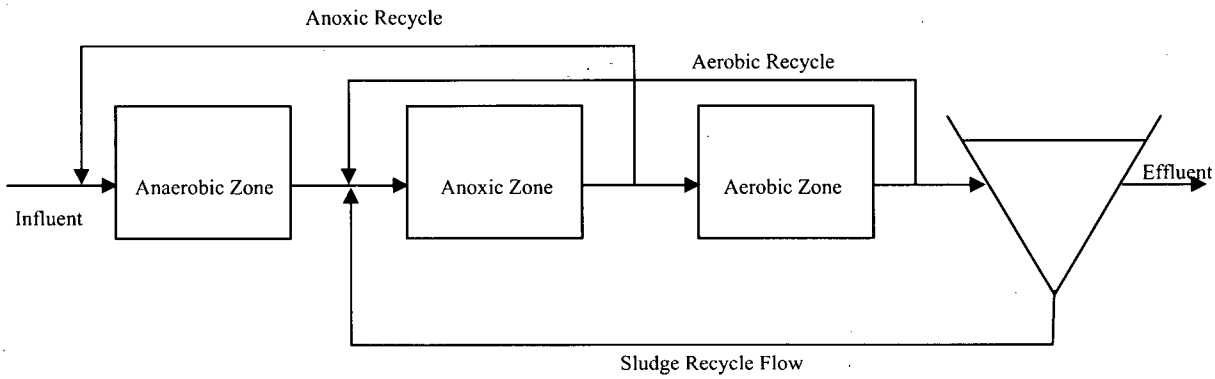

Figure 1.3 Schematic representation of a University of Cape Town (UCT) process.

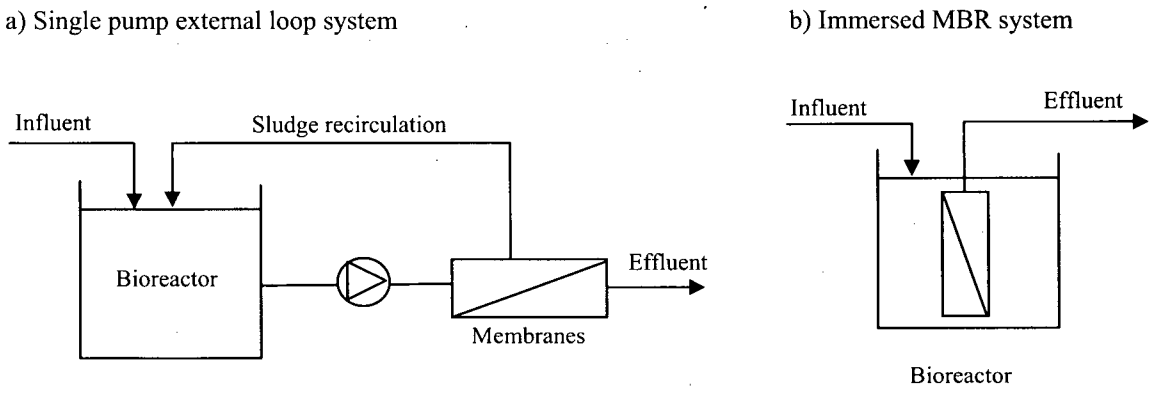

Figure 1.4 Schematic diagram of (a) an external and (b) submerged membrane module.

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Chapter 2 A comparative study on the performance of biological nutrient removal processes with sedimentation and membrane-based separation*

2.1 Introduction

During the 1980s and 1990s, Western Canada witnessed a proliferation of biological nitrogen and phosphorus removal (BNR) wastewater treatment plants (WWTPs), with capacities ranging between 2.0 and 500 ML/d, to safeguard quiescent and sensitive water bodies from algae blooms (Oldham and Rabinowitz, 2001). In recent years, the increasing pressure to expand existing BNR WWTPs and the implementation of more stringent requirements on effluent quality have prompted municipalities to consider membrane bioreactor (MBR) technology as an innovative solution to the problems. MBR processes couple biodegradative treatment to a physical solid-liquid separation process, in a manner that harnesses the advantages of both unit operations to produce ultra-high quality treated effluent. The main advantages of an MBR system, over conventional activated sludge (CAS) processes, are: the possibility of operating the bioreactor at elevated concentrations of biosolids to maintain high levels of biological activity (Buisson *et al.*, 1998); the complete uncoupling of solids retention time (SRT) and hydraulic retention time (HRT), allowing for the possible retention and degradation of slowly biodegradable contaminants (Manem and Sanderson, 1996; Cicek *et al.*, 1999) and; the production of a suspended solids-free effluent and operation of the system independent of the settleability of the biomass (Krauth and Staab, 1993; Winnen *et al.*, 1996). The characteristics of MBR processes make them particularly attractive for applications in which a small treatment

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plant footprint and a high quality treated effluent are required (Côté *et al.*, 1998). Due to the expected reduction in capital costs, together with the significant improvement in effluent quality, MBR technology is becoming a competitive solution with new installations being completed at locations around the globe (Trussel *et al.*, 2005).

Recent attempts have been made to couple membrane technology to the enhanced biological phosphorus removal (EBPR) process both in a flow-through mode (Fleischer *et al.*, 2005; Lesjean *et al.*, 2003; Adam *et al.*, 2002) and in a sequencing batch reactor system (Ahn *et al.*, 2003). Even though these feasibility studies have confirmed that the MBR process can achieve satisfactory total phosphorus removal, little research has been conducted with a membrane and a conventional EBPR system working in parallel under identical conditions to uncover fundamental differences. In the past two decades, a great deal of knowledge has been accumulated on the design and operation of conventional EBPR (CEBPR) systems (Van Loosdrecht *et al.*, 1997; Mino *et al.*, 1998; Mulkerrins *et al.*, 2004). However, it would be simplistic to blindly transfer this knowledge to the new membrane EBPR (MEBPR) process given the radical differences that arise when membrane-based solids-liquid separation technology is employed. In MBR processes, all the microbial species present will have the same residence time, governed by the sludge wasting rate. This is also true for colloids and macromolecules retained by the membrane: their residence time no longer will be equivalent to the HRT but, rather, to the SRT (Ben Aim and Semmens, 2003). In addition, the strong coarse-bubble aeration or the high shear employed to reduce the membrane fouling tends to hinder the formation of flocs, resulting in a biomass particle distribution that is finer than that in the conventional counterpart (Zhang *et al.*, 1997; Cicek *et al.*, 1999).

A number of previous MBR studies in the broad area of carbon and/or nutrient removal have described the impact of membrane separation on the mixed liquor and on the process performance relative to a conventional system (Muller *et al.*, 1995; Zhang *et al.*, 1997; Liebig *et*

al. 2001; Adam *et al.*, 2002; Soriano *et al.* 2003; Wei *et al.*, 2003; Clara *et al.*, 2004; Gao *et al.*, 2004; Yoon *et al.*, 2004; Fleischer *et al.*, 2005). However, the operating conditions and/or the configuration of the parallel processes used in these studies were far from identical. Although these studies contributed indisputably to the advancement of the MBR concept, only limited information has been obtained on the fundamental differences between membrane and conventional treatment processes. A significant step toward parallel operation was made by Cicek *et al.* (1999) and Holbrook *et al.* (2005), who investigated the performance and biomass properties of a membrane and conventional system operated under identical conditions, with the exception of the prevailing SRT (10 days apart) for the former and the process configuration for the others. More recently, the first comparative study under strictly identical conditions was documented, with the objective of exploring performance and biomass differences under short SRTs and HRTs (Ng and Hermanowicz, 2005). These comparative studies under aerobic-only conditions (i.e. no nutrient removal) indicated that significant differences exist in the performance, particle size distribution, enzymatic activities, observed sludge yields, and sludge filterability.

The objective of the present investigation was (1) to evaluate whether MEBPR process performance is similar to that of a CEBPR, under hydraulically challenging conditions and, at the same time, (2) to uncover the fundamental differences that arise on the BNR performance when membrane solid-liquid separation technology is employed. To achieve the overall goal of the study, a MEBPR pilot train was run in parallel to a CEBPR process, under identical operating conditions and with the same municipal primary wastewater feed. The only difference permitted was the technology adopted to separate the treated water from the activated sludge: membrane-based filtration and gravity-based sedimentation. Following the completion of the parallel evaluation, the CEBPR process was retrofitted as a second MEBPR train and, while keeping all the operating conditions unchanged, its performance was assessed during the transition period.

Carbon, nitrogen and phosphorus profiles in the bioreactor, together with sludge inventory and observed sludge yields, were calculated and compared for the two systems.

2.2 Materials and methods

The present research study was conducted at the University of British Columbia (UBC) wastewater treatment pilot plant facility between May 2003 and October 2004.

2.2.1 Description of the test site

The UBC pilot plant is a dual train facility, in which two 2500 liter activated sludge systems can be operated in parallel for comparison of different experimental treatments or process designs. The feed to both trains was primary effluent produced on site by clarification of municipal wastewater pumped continuously from two 15,000 liter external storage tanks. The wastewater was collected two to four times a day from the trunk sewer that runs nearby and was temporarily stored in the external storage tanks. The primary effluent, always in excess of the required process flow rate, was collected in a 20 liter holding tank that provided a constant head to the process feed pumps in order to assure minimum variation of flow. The characteristics of the wastewater used for this research study are reported in Table 2.1. The relatively low pH and the limited amount of calcium, iron and aluminum in the incoming wastewater (data not shown) assured that the P removal mechanism would be predominantly due to biological activity with negligible contribution from natural chemical precipitation.

2.2.2 MEBPR and CEBPR process configuration

A standard University Cape Town (UCT) type process was selected for the comparative study. The process included a series of three separated compartments: an anaerobic zone, followed by an anoxic zone and an aerobic zone (Tchobanoglous *et al.*, 2003). An aerobic internal recycle was used to return nitrates from the aerobic zone back to the mid-anoxic zone for denitrification. A second internal recycle, the anoxic recycle, returned biomass from the anoxic

to the anaerobic zone in order to stimulate the growth of phosphorus-accumulating organisms (PAOs) responsible for the EBPR process (Comeau *et al.*, 1986).

One of the two parallel trains of the UBC pilot plant was initially retrofitted as an membrane-assisted bioreactor, by installing a membrane module in the existing aerobic tank. The new membrane-UCT differed from the standard UCT process in the suspended solids distribution among the three zones. This was because the process effluent was extracted from the membrane in the aerobic zone, which concentrated the sludge in this zone relative to the upstream zones. In addition to hosting a higher biomass level in the aerobic compartment, an MEBPR process distributes the sludge differently in the other two zones for the same internal recycling rates. An elegant numerical elaboration of this phenomenon was recently presented by Ramphao *et al.* (2005).

To fulfill the objective of the present study, which required identical operating conditions for both treatment trains, it was decided to circumvent the biomass distribution obstacle by returning the settled sludge from the conventional train clarifier back to the aerobic zone, rather than to the anoxic zone. Even though this modification would not make any sense in the real application of conventional BNR plants, here it was used to insure an acceptable comparison. Figure 2.1 illustrates the final configuration of the MEBPR and the CEBPR processes adopted for the present comparative study. Two secondary clarifiers were employed to add some excess clarification capacity in the event of bulking problems and increasing hydraulic loads. The range of HRT and overflow rate values applied to each clarifier was 2.02-1.41 h and 5.8-8.12 $m^3/(m^2 \cdot d)$, respectively, depending on the influent flow rate to the pilot plant. Both systems utilized the same total reactor volumes (2228 L) and volume fractions (0.11, 0.28 and 0.61 for the anaerobic, anoxic and aerobic, respectively). Complete mixing conditions in the two unaerated compartments were established by mechanical mixers, whereas the aeration in the aerobic zone functioned as oxygenation and a mixing device.

The custom-made membrane module used for this investigation was a ZeeWeed® hollow fiber membrane, provided by Zenon Environmental Inc. (Oakville, Ontario, Canada). The membrane fiber had a nominal pore size of 0.04 μm and the module featured 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 the permeate stored in the permeate tank. It follows that the membrane module was extracting permeate at a slightly higher flow rate than the influent flow rate, to compensate for the backflush operation. Permeate was also recycled back to the aerobic compartment as required, to maintain a constant water level at all times. Intermittent coarse-bubble aeration (10 sec ON and 10 sec OFF) was provided to the module, at an air flow of 0.34 m^3/min (12 scfm). This helped to reduce the cake of solids on the membrane surfaces and to prevent the packing of solids between the membrane fibers. The amount of air provided to agitate the membranes was also sufficient to satisfy the biochemical oxygen demand, while keeping the aerobic zone dissolved oxygen (DO) level between 2.5 and 3.5 mg/L (the DO was kept comparable in both systems). When the transmembrane pressure exceeded the recommended value of 65 kPa (0.65 bar), the module was replaced by a module that had been previously restored through soaking in solutions of concentrated citric acid and sodium hypochlorite.

2.2.3 Operating conditions of the comparative study

The two processes were investigated at operating HRTs of 10 and 7 hours, corresponding to an influent flow rate of 5.34 and 7.63 m^3/d respectively, while keeping the SRT fixed at 12 days. The selection of the first experimental set point (i.e. HRT of 10 h and SRT of 12 days) was representative of the current design practice for conventional BNR processes in Western Canada. With the research objective of demonstrating the MEBPR process performance relative to that of CEBPR counterpart, this set of conditions was deemed to be the most appropriate with which to

start the comparative study. The second experimental run, at a 7 hour HRT, aimed to assess the response of both processes when challenged with higher hydraulic loads (which represent an attractive operating window for membrane systems).

If the definition and control of the SRT in MBR systems are clear-cut, the same is not true for conventional processes, where the ambiguity lies on whether or not to include the biomass inventory carried at the bottom of the clarifier (i.e. sludge blanket). For the present study, it was critical to carefully determine which approach would align best with the requirement of identical operating conditions. The portion of biomass held in the sludge blanket undergoes hydrolysis and endogenous biological activity, helping to sustain the viability of the microorganisms (Van Loosdrecht and Henze, 1999). Therefore, it seemed more appropriate to incorporate the clarifier sludge blanket biomass in the calculation of the CEBPR SRT. The amount was estimated by measuring the solids concentration after complete mixing of the clarifier and from measurement of the working volume of the clarifier. The final component of the CEBPR SRT calculation was the biomass unintentionally lost with the secondary effluent. The following expressions were applied for the calculation of the daily wasting volume (Q_W) withdrawn from the aerobic zone of the two systems:

MEBPR: $$Q_W = \frac{\sum_{i=1}^{3} V_i \cdot X_i}{X_{AER} \cdot SRT}$$

CEBPR: $$Q_W = \frac{\sum_{i=1}^{3} V_i \cdot X_i + V_{CL} \cdot X_{CL}}{X_{AER} \cdot SRT} - \frac{Q_{IN} X_{EFFL}}{X_{AER}}$$

where V_i and X_i are the volume and solids concentration in each reactor zone, V_{CL} and X_{CL} the volume and solids concentration of the clarifier, X_{AER} the solids concentration in the aerobic zone, Q_{IN} the process influent flow rate, and X_{EFFL} the suspended solids concentration in the secondary effluent.

In addition to sharing the same primary effluent, the MEBPR and CEBPR processes were operated at identical HRT, SRT, internal recycle ratios, dissolved oxygen concentrations, and ambient temperatures. The only differences permitted were the solids-liquid separation technology employed and the air bubble size generated by the two different aerator devices.

2.2.4 Transition period

When the one-year long comparative study was completed, the CEBPR train was retrofitted to become a second MEBPR system, according to the methodology described above. Without changing the operating conditions, the new process differed only in the way the treated water was separated from the mixed liquor. By monitoring the performance of the conventional EBPR sludge over a 4-month period as it evolved toward a membrane sludge, an ideal experimental point was created to further explore the impact of solid-liquid membrane separation on the BNR process.

2.2.5 Monitoring and analytical procedures

The following operating data were monitored daily: influent and effluent temperature, pH in the influent and in each reactor zone, wasting volumes, and dissolved oxygen (DO) levels in the aerobic zones. Temperature and pH were measured using the portable VWR probe, whereas dissolved oxygen concentration was monitored by a dedicated *in situ* YSI probe. Approximately 1.5 Kg of sodium bicarbonate was added once a day to each external storage tank to prevent the aerobic pH from dropping as a result of the nitrification activity and related alkalinity consumption. In addition, all flow rates on each process were checked at least every 10 days with the help of a graduated cylinder and a timer.

A systematic sampling and analytical program was developed for long-term monitoring of process performance. Grab samples of the influent and effluent (i.e. permeate for MEBPR, and clarifier effluent for CEBPR process) were collected 5 days per week and analyzed for total

and volatile suspended solids (TSS and VSS), total chemical oxygen demand (COD), soluble COD, volatile fatty acids (VFA) comprising acetate and propionate (only for influent), total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH_4-N), nitrite plus nitrate nitrogen (NO_X-N), total phosphorus (TP), and orthophosphate phosphorus (PO_4-P). Mixed liquor samples were collected three times a week for each compartment for the entire investigation period and analyzed for TSS, VFA (only anaerobic zone), NH_4-N, NO_X-N, and PO_4-P. During selected periods, the measurement of sludge total COD, TKN and TP was carried out for mass balance calculations. All analyses were performed according to Standard Methods (APHA *et al.*, 1998).

With the only exception of the MEBPR effluent, all other samples required filtration to generate samples for the measurement of soluble parameters. The filtration method employed a two liter reactor in which a ZeeWeed® membrane module (ZW-1, nominal pore size of 0.04 μm) was immersed with a vacuum applied by means of a peristaltic pump. Air or nitrogen gas was bubbled through the sample to keep the suspension mixed and to control the formation of cake solids on the membranes. Before collecting a representative sample, a filtrate volume of 500 mL was discarded to flush away the old sample entrapped in the membrane fibers, module header, and pump tube. This filtration method requires between 5 to 10 minutes per sample and was found to be economically advantageous over the use of filter papers with pore size of 0.45 μm or less.

2.3 Results and discussion

The comparative pilot-plant study was completed at two distinct HRTs of 10 and 7 hours. For the purpose of discussing the performance of the parallel MEBPR and CEBPR processes, it was useful to divide the operation into five periods as illustrated in Table 2.2. At the onset of the comparative study, both trains relied on the VFA naturally present in the incoming wastewater and the processes were operated with an aerobic recycle ratio fixed at 2, to take full advantage of

the denitrification potential (Period I). At the second instance of phosphorus breakthrough (see below), it was decided to insure complete removal of nitrates in the anoxic zone by reducing the aerobic recycle ratio from 2 to 1 (Period II). As a result of a period during which both processes failed to remove phosphorus satisfactorily, the ratio of VFA to total phosphorus in the incoming wastewater was raised from 5 to 10 by adding external sodium acetate (corresponding to a concentration of 20 mg COD/L) (Period III). During this period, the aerobic recycle ratio was re-set back to 2. After 186 days, the research program entered the 7-hour HRT phase with all other operating parameters kept constant (Period IV). Following a third instance of EBPR failure, it was decided to further raise the external acetate addition to 30 mg COD/L and to bring the aerobic recycle ratio back to 1, to protect the EBPR mechanism from the confounding effect of nitrates (Period V). A transition period followed the comparative study in which the secondary clarifier on the conventional train was decommissioned and a membrane module was put into operation in the aeration tank.

2.3.1 Solids profile and sludge inventory

As discussed earlier in the text, the presence of a membrane filtration unit in the aerobic compartment of a UCT-type process caused the biomass to distribute differently from that of the conventional UCT train. To maintain a similar biomass distributed among the three compartments of both trains, the return activated sludge (RAS) of the CEBPR system was discharged to the aerobic zone. Figure 2.2a presents the average TSS concentration profiles for Period I, when the anoxic and aerobic recycle ratios were set at 1 and 2, respectively. It can be noted that the two processes exhibited similar biomass concentration profiles, with the highest TSS levels in the aerobic zones and the lowest in the anaerobic zones, in accordance with the solids mass balance of the system. The ratio of the aerobic VSS/TSS was found to be comparable in the two systems over the entire duration of the study, with an average value of 0.83. The

MEBPR train carried a slightly higher solids concentration in each reactor zone than did the CEBPR counterpart. Since the sludge was intentionally included in the secondary clarifier for the calculation of the daily CEBPR sludge wasting, the three CEBPR reactor zones hosted a resulting total mass of solids which turned out to be 80% of that on the MEBPR side. The last bar of Figure 2.2a shows the average TSS concentration of both clarifiers after complete mixing, and this mass accounted for the remaining 20% of the total biomass in the CEBPR system. It follows that, while the MEBPR bioreactor SRT was somewhat close to setpoint SRT value, the CEBPR bioreactor SRT was only about 80% of the setpoint 12 day value. This first fundamental process difference had an impact on P removal, as well as on the observed sludge yield as described in the following sections. A complete comparison of the TSS measurements in the bioreactors of the two systems is presented in Figure 2.2b. It can be noted that nearly all the points lie below the diagonal line, indicating that the MEBPR process carried higher TSS levels at almost any given time. The significant differences in the suspended solids concentrations were confirmed through paired *t*-tests (Gilbert, 1987) at a 99% confidence level. The scatter plot of Figure 2.2b gives an indication of the level of association between the TSS of the two reactors. This is quantified with the *Pearson product-moment coefficient of linear correlation* which is normally referred to as correlation coefficient (Wilks, 1995). In this study, the correlation coefficient was calculated for each reactor zone and it was found to range between 0.72 and 0.80, with the higher correlation for the aerobic TSS measurements.

A complete sludge inventory for each system was estimated over the entire comparative study (Fig. 2.3). The graph clearly illustrates that the CEBPR process carried anywhere from 20 to 25% less biomass in the three reactor zones, than did the MEBPR train. The remaining biomass was located in the sludge blanket in the clarifier, bringing the total sludge inventory to similar levels in both systems. Of the total mass present in the MEBPR and CEBPR bioreactors, about 75% was present in the aerobic zone, 21% in the anoxic zone and the remaining 4% in the

anaerobic zone. For the MEBPR process operated at an SRT of 12 days, the above mass fractions translate into aerobic, anoxic and anaerobic zone SRTs of 9, 2.5, and 0.5 days, respectively. On the other hand, the CEBPR process SRT was set around 9.5 days (80% of the total 12 days) which is distributed as 7.1 days aerobic, 2 days anoxic, and 0.4 days anaerobic. The sludge mass fractions for the present comparative study and the corresponding zone SRTs are skewed toward high aerobic values, thus allowing stable nitrification even at low temperature. As reported by Grady *et al.* (1999), an anaerobic SRT of about 0.5 days at 20 °C represents the minimum value for EBPR processes that receive an influent wastewater with a sufficient concentrations of VFA and readily biodegradable organic matter. In addition, a total anaerobic and anoxic SRT of 2 to 3 days should favor the hydrolysis of slowly biodegradable substrate which is beneficial to the denitrification process. If, however, the total anaerobic and anoxic SRT for the present study was within the recommended range, the anaerobic SRT only was close to the minimum value of 0.5 days which can be problematic when the VFA/P ratio in the influent is low.

The two parallel processes exhibited a clear difference in the propensity to form a layer of foam in the anoxic compartment. Although the surface of the CEBPR anoxic zone was basically free from any floating material at all times, the MEBPR counterpart was consistently covered by a thick layer of sludge that would accumulate after re-suspending it daily by mechanical mixing. The foam appeared as a concentrated floating sludge with TSS concentrations reaching values as high as 60 g/L. This dense mass was estimated by individually measuring the TSS in the three compartments before and after re-suspending the foam in the anoxic zone (Monti *et al.*, 2005a, see Chapter 7), and it accounted for about 8% of the overall biomass present in the MEBPR system (Fig. 2.3).

2.3.2 COD removal

2.3.2.1 Mass balances

In a COD mass balance of an activated sludge system, the COD entering via the influent should be equal to the summation of the COD leaving with the effluent, associated with the wasted sludge, oxidized through denitrification, and oxidized with oxygen consumption in the aerobic zone. The latter was estimated from the total oxygen utilization rate (OUR), after deducting the oxygen required for nitrification. The total OUR was calculated from measurement of the dissolved oxygen decline over time after stopping the air supply in the aerobic zone. The average results of the COD mass balances for the parallel MEBPR and CEBPR, over the five investigated periods, are presented in Table 2.3.

The COD recovery ranged between 78 and 92%, with the lower values being associated with the 10 h HRT periods (I, II, III). Similar recoveries have been documented in the literature for BNR processes (Barker and Dold, 1995, Hu *et al.*, 2003) with the "missing" COD being attributed to fermentation processes occurring in the anaerobic zone. Although direct evidence to support this hypothesis was not generated in the present study, the COD recovery improved during the higher rate operating conditions. As the anaerobic HRT was reduced, it was expected that less fermentation would take place in the first zone, therefore reducing the loss of COD under anaerobic conditions.

The particulate COD leaving with the CEBPR clarifier overflow was included in the category "waste" in the mass balance. The rationale was that the CEBPR wasting volume was calculated with the inclusion of the solids unintentionally lost with the effluent. In addition, and more importantly, a fair comparison of the effluent quality between the two systems required the elimination of the suspended solids bias on the CEBPR side. Having said that, the MEBPR process exhibited a consistent tendency to produce a significantly lower COD in the sludge waste stream, which was related to a smaller observed yield value (see below). The reduced sludge

production, together with the lower mass of COD consumed for denitrification, resulted in a greater amount of COD being oxidized in the MEBPR aerobic zone (i.e. oxygen). As it will be presented in the N removal section, the CEBPR process benefited from the extra denitrification potential occurring in the secondary clarifier. The COD leaving with the soluble portion of the effluent was found to be comparable in the two systems.

2.3.2.2 Performance and correlation

Figure 2.4 presents the total influent COD and the soluble effluent COD for both processes, over the entire research program. The significant decrease in the influent COD on day 100 resulted from a change in the filling frequency of the external storage tanks. During Period I, fresh sewage was pumped into the tanks twice a day, at 7 am and at 7 pm. In anticipation of the planned lower HRT operation, it was necessary to increase the filling frequency to four times a day, to provide sufficient feed to the pilot plant. The new late night and early afternoon filling cycles served to dilute the wastewater and it negatively affected the COD/TKN and COD/TP ratios. The MEBPR soluble COD taken straight from the permeate tank was compared with the CEBPR COD sample filtered off-line through a membrane with the same pore size. From the COD profiles shown in Figure 2.4, it was calculated that both processes achieved an average COD removal above 90% at all times, with the exception of Period V, when the COD in both effluents was measured at higher levels. With the replacement of the secondary clarifier with a membrane module (i.e. transition), the effluent quality (expressed as soluble COD) stabilized at lower levels. It remains difficult to explain why the same removal efficiency was not observed in the MEBPR train during the previous Period V. It is possible that large COD measurement errors may have characterized the batch of samples collected in this experimental run.

A more detailed comparison of the COD effluent data for the 10-hour and the 7-hour HRT is illustrated in Figure 2.5a. For the longer HRT, the box-plot graph shows that the

distributions of the measured soluble COD data were similar, though the CEBPR samples exhibited higher maximum values. A paired *t*-test confirmed a significant difference in the average soluble COD concentrations at 99% confidence level. Similar conclusions were reached also by other authors (Ng and Hermanowicz, 2005; Cicek *et al.*, 1999) who pointed to the smaller particle size of the MBR sludge and the consequent improved mass transfer as a probable reason for the better substrate removal. On the other hand, when both systems were operated at the shorter HRT of 7 hours, the two average soluble COD concentrations in the effluents were comparable. When the effluents are compared using the total COD data, the MEBPR significantly outperformed the CEBPR system, due to the complete retention of the suspended solids by the membrane module.

To assess the level of correlation between the two time series of soluble effluent COD data, the CEBPR values were plotted against the MEBPR values in the scatter plot of Figure 2.5b. As can be observed, the paired data distributed almost evenly between the two halves of the graph with a relatively weak correlation ($r = 0.52$). Therefore, it can be concluded that, although the two data sets generated comparable averages and ranges over the entire period, the actual removal pattern is not quite the same. This seems to indicate that the system dynamics in COD removal were different in the MEBPR and CEBPR processes, likely due to distinct microbial communities that catalyze the reactions.

2.3.2.1 Sludge yield

From the extensive data set available for each experimental run, it was possible to calculate the observed sludge yield with the cumulative method. The total mass of suspended solids generated with the wasting, and corrected for the accumulation in the system, was divided by the total mass of COD removed over the duration of each experimental period. The results and the comparison between the two systems are illustrated in Figure 2.6. It is worth pointing out

that, for an objective comparison, it is imperative to include the total mass of suspended solids generated with the secondary effluent, as a part of the CEBPR sludge production calculation. Although this portion of sludge may not always be considered when calculating the sludge yield in a full scale WWTP, it has been included here to produce an exact estimate of the sludge production in both systems. From Figure 2.6, it can be seen that the MEBPR process exhibited values in the range of 0.23 to 0.28 g VSS/g COD, and that these values were about 15% lower than those observed in the CEBPR counterpart, during the five experimental periods. In addition, a decrease in the sludge yield was measured at the end of the CEBPR to MEBPR transition period, with the final value falling in the same range as that previously observed on the MEBPR train. As reported earlier, the MEBPR bioreactor operated at an SRT close to the nominal value of 12 days vs. the 9.5 days of the CEBPR counterpart; this had a clear impact on the food to microorganisms ratio in the two processes, therefore, to the respective growth yield. It was estimated that the contribution of this difference to the observed sludge yield might amount to about 10% of the measured 15%. In addition, the larger aerobic mass fraction of the MEBPR system compared to the CEBPR system was hypothesized to have increased the overall biomass decay rate (Siegrist *et al.*, 1999), hence reducing the observed sludge production to the remaining 5%. Some research studies reported in the technical literature have also observed larger sludge yields for CAS systems than for MBRs (Ghyoot and Verstraete, 1999), while other comparative studies have demonstrated just the opposite (Cicek *et al.*, 1999; Wei *et al.*, 2003; Holbrook *et al.*, 2005). The source of the contradiction could be attributed to the different system configurations and operating conditions used in these studies. Moreover, the decision of whether or not to include the suspended solids associated with the secondary effluent, in the calculation of the sludge yield, may lead to divergent conclusions.

2.3.3 N removal

2.3.3.1 Mass balances

In the N mass balance, the total N entering an activated sludge system via the influent as TKN should be equal to the total N leaving with the effluent as TKN and NO_X-N, the NO_X-N denitrified to N_2 gas, and the N in the waste sludge stream. The average results for the parallel MEBPR and CEBPR processes over the five experimental periods are presented in Table 2.4. A very high recovery was obtained for the N balances around both systems, with values spanning from 95 to 99%.

In any single experimental period, the CEBPR process exhibited a greater potential for the nitrogen removal than did the MEBPR. Through mass balance measurements around the clarifier, it was concluded that an extra sink for nitrate existed in the clarifier sludge blanket, in which about 10% of the total incoming N was denitrified. Denitrification in secondary clarifiers has been well documented in the technical literature (Siegrist and Gujer, 1994) and it is attributed to anoxic endogenous respiration. In a UCT-type process, the replacement of the secondary clarifier with membrane technology entails the loss of this additional N removal; this can be desirable to safeguard the anaerobic zone from accidental nitrate leaking. The amount of effluent N presented in Table 2.4 includes soluble TKN and the NO_X-N. The variation of this quantity over the experimental periods is related to the extent of denitrification achieved in the process. As a result of the extra N removal in the clarifier, the CEBPR process generated a consistently lower mass of soluble N in the effluent, compared to the MEBPR counterpart.

As in the case of COD balance, the N associated with the suspended solids escaping in the secondary effluent were accounted for in the "waste" category of the mass balance. Similarly to the observation above for COD, the MEBPR process exhibited a lower removal of N through the daily waste sludge stream, confirming the earlier observation of reduced sludge yields.

2.3.3.2 Performance and correlation

The time series of influent TKN and the effluent nitrate and ammonia concentrations leaving both systems are presented in Figure 2.7. It can be noted that both the MEBPR and the CEBPR processes achieved complete nitrification with measured effluent NH_4-N concentrations that were below the detection limit. The large fraction of aerobic sludge favored the establishment of a robust nitrifying community at all times, even during periods of colder temperatures (14-15 °C) and at the shorter setpoint HRT. Stable nitrification in MBR systems has also been reported in other studies over a wide range of operating conditions (Muller *et al.*, 1995; Fan *et al.*, 1996; Liebig *et al.*, 2001; Fleisher *et al.*, 2005). The only significant difference in ammonium profiles was noticed during the coldest week of the study, when the MEBPR effluent ammonia exhibited a spike with values ranging from 0.45 to 2.10 mg N/L. With the use of off-line nitrification batch tests, it was observed that the MEBPR sludge exhibited a significantly lower potential to nitrify (Monti *et al.*, 2005b, Chapter 4), which may explain the temporary reduction in the nitrification performance. The larger amount of N denitrified in the CEBPR system produced a consistently lower nitrate concentration in the treated effluent over the entire comparative study. The subsequent transition period confirmed that the introduction of the membrane module in the CEBPR system did not compromise the ability of the activated sludge to achieve complete nitrification under pseudo-steady state operating conditions. However, a sudden increase in the nitrate levels was observed in the treated effluent, confirming the inherent loss of additional denitrification that comes with membrane solids-liquid separation equipment.

A summary of the nitrate data during each experimental run during the comparative study is illustrated in Figure 2.8a. A paired *t*-test confirmed that the difference in the MEBPR and CEBPR nitrate levels was significant at a 99% confidence level at both operating HRTs. In general terms, the denitrification of nitrates in the sludge blanket depended upon the operating conditions in the secondary clarifier (i.e. RAS). In this study, an average of 3 mg N/L reduction

was observed in the clarifier, relative to the aerobic compartment. Slightly higher nitrate concentrations in MBR effluent have also been reported by other researchers (De Wever *et al.*, 2004; Ng and Hermanowicz, 2005), although, in those studies, no measurements were made to quantify the N removal in the sludge blanket. The bonus of extra denitrification in the clarifier is counterbalanced by the presence of suspended solids in the clarifier overflow, which elevates the level of total nitrogen (TN) in the secondary effluent. During the 10 hour HRT run, the TN was comparable in the two final effluents, whereas it was significantly lower in the CEBPR effluent at the shorter HRT thanks to the larger denitrification achieved in the last phase (Fig. 2.8a).

The effluent nitrate concentration data for the MEBPR and CEBPR system are plotted in Figure 2.8b, to indicate the degree of correlation between the parallel processes over the course of the study. The large majority of the paired data occupies the lower portion of the graph and the points are distributed in a narrow region with a relatively high correlation ($r = 0.85$). It can then be concluded that the MEBPR and CEBPR systems display similar behavior in nitrogen removal, with lower effluent concentrations in the CEBPR train, due to the extra nitrate sink in the secondary clarifier.

From the measurement of ammonia and nitrate concentrations within the anaerobic and anoxic compartments of the two bioreactors, it was possible to gain additional insights on the level of similarity of these nitrogenous compounds between the two systems. From Figure 2.9a, it can be observed that the ammonia concentrations in the two processes were highly correlated ($r = 0.93$) with the majority of the points sitting marginally above the diagonal. Paired *t*-tests confirmed that the average concentrations in the CEBPR anoxic and aerobic zones were significantly greater than those of the MEBPR at a 99% confidence level. The foregoing seems to indicate that subtle, but consistently significant differences, exist in the unaerated zones (e.g. ammonification process) that affect the level of ammonia. For the nitrates in the anoxic zone, Figure 2.9b shows that most of the measured data were below 0.5 mg N/L, which is

recommended if an efficient bio-P process is sought. However, several instances of higher nitrate levels were experienced in both systems, particularly when the aerobic recycle ratio was set at 2. Under these circumstances, the MEBPR process was observed to operate with higher residual nitrate concentrations in the anoxic zone than the CEBPR, likely due to the overall reduced dentrification potential (as discussed earlier in the text).

2.3.4 Phosphorus removal

2.3.4.1 Performance comparison

In contrast to the consistent removal of COD and N that was observed, the removal of P was characterized by periods of good performance, followed by breakthroughs. Figure 2.10 presents the time series of influent TP and the effluent PO_4-P of the MEBPR and CEBPR processes over the five experimental periods. It can be noted that the prolonged periods of P-removal "failure" (labeled as 1, 2, and 3 in the graph) were observed concomitantly for both systems, though at different magnitudes. The similar trends in the MEBPR and CEBPR effluent phosphorus concentrations indicated that an external, rather than internal, cause contributed to the temporary suboptimal P performance.

Bio-P failure 1 & 2. From Figure 2.10, it transpires that the nature of the first two process upsets was linked to the unfavorable VFA to TP ratio in the incoming wastewater. As far as the first observation of P breakthrough is concerned, the subsequent increase of the ambient temperature (summer time) contributed to enhance the natural formation of VFA through the fermentation process occurring in the sewer line and in the external storage tank; this resulted in an overall improve in the performance of both systems. The second observation of process failure was recorded when the pattern of wastewater collection (in the external storage tanks) was changed to four filling cycles (from two) per day. The new early afternoon and late night pumping cycles resulted in a significant overall dilution of the wastewater and in a reduced

amount of available VFA. In addition, the smaller amount of biomass produced and the consequent reduced utilization of P for cell growth, left more P to be removed through the EBPR mechanism. At the end of Period II, it was decided to supplement the influent with 20 mg COD/L of external carbon in the form of sodium acetate, bringing the VFA to TP ratio to more favorable levels. Once this modification was implemented (Period III), both the MEBPR and CEBPR processes responded very quickly by delivering similarly low PO_4-P concentrations in the treated effluent. The relatively small anaerobic mass fraction of both process trains might have also contributed to the instability of the P-removal, when faced with a sudden decrease of carbon concentrations in the influent. Although instances of process failure were observed for both systems at similar times, the CEBPR process exhibited an average effluent PO_4-P that was significantly lower than the MEBPR counterpart. Assuming a value of 0.02 g P/g COD for biomass growth (Henze *et al.*, 2002), it was estimated that 55% of this difference was attributed to the higher sludge yield of the CEBPR, relative to the MEBPR system. Moreover, the tendency of the anoxic MEBPR zone to leave higher concentrations of nitrates (see Fig. 2.9b) resulted in some acetate being oxidized in the upstream anaerobic zone which, therefore, was unavailable for bio-P organisms for further P removal. This phenomenon is illustrated in Figure 2.11 where the concentration of PO_4-P and VFA in the anaerobic zone, corrected for the different biomass contents, are compared in the two systems. From the two scatter plots, it can be seen that the MEBPR sludge was exposed to lower levels of VFA (Fig. 2.11a), resulting in more limited specific P release than the CEBPR sludge. It can then be concluded that the reduced sludge yield and the limited denitrification in the MEBPR process are two significant aspects that require proper consideration, when a carbon limited wastewater is to be treated for biological phosphorus removal.

Bio-P failure 3 & 4. A third major P breakthrough was recorded for both systems after three weeks of operation at 7 h HRT, resulting in a total loss of EBPR activity. This observation

was recorded in conjunction with a period of abnormally cold weather that brought the process temperature suddenly from 17 to below 14 °C. The sensitivity of the EBPR kinetics to short-term temperature transients is well documented in the literature (Brdjanovic *et al.*, 1997). As it can be observed from Figure 2.10, the MEBPR system showed a much faster recovery (40 vs. 60 days) in activity, relative to the CEBPR system, likely caused by the ability of the membrane to retain all the organisms and by the slightly longer bioreactor SRT. This significant result suggests that a membrane-based EBPR process could also be characterized by a shorter start-up time, compared to the conventional system. The only event of clear dissimilarity in the P removal was recorded toward the end of the comparative study, when spikes of PO_4-P in the MEBPR effluent were not detected in the CEBPR counterpart. This process upset quickly passed and the lack of a reasonable explanation for it led to a further assessment of the MEBPR process under 7 h HRT, in the subsequent transition period.

The overall P removal performance during the comparative study suggested that the MEBPR process behaved very similarly to a CEBPR, with minor but significant differences only arising when carbon limited wastewater was treated. When the effluent quality was compared with total P data, the complete retention of solids in the final effluent gives a competitive advantage in achieving values as low as 0.1-0.2 mg P/L, compared to 0.7-1.0 mg P/L normally measured in the secondary effluent of conventional EBPR systems. A comparison of the PO_4-P and TP effluent data collected in this study is shown in Figure 2.12. It can be seen that, unlike the case of the CEBPR effluent, the TP of the MEBPR effluent is essentially the same as the PO_4-P. When a satisfactory performance of the EBPR process is achieved in an MBR reactor, the final effluent boasts a superior quality that is only possible with tertiary treatment in a conventional plant.

2.3.4.2 Transition period

In the context of P removal, the objective of the transition period was two-fold: (1) to evaluate the adaptation of a conventional EBPR process when upgraded with membrane technology and (2) to determine whether an MEBPR system can sustain long-term satisfactory bio-P performance at the HRT of 7 hours, or temporary instabilities such as "failure 4" are reproducible.

As can be observed in Figure 2.10, the replacement of the secondary clarifier with membrane filtration did not impact whatsoever the excellent bio-P performance of the EBPR sludge. It is, therefore, expected that the upgrading of full scale conventional BNR plants with membrane technology should result in a smooth transition and with similar performance. The stable performance observed throughout the four-month duration of the transition phase provided a clear demonstration that the MEBPR process is a reliable technology at an HRT of 7 hours which is considered somewhat impractical for a conventional EBPR process. Adam *et al.* (2002) and Lesjean *et al.* (2005) were among the first to demonstrate the successful operation of the EBPR process with membrane technology. However, the hydraulic loads under which their process was subjected were within the high range of 14-18 hours. In addition, they reported a significant portion of P (up to 45%) being sequestered through natural chemical precipitation, therefore, limiting the understanding of the full potential of the EBPR mechanism when coupled with a membrane process. More recently, Fleisher *et al.* (2005) reported a pilot-scale study on the coupling of MBR with the EBPR process, under an HRT of 9 hours. In this case, only the combination of biological and chemical precipitation (through alum addition) could guarantee a consistent effluent total P as low as 0.1 mgP/L.

2.4 Conclusions

The present study demonstrated that, in the absence of carbon-limiting conditions, an MEBPR system was capable of generating an effluent with PO_4-P levels that are comparable to those of a CEBPR and with a TP content that is drastically reduced, because of the complete retention of suspended solids. For the first time, it was also demonstrated that a membrane process can reliably sustain satisfactory EBPR performance at an HRT of 7 hours, conditions which are considered to be challenging for conventional BNR plants. According to this experimental work, the following additional conclusions were generated.

- The observed sludge yield of the MEBPR process was between 0.23 and 0.28 g VSS per gram of COD removed, 15% lower that that measured in the CEBPR at the operating condition assessed. It was estimated that a 10% reduction was associated with the slightly longer bioreactor SRT of the MEBPR system, whereas the remaining 5% was hypothesized to result from extended biomass decay, due to the larger aerobic mass fraction.
- Both the MEBPR and CEBPR processes were capable of achieving complete nitrification at all times. The CEBPR process benefited from the extra anoxic zone of the clarifier sludge blanket to produce an effluent with a significantly lower nitrate concentration. As a consequence, the main anoxic zone of the CEBPR train had the capacity to resist nitrate spikes more than the MEBPR train, limiting any carry over in the critical upstream anaerobic zone.
- When the influent VFA became limiting, both processes experienced difficulty in maintaining low PO_4-P concentration in the final effluent. However, overall, the CEBPR system achieved a significantly better P removal than the MEBPR due to the greater observed sludge yield and denitrification capacity; this led to a more efficient utilization of VFAs in the anaerobic zone. When a severe loss of bio-P was experienced, likely due to a

short-term temperature shock, the MEBPR train demonstrated a faster recovery of the bio-P activity than the CEBPR counterpart.

- Average COD removal efficiencies above 90% were achieved in both processes, with the MEBPR train exhibiting a better performance because of the effluent polishing. A weak correlation was found in the soluble COD of the two systems effluent.
- Unlike the CEBPR, the MEBPR anoxic zone consistently formed a thick layer of foam with suspended solids concentrations as high as 60 g/L and accounting for 8% of the total sludge inventory.

Table 2.1 UBC Pilot Plant primary effluent wastewater.

Parameter	Mean	95% Conf. Int.	Min - Max
TSS (mg/L)	90.5	±4.7	18 – 216
CODtot (mg/L)	307.3	±9.5	182.1 – 584.6
CODsol (mg/L)	96.5	±4.3	50.5 – 265.0
Acetate (mg/L)	26.8	±1.4	6.6 – 46.4
Propionate (mg/L)	3.6	±0.2	1.0 –12.7
Tot VFAs (mg COD/L)	34.1	±1.5	8.9 – 63.0
TKN (mg N/L)	33.6	±0.5	23.6 – 43.7
NH_4-N (mg N/L)	25.6	±0.3	19.5 – 34.9
NO_3-N (mg N/L)	Not detec.		
TP (mg P/L)	4.2	±0.1	2.4 – 6.9
PO_4-P (mg P/L)	2.4	±0.05	1.4 – 5.0
T (°C)	20.4	±0.3	14.0 – 23.6
pH	7.2	±0.03	6.6 – 7.9

Table 2.2 Experimental periods and relative operating conditions.

Period	Day	Temp.[1] (°C)	Operating paramters				
			HRT (h)	SRT (d)	Anx rec. ratio[2]	Aer rec. ratio[2]	HAc Addition (mg COD/L)
I	1 - 109	21.9 (0.21)	10	12	1	2	0
II	110 - 147	21.1 (0.45)	10	12	1	1	0
III	148 - 185	18.1 (0.45)	10	12	1	2	20
IV	186 - 236	16.3 (0.42)	7	12	1	2	20
V	237- 353	18.5 (0.36)	7	12	1	1	30
Transition	354 - 458	21.8 (0.26)	7	12	1	1	30

[1] Temperature expressed as average value with 95% confidence interval in parenthesis.

[2] Anx rec.: anoxic recycle; Aer rec.: aerobic recycle. The term ratio is defined as the recycle flow rate divided by the influent flow rate. The return activated sludge (RAS) from each clarifier was set to half the influent flow rate, therefore a total RAS recycle ratio of 1.

Table 2.3 **Averaged COD mass balances for the MEBPR and CEBPR systems over the five periods. Values in parenthesis are the 95% confidence intervals.**

	% of the total incoming COD				Recovery
	Oxygen	Denitrification	Waste	Effluent	%
MEBPR	27.2 (±4.5)	13.8 (±3.0)	38.4 (±4.0)	8.4 (±3.3)	87.8 (±6.1)
CEBPR	18.0 (±3.8)	15.5 (±2.8)	43.4 (±4.4)	9.6 (±3.6)	86.5 (±6.5)

Table 2.4 **Averaged N mass balances for the MEBPR and CEBPR systems over the five periods. Values in parenthesis are the 95% confidence intervals.**

	% of the total incoming N			Recovery
	Denitrification	Waste	Effluent	%
MEBPR	41.3 (±6.5)	19.9 (±2.5)	34.7 (±6.5)	95.9 (±1.6)
CEBPR	46.6 (±6.1)	22.5 (±2.9)	28.6 (±6.5)	97.7 (±1.4)

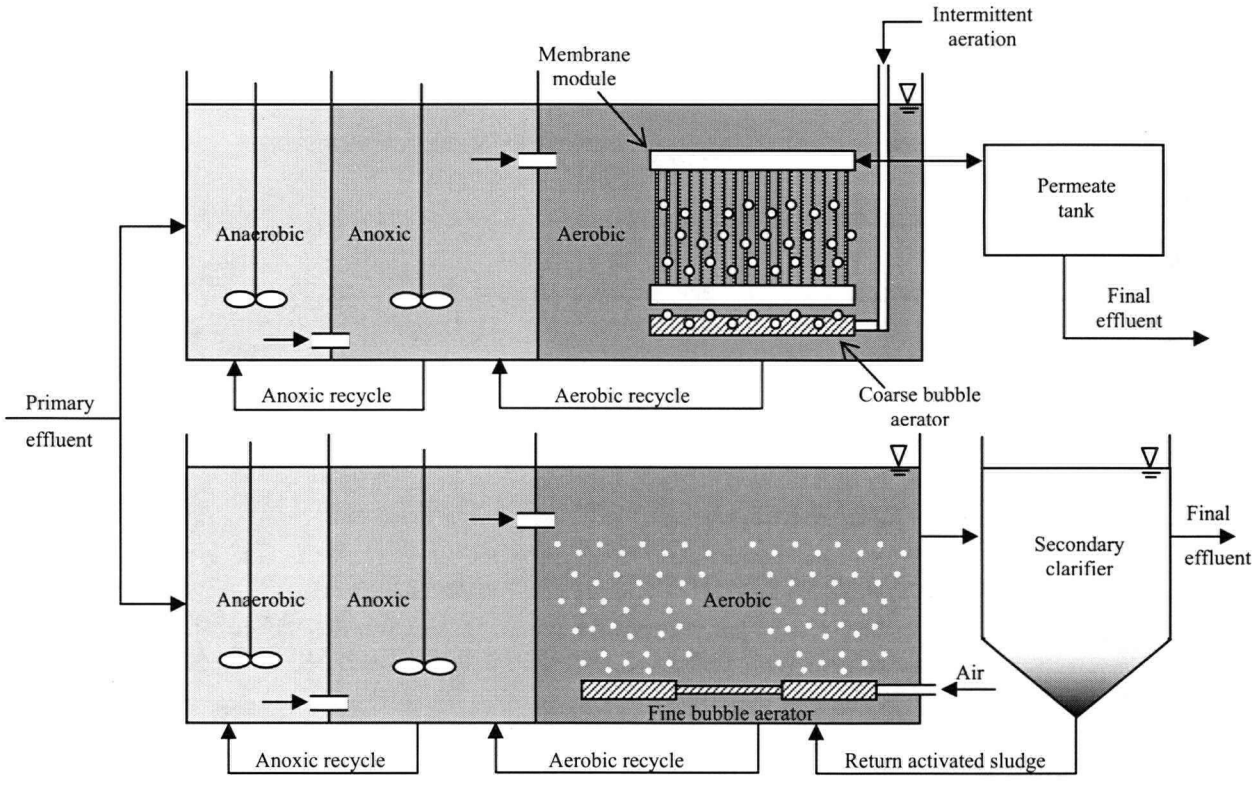

Figure 2.1 **Schematic of the parallel MEBPR (above) and CEBPR (below) system. Pilot plant tank dimensions: height, 130 cm; width, 60 cm; length, 30 cm for the anaerobic zone, 75 cm for the anoxic zone, and 170 cm for the aerobic zone.**

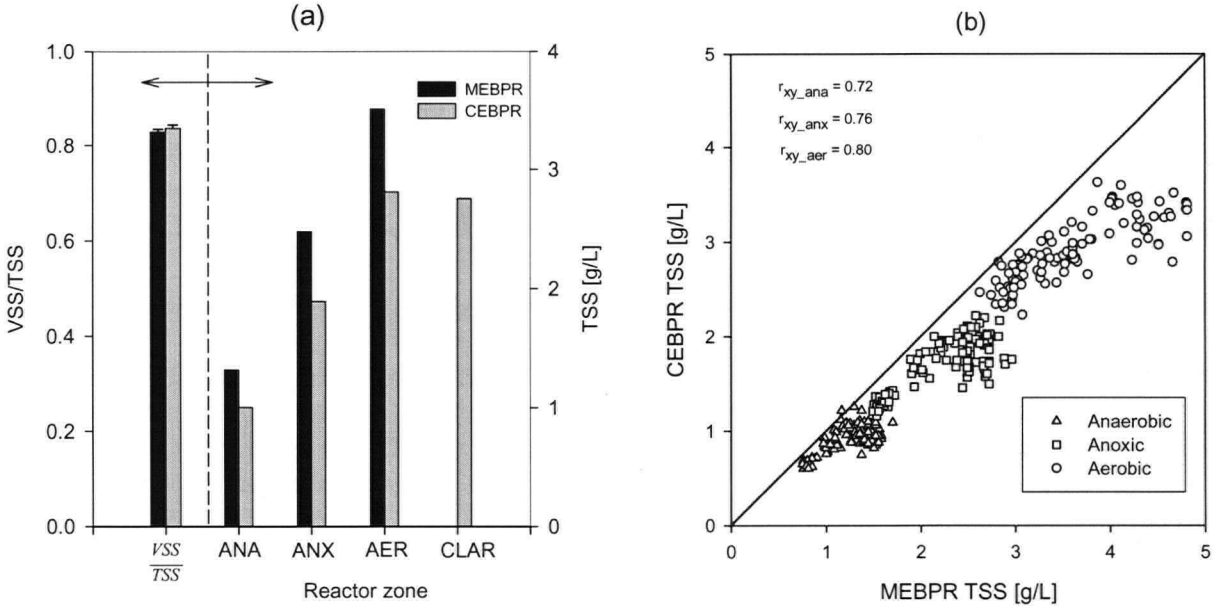

Figure 2.2 **(a) Average ratio of VSS to TSS of the aerobic mixed liquor with 99% confidence interval and TSS concentration in the three reactor zones, and clarifier during Period I. (b) Comparison of TSS level between the MEBPR and CEBPR systems over the entire duration of the comparative study.**

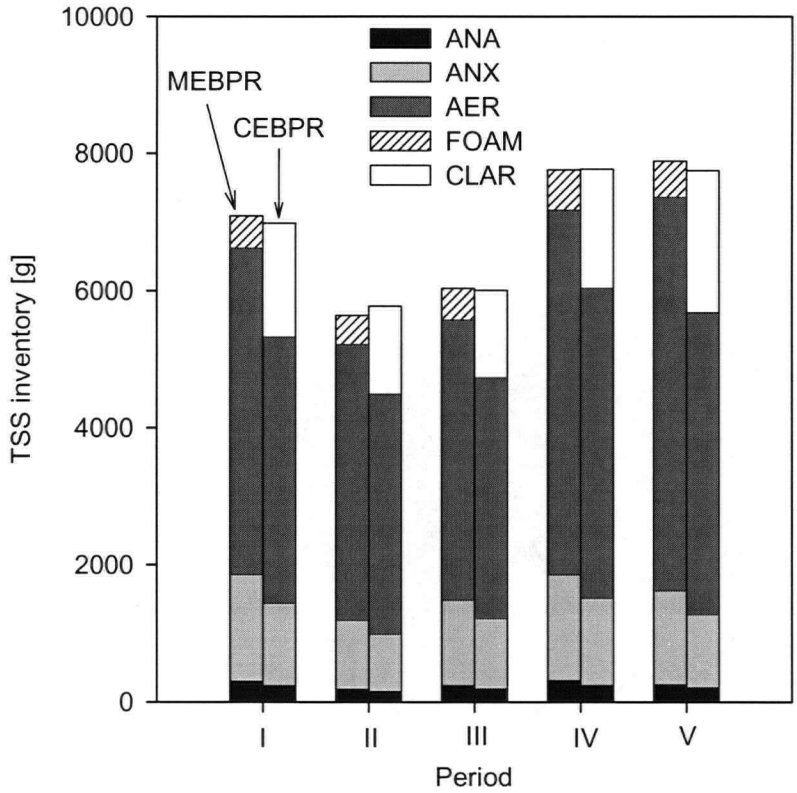

Figure 2.3 **Total suspended solids inventory in each compartment over the investigated periods. Ana: anaerobic, Anx: Anoxic; Aer: Aerobic; Clar: clarifier sludge blanket.**

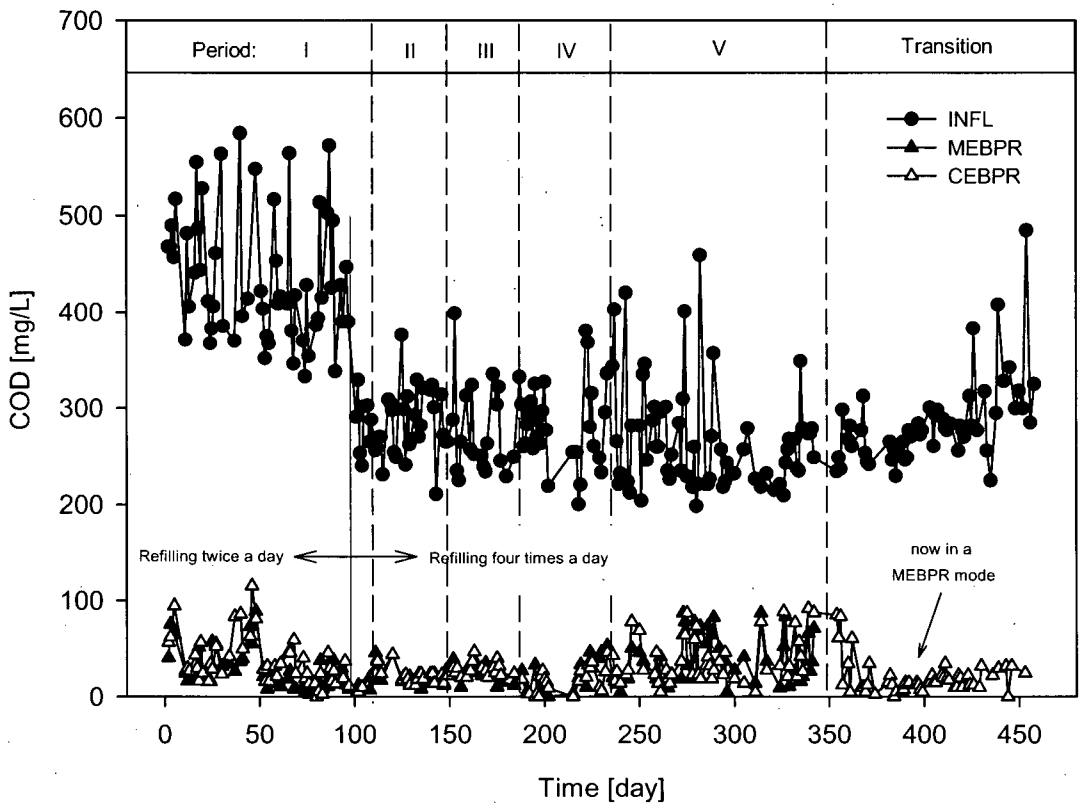

Figure 2.4 Total COD in the influent and soluble COD for the MEBPR and CEBPR effluent.

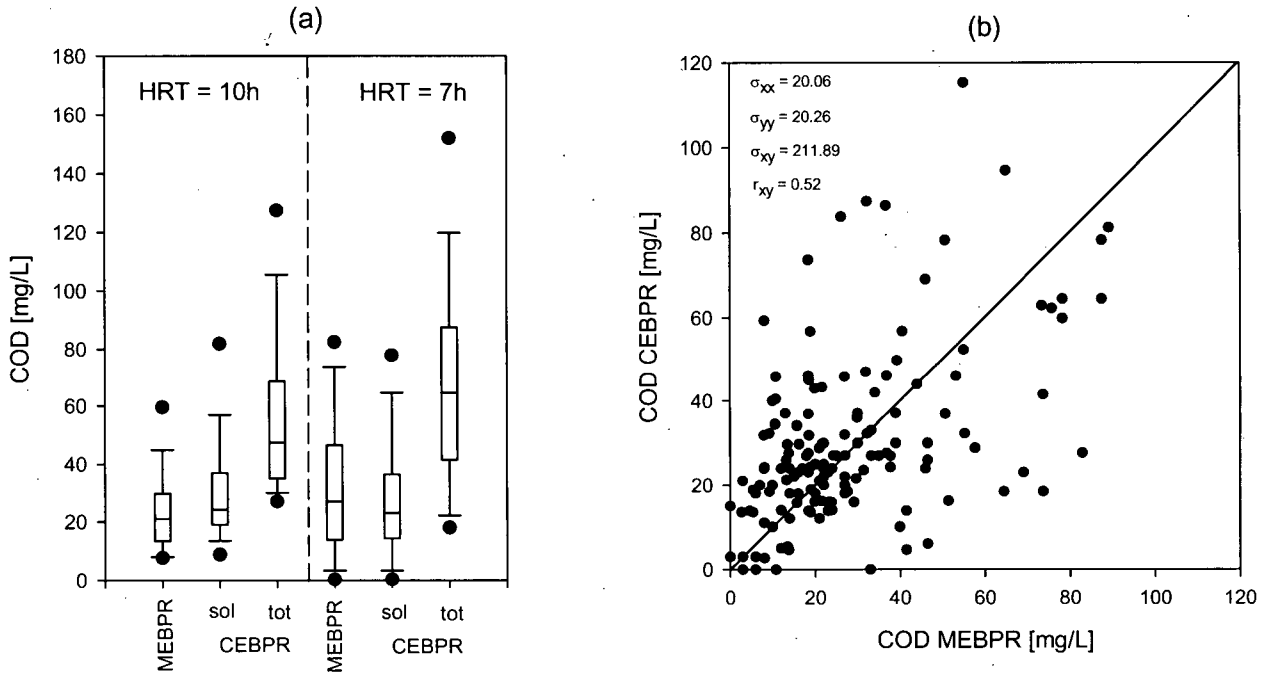

Figure 2.5 **(a) Box-plots of effluent COD. Percentile shown: 5th, 10th, 25th, 75th, 90th and 95th. The horizontal line inside the box represents the median (b) Comparison of soluble COD level in the effluent between the MEBPR and CEBPR systems over the entire duration of the study.**

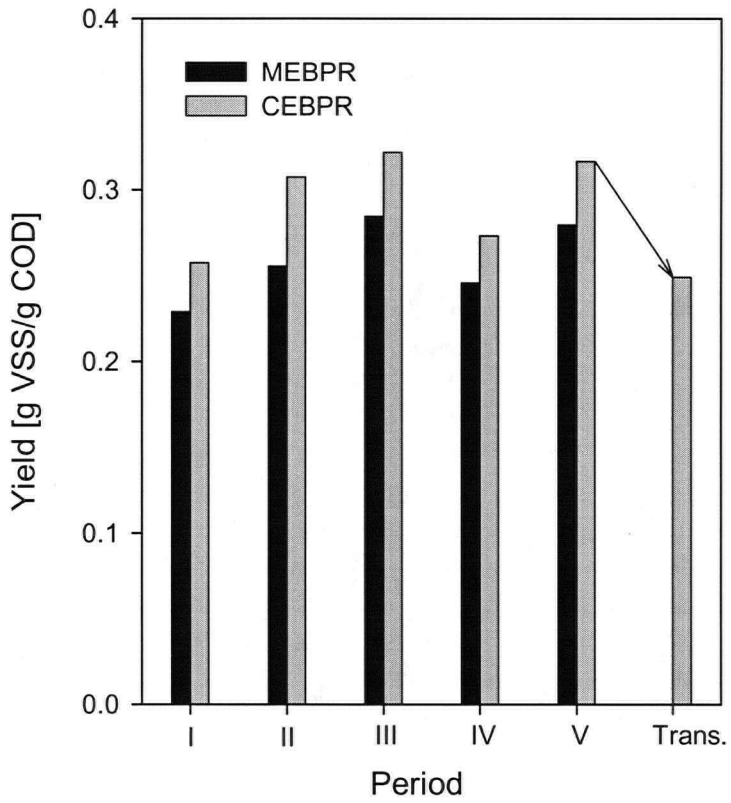

Figure 2.6 Comparison of observed sludge yield between the two systems and at end of the CEBPR to MEBPR transition Period.

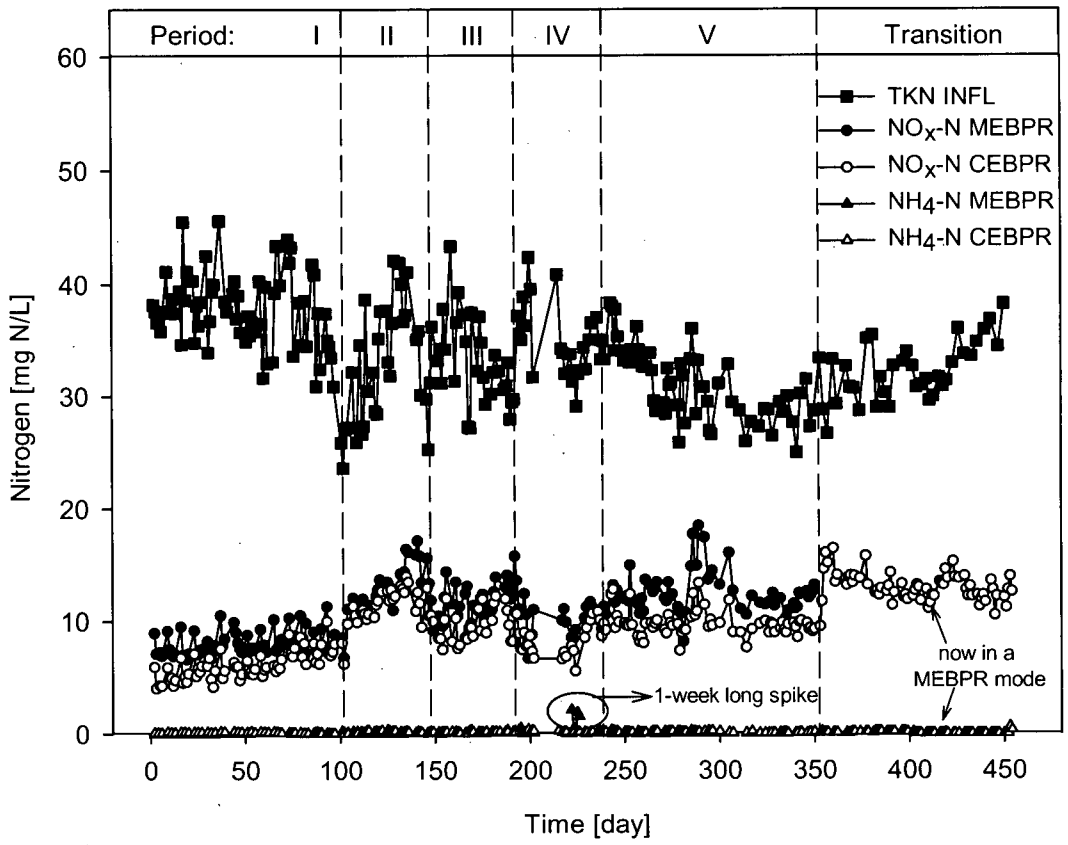

Figure 2.7 **Total nitrogen in the influent and ammonium and nitrate in the MEBPR and CEBPR effluent.**

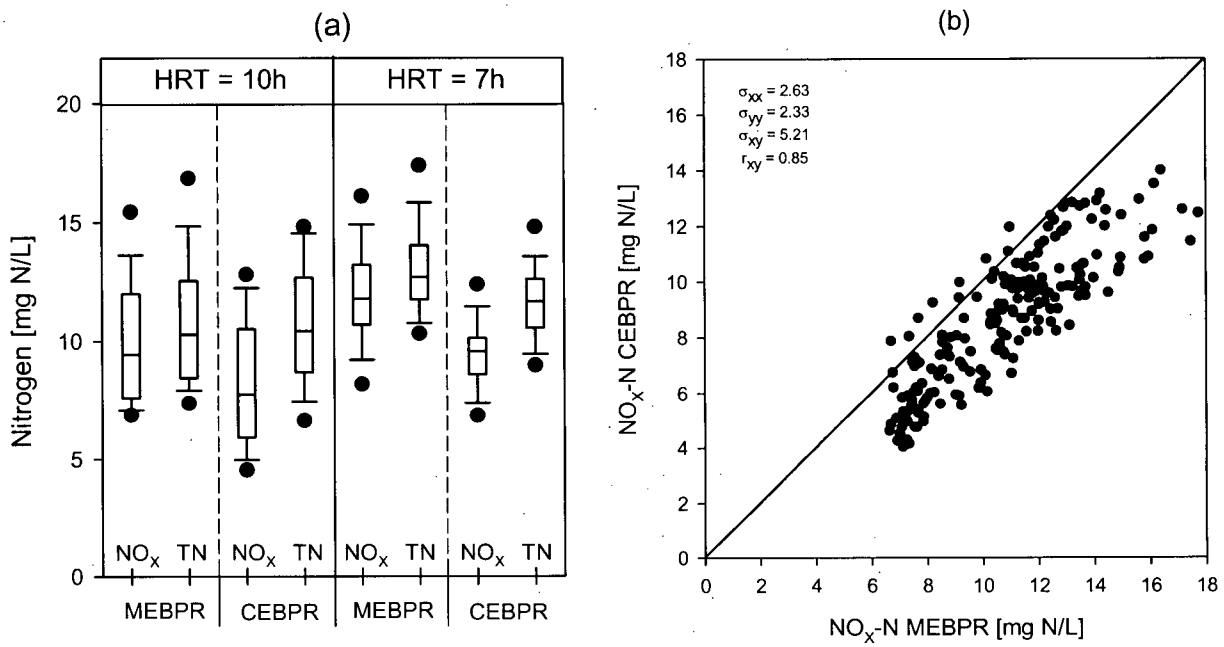

Figure 2.8 **(a) Box-plots of effluent nitrates and total nitrogen. Percentiles shown: 5th, 10th, 25th, 75th, 90th and 95th. The horizontal line inside the box represents the median (b) Comparison of nitrate level in the effluent between the MEBPR and CEBPR systems over the entire duration of the comparative study.**

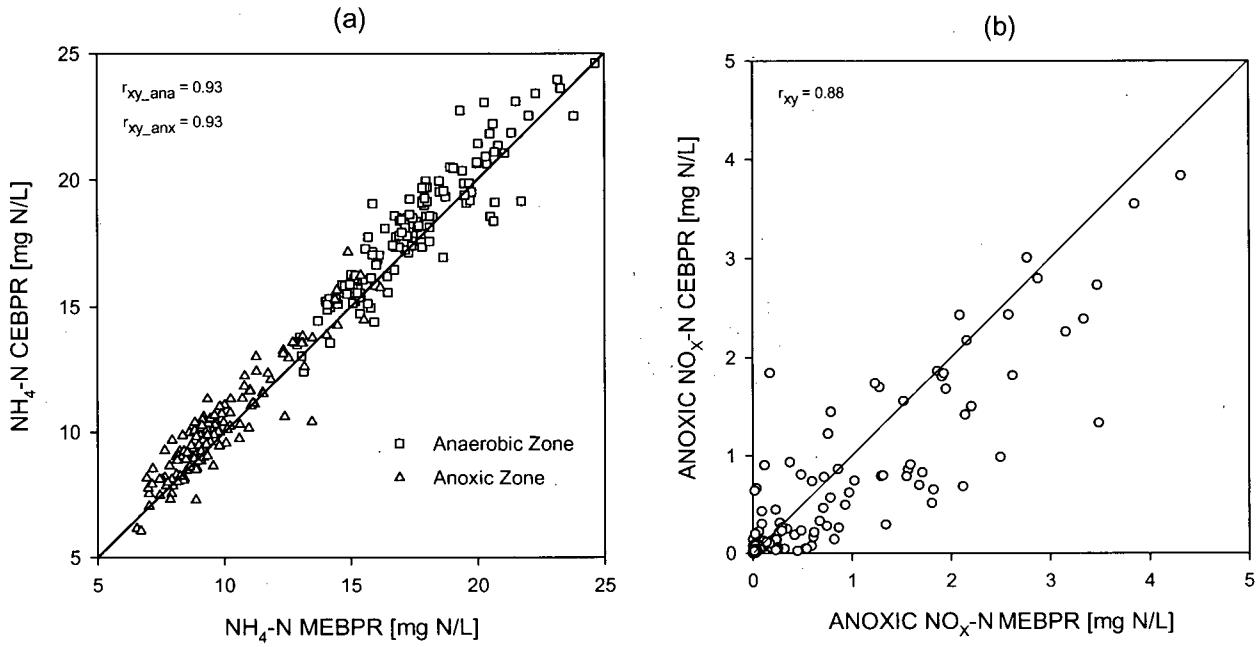

Figure 2.9 **Comparison of (a) ammonium concentrations in the anaerobic and anoxic zone and of (b) nitrates in the anoxic zone of the MEBPR and CEBPR systems.**

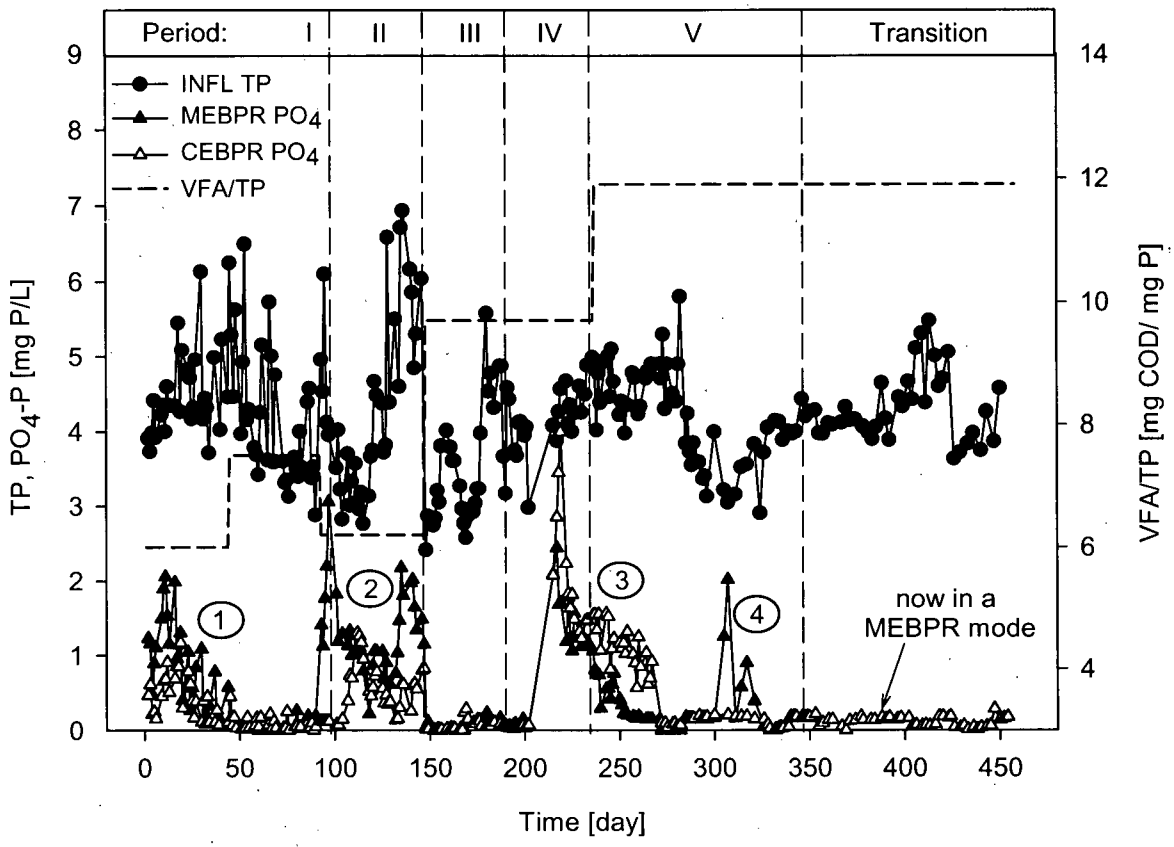

Figure 2.10 **Total phosphorus (TP) in the influent and orthophosphate (PO_4-P) in the MEBPR and CEBPR effluent. The dash line represents the VFA to TP ratio prevailing in the incoming wastewater.**

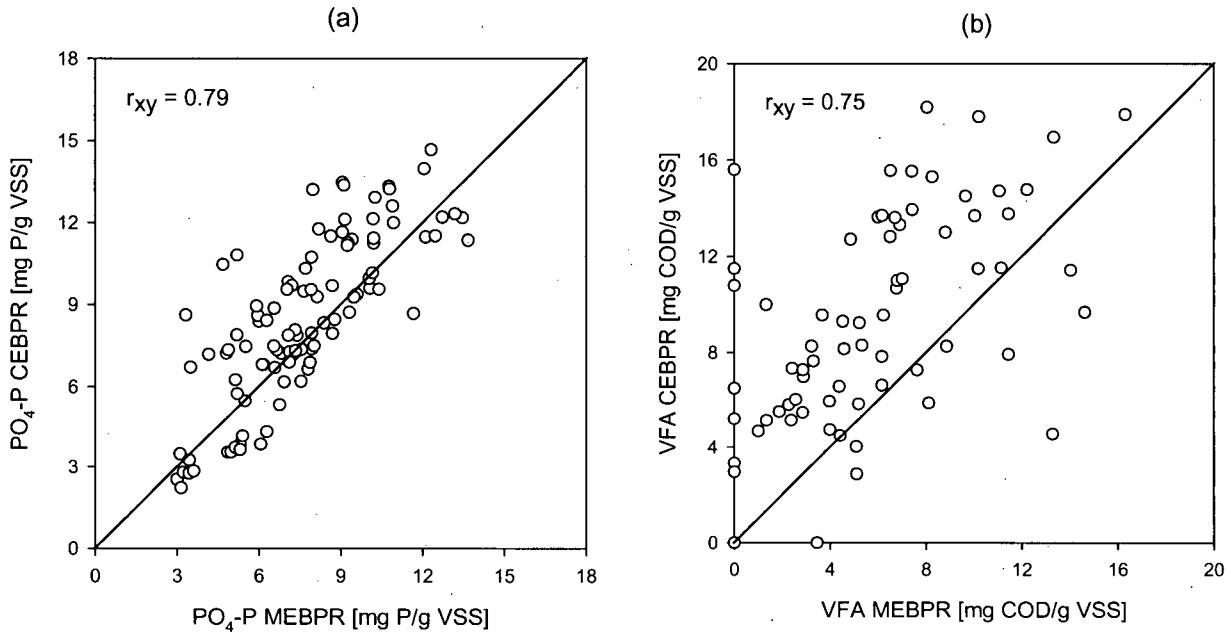

Figure 2.11 Comparison of (a) PO_4-P (b) VFA concentrations in the anaerobic zones of the CEBPR and MEBPR train per g VSS/L.

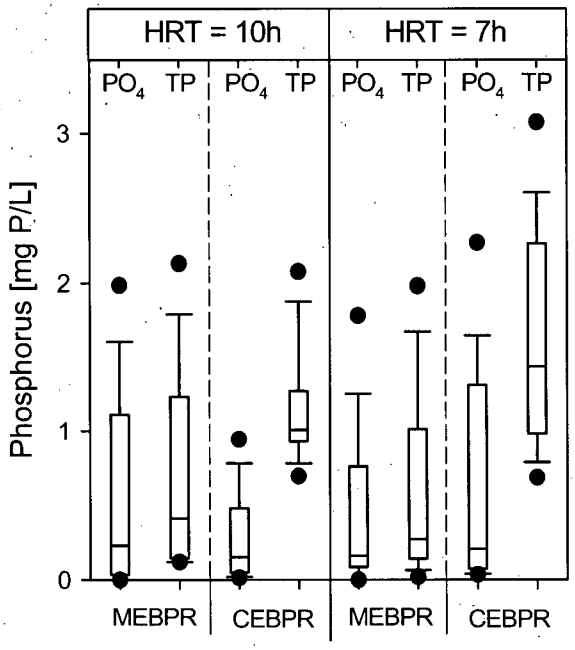

Figure 2.12 **Box-plots of effluent PO_4-P and total P at the two HRT set points. Percentiles shown: 5^{th}, 10^{th}, 25^{th}, 75^{th}, 90^{th} and 95^{th}. The horizontal line inside the box represents the median.**

2.5 References

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Chapter 3 Reaction kinetics in a membrane and conventional biological nutrient removal system. Part I: EBPR*

3.1 Introduction

Membrane bioreactor (MBR) technology represents an innovative wastewater treatment solution to respond to increasingly stringent discharge limits, particularly for sensitive receiving water bodies. For this reason, together with a drastic reduction in capital and operational costs, an increasing number of MBR plants are coming into operation around the globe for the treatment of municipal wastewater (Kraume *et al.*, 2005; Trussel *et al.*, 2005). When the secondary clarifier is replaced with membrane filtration for the final solids-liquid separation, the following significant modifications emerge in the treatment process: (1) the uncoupling of the hydraulic retention time (HRT) from the solids retention time (SRT), allowing for a wide range of operating conditions and for better process control; (2) the operation of the system at high suspended solids concentration which reduces the bioreactor volumes and the overall plant footprint (Côté *et al.*, 1998), and; (3) the production of a suspended solids-free effluent with superior quality, independently from the settleability property of the mixed liquor (Krauth and Staab, 1993).

Most MBR applications reported in the technical literature were conceived to achieve carbon removal (with or without nitrification) and nitrogen removal from wastewater. More recently, the first demonstrations of the coupling of membrane solids-liquid separation with

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enhanced biological phosphorus removal (EBPR) have been documented (Adam *et al.*, 2002; Fleisher *et al.*, 2005; Patel *et al.*, 2005). The combination of MBR technology with the EBPR process permits the design of a treatment plant with reduced reactor volumes compared to the conventional EBPR scheme, and the production of an effluent quality with a total phosphorus (P) concentration slightly above the othophosphate concentration. Adam *et al.* (2002) first, and Patel *et al.* (2005) later, indicated that biological P removal (bio-P) can be successfully accomplished in MBR systems operated at relatively long SRTs of about 20 days and with a high ratio of short chain volatile fatty acids (VFA) to total P in the feed. On the other hand, satisfactory P removal in the study of Fleisher *et al.* (2005) could only be achieved with addition of alum, therefore through a combination of biological and chemical treatment methods.

Even though these investigations have offered an important preliminary assessment of the feasibility of biological phosphorus removal (bio-P) in MBR systems, little is still known on the differences between membrane EBPR (MEBPR) and conventional EBPR (CEBPR) removal mechanisms. The replacement of the secondary clarifier with membrane solids-liquid separation entails that all the solids are retained in the bioreactor, including non-floc-forming bacteria that may be capable of EBPR activity. In addition, the sludge blanket which forms at the bottom of the clarifier is absent in MBR systems, therefore eliminating a cost-free sink of oxygen and nitrate that may be beneficial to biological nutrient removal processes. Finally, the strong course-bubble aeration employed to reduce the membrane fouling tends to hinder the formation of flocs, resulting in a biomass particle distribution that is finer than that in the conventional counterpart (Zhang *et al.*, 1997; Cicek *et al.*, 1999). All these aspects are hypothesized to impact the EBPR process kinetics and, consequently, the ultimate P removal from the treated wastewater. Both Adam *et al.* (2002) and Patel *et al.* (2005) measured the soluble P profiles in the three zones of an MBR system, with strong indication of anaerobic P release followed by anoxic and aerobic P uptake. However, specific P release and uptake rates, mass of P released to VFA utilized, the

contribution of denitrifying P-removing bacteria, and understanding of how all this basic EBPR information compares with a conventional bio-P process, are clearly missing. These represent essential engineering aspects for the design and, possibly modeling, of membrane biological nutrient removal processes.

The objectives of the present study were (1) to compare the extent of P release and subsequent P uptake in an MEBPR and CEBPR system operated in parallel, and (2) to measure the bio-P specific rates and stoichiometric coefficients in the two mixed liquors with the use of off-line batch tests. An MEBPR pilot train was run in parallel to a CEBPR process under identical operating conditions and with the same municipal primary wastewater feed. The only difference permitted was the technology adopted to separate the treated water from the activated sludge and the air bubble size of the aerator device. Off-line batch tests were executed on a regular basis over a period of one-year, whereby the EBPR activity of membrane and conventional sludge could be simultaneously evaluated.

3.2 Materials and methods

3.2.1 Description of the pilot plant

The University of British Columbia (UBC) pilot plant is a dual train facility, in which two 2,500 liter activated sludge systems can be operated in parallel for comparison of different experimental treatments or process designs. For the present study, the feed to both trains was primary effluent produced on site by clarification of municipal wastewater pumped continuously from two 15,000 liter external storage tanks. The primary effluent, always in excess of the required process flow rate, was collected in a 20 liter holding tank that provided a constant head to the process feed pumps in order to assure minimum variation of flow. The characteristics of the wastewater used for this research study are reported in Table 3.1. The pilot plant was also equipped with a sodium acetate dosing system to supplement readily biodegradable substrate in

case of limitation in the incoming wastewater. A University of Cape Town (UCT) type bioreactor (Tchobanoglous *et al.*, 2003) was selected for the comparative study and divided into an anaerobic (11%), anoxic (28%), and aerobic zone (61% of the total volume). One of the two parallel trains of the UBC pilot plant was retrofitted as a membrane-assisted bioreactor by installing a ZeeWeed®-140 membrane module (see Table 3.2 for specifications and operation) in the existing aerobic tank. The new membrane-UCT differed from the standard UCT process in the suspended solids distribution among the three zones. This occurred because the process effluent was extracted from the membrane in the aerobic zone, which concentrated the sludge in this zone relative to the upstream zones. To fulfill the objective of the present study, which required identical operating conditions for both treatment trains, it was decided to minimize the biomass distribution difference by returning the settled sludge from the conventional train clarifier back to the aerobic zone, rather than to the anoxic zone. Two secondary clarifiers were employed to provide excess clarification capacity in the event of bulking problems and increasing hydraulic loads. The range of HRT and overflow rate values applied to each clarifier was 2.02-1.41 h and 5.8-8.12 $m^3/(m^2 \cdot d)$, respectively, depending on the influent flow rate to the pilot plant. ranged between Figure 3.1 illustrates the final configuration of the MEBPR and the CEBPR processes adopted for the present comparative study. The two systems were investigated at an SRT of 12 days. The required sludge wasting volume in the conventional train was calculated by including both the mass of sludge in the secondary clarifier and the suspended solids leaving with the final effluent. In addition to sharing the same primary effluent, the two processes were operated at identical HRTs, total SRT, internal recycle ratios, dissolved oxygen concentrations, and ambient temperatures. The only differences permitted were the solids-liquid separation technology employed and the air bubble size generated by the two different aerator devices.

3.2.2 System operating conditions

The comparative pilot-plant study was conducted for almost one year at an SRT of 12 days and at two distinct HRTs of 10 and 7 hours. The structure of the overall research program is outlined in Table 3.3, and it was explained in details in Monti *et al.* (2005) (Chapter 2). Following the five periods of the comparative research program, the CEBPR train was retrofitted to become a second MEBPR system. Without changing the operating conditions, the new process differed only in the way the treated water was separated from the mixed liquor. By monitoring the EBPR activity of the conventional sludge over a four-month period as it evolved toward a membrane sludge, ideal experimental conditions were created to further explore the impact of solid-liquid membrane separation.

3.2.3 Pilot plant process monitoring and analyses

Influent and effluent temperature, pH in the influent and in each reactor zone and dissolved oxygen (DO) levels in the aerobic zones were recorded daily at the pilot plant. All flow rates on each process were checked at least every 10 days. A systematic sampling and analytical program was developed for long-term monitoring of process performance. Grab samples of the influent and effluent were collected 5 days per week and analyzed for total and volatile suspended solids (TSS and VSS), total chemical oxygen demand (COD), soluble COD, volatile fatty acids (VFA) comprising acetate and propionate (only for influent), total Kjeldahl nitrogen (TKN), ammonium-nitrogen (NH_4-N), nitrite plus nitrate nitrogen (NO_X-N), total phosphorus (TP), and orthophosphate phosphorus (PO_4-P). Mixed liquor samples were collected three times a week for each compartment for the entire investigation period and analyzed for TSS, VFA (only anaerobic zone), NH_4-N, NO_X-N, and PO_4-P. All analyses were performed according to Standard Methods (APHA *et al.*, 1998).

For a fair comparison of the two processes, the CEBPR effluent soluble sample was generated from a filtration method whose pore size was exactly the same as the ZeeWeed®-140 membrane unit. The filtration employed a two liter reactor in which a ZeeWeed®-1 (surface area of 0.09 m^2, and nominal pore size of 0.04 μm) was immersed in the sample with a vacuum applied by means of a peristaltic pump. Before collecting a representative sample, a filtrate volume of 500 mL was discarded to flush away the old sample entrapped in the membrane fibers, module header, and pump tube.

3.2.4 Batch test procedures and analysis

Off-line batch experiments were performed in a 6-liter reactor stirred with a magnetic bar, and a mechanical mixer at 60 rpm. The reactor was equipped with a portable VWR probe for temperature and pH measurement and with a YSI probe for monitoring the dissolved oxygen (DO) concentration. Five liters of mixed liquor from the aerobic zones of the MEBPR and CEBPR processes were manually transferred to two parallel batch reactors located in the immediate vicinity. The experiments were conducted at the constant pH of 7, by dosing 0.1N HCl and 0.1N NaOH, and at ambient temperature (not controlled). The equilibrium temperature in the parallel batch tests ranged between 18 and 22 °C during the entire investigation period, depending on the time of the year. For a pair-wise comparison of the specific EBPR rates in the two systems, it was decided not to correct the rates for temperature. However, the temperature effect was included in the discussion of the P-release rate in the transition period, to allow for an objective analysis of the EBPR characteristics over time. A temperature dependency coefficient θ of 1.075 was used for normalizing the P-release rate to 20 °C (Brdjanovic *et al.*, 1998). Each bio-P batch test was characterized by a pre-treatment phase of the sludge followed by anaerobic, anoxic and aerobic phases. Mixed liquor samples required for sampling were centrifuged for 3 minutes at 550 rcf and the supernatant was filtered through a 0.45 μm pore size filter, to obtain

10 mL of soluble sample for subsequent measurements. Triplicate samples for total and volatile suspended solids (TSS and VSS, respectively) were collected from each reactor at the beginning of the anaerobic phase and at the end of the experiment, and the 6 final values were averaged to obtain the suspended solids concentration of each batch test.

3.2.4.1 Pre-treatment phase

Before subjecting the sludge to anaerobic conditions, it was necessary to eliminate the nitrates present in the solution. The mixed liquor suspension was kept unaerated for 7 to 10 hours, depending on the initial nitrate concentration, to denitrify through endogenous respiration. Subsequently, the mixed liquor was aerated for about one hour to permit uptake of the orthophosphates that were released to the solution due to endogenous activity of phosphorus-accumulating organisms (PAOs). N_2 gas was then introduced to the reactor, to establish anaerobic conditions for the following phase.

3.2.4.2 Anaerobic phase

At time zero, 696 mg of sodium acetate were added to each reactor to establish an initial acetate concentration of roughly 60 mg COD/L. After 1-2 minutes from substrate addition, a sample was collected from each reactor to measure the initial concentration of acetate (HAc), NH_4-N, NO_X-N, PO_4-P. Anaerobic conditions were maintained for 120 minutes and samples were collected every 15 minutes until the completion of this phase. The maximum specific P release rate and the HAc uptake rate were calculated for the first 45 minutes of the experiment. In addition, the NH_4-N release rate was determined by including all the measurement points collected during the whole cycle.

3.2.4.3 Anoxic and anaerobic phase

At time 120 minutes, 455 mg of sodium nitrate were added to achieve an initial concentration of 15 mg NO_3-N/L in each reactor. With continuing flushing of N_2 gas in the

reactor, samples for NH_4-N, NO_X-N, and PO_4-P were collected every 20 minutes during the 80 minute long anoxic phase. The maximum specific anoxic P uptake rate, denitrification rate, and NH_4-N release rate were calculated for this phase.

At time 200 minutes, N_2 gas was replaced with air sparging to establish aerobic conditions in the batch reactor. The dissolved oxygen concentration was maintained above 3.5 mg/L at all times. Samples were taken for NO_X-N, and PO_4-P every 15 minutes during the 180-minute aerobic phase. The maximum specific aerobic P uptake rate was calculated for the first 45 minutes of straight slope. The NO_X-N production rate was also determined in the second half of the aerobic phase, to estimate the amount of NH_4-N being generated through biomass decay. From the ratio of P uptake rates under anoxic and aerobic conditions, the relative proportion of denitrifying dephosphatation activity in the PAOs was estimated (Wachtmeister *et al.*, 1997).

All the maximum reaction rates were determined as initial rates during the first 40-45 minutes of the experiments, using linear regression methods.

3.3 Results and discussion

The comparative pilot-plant study was completed at two distinct HRTs of 10 and 7 hours. During the two experimental runs, further modifications on the aerobic pilot plant recycle ratio and/or the addition of an external carbon source in the anaerobic zone were necessary to insure satisfactory performance in the overall BNR process.

3.3.1 Phosphorus removal performance

The evaluation of the phosphorus removal performance in the membrane and conventional pilot-scale system was essential to best interpret the outcomes of the EBPR kinetic study. A comparison of soluble P concentration in the MEBPR and CEBPR influent and effluents is illustrated in Figure 3.2. It can be noted that the two parallel processes exhibited a similar P removal behavior during the course of the comparative study, with the only exception

being the period between days 300 and 320. Starting from near the end of period I, 18 off-line batch tests were completed on the mixed liquors collected simultaneously from both treatment trains. A detailed discussion on the P removal performances was presented in Monti *et al.* (2005) (Chapter 2), with the main results summarized as follows.

- The CEBPR process achieved significantly greater P removal than the MEBPR counterpart during the first two instances of P breakthrough, when the VFA in the influent was limiting. It was found that the higher observed sludge yield and the greater denitrification capacity of the CEBPR system explained to a very large extent, the better P removal performance.
- The sudden drop of temperature (from 17.5 to 13.2 °C) encountered during the coldest period of the research program was hypothesized to have triggered the process upset observed after day 200. It was noticed that the MEBPR process exhibited a faster recovery of the bio-P activity than the CEBPR counterpart, likely caused by the ability of the membrane to retain all the organisms and by the slightly longer bioreactor SRT.
- The smooth transition of pilot train side B from CEBPR to MEBPR mode, together with the long-term satisfactory P removal demonstrated that, in the absence of carbon-limiting conditions, the MEBPR process was capable of performing at least as well as the conventional technology, under the operating conditions imposed.

3.3.2 P release and uptake in the main system

The EBPR mechanism responsible for excess P removal from the wastewater in conventional systems is characterized by P release and uptake in and from the wastewater, depending on the prevailing environmental conditions (Van Loosdrescht, 1997). It was critical to determine whether the EBPR process in a membrane-assisted treatment system would behave differently from the conventional counterpart. Mass balance calculations were performed on a daily basis to determine the amount of soluble P (measured as PO_4-P) released in the anaerobic

zone and the amounts taken up in the subsequent anoxic and aerobic zone. The calculated P-release and P-uptake masses per day were divided by the influent flow rate, to express the release and uptake in milligrams of P per liter of influent flow (Hu *et al.*, 2003). The results for the MEBPR and CEBPR process are presented in Figure 3.3.

The three profiles for both processes exhibited a highly dynamic pattern, which suggests that the EBPR activity never reached pseudo-steady state conditions at any given time. In addition, the anaerobic P release and the subsequent total P uptake were highly correlated in each system, with the curve peaks corresponding to periods of best P removal. Wentzel *et al.* (1985) were among the first to show a close linear relationship between daily P release and P uptake, which they empirically found by analyzing several bio-P processes. As later explained by other authors (Comeau *et al.*, 1986; Wentzel *et al.*, 1986; Arun *et al.*, 1987; Smolders *et al.*, 1995), PAOs utilize VFA in the anaerobic phase, resulting in P release to the liquid. The released P is subsequently sequestered and accumulated intracellularly under anoxic and/or aerobic conditions. The greater the mass of P being cycled in and out of the solution, the larger is the number of PAOs in the mixed liquor. Owing to a net growth of the cells, the accumulated PO_4 (poly-P) will be removed from the treatment process with the waste sludge and a significant amount of P will have been removed from the influent wastewater.

From Figure 3.3, it can be seen that, in this study, the anoxic P uptake contributed substantially to the total uptake during periods of good performance, whereas this activity decreased significantly when the process exhibited instances of P breakthrough. The only exception to this phenomenon was the second half of Period V in the CEBPR train (Figure 3.3b). Here, good performance of the bio-P process was not accompanied by an increase in the anoxic P uptake activity, indicating that the PAO community was capable of sequestering phosphate from the solution only under aerobic conditions. When the CEBPR train was subsequently retrofitted to be a second MEBPR process (transition period), the anoxic P uptake activity

accelerated, together with a further increase in the total P release and uptake. Hu *et al.* (2002) indicated that the nitrate load to the anoxic zone is a major factor in stimulating the growth of denitrifying PAOs and, therefore, their capacity for anoxic P uptake. In Period V, the anoxic recycle ratio in both systems was set at 1, to safeguard the anaerobic compartment from the deleterious effect of nitrates (Table 3.3). Under these conditions, the nitrate load to the anoxic zone was at the minimum level imposed throughout the whole study. However, due to the anoxic endogenous respiration occurring in the clarifier sludge blanket (Monti *et al.*, 2005, Chapter 2), the CEBPR process exhibited a greater overall denitrification efficiency than the membrane counterpart, which in turn, resulted in a reduced nitrate load to the CEBPR anoxic compartment. This may explain the significantly weaker CEBPR anoxic P uptake activity relative to the MEBPR counterpart observed in Period V, and the subsequent re-activation when the CEBPR train was retrofitted as an MEBPR system.

For a reasonable comparison between the parallel processes, the anaerobic P release and the anoxic P uptake were normalized with the concentration of suspended solids present in the corresponding zones. As discussed in Chapter 2 (Monti *et al.*, 2005), the CEBPR process carried less biomass in the bioreactor than the MEBPR counterpart, with the remaining biomass inventory found in the sludge blanket of the secondary clarifier. Figure 3.4 shows the comparison of EBPR activity in the two parallel processes. As far as the anaerobic P release is concerned (Figure 3.4a), it can be noted that the profiles for each process followed a remarkably similar pattern, with a very close match during periods of equally good P removal. The two curves depart during periods when the effluent PO_4 concentrations of the two systems clearly differed. Similar results were also observed for the anoxic P uptake (Figure 3.4b). As reported above, the two systems exhibited different patterns during the central part of Period V, at which time the measured anoxic P uptake activity of the MEBPR sludge was significantly higher than that of the CEBPR sludge. From the forgoing discussion, it can be concluded that the two systems,

operating under comparable conditions, exhibited an overall highly similar behavior in the EBPR mechanism. The early appearance of a P breakthrough in the MEBPR process at the end of Period I (see Figure 3.2) was clearly reflected in both the anaerobic P release and anoxic P uptake activities. Similarly, the isolated peak in the MEBPR effluent P concentration at the end of Period V caused a mismatch between the two anaerobic P release curves. These results suggest that the biological phosphorus process, established in a membrane-assisted bioreactor, shared comparable properties of the well-known EBPR process taking place in conventional wastewater treatment plants.

3.3.3 Bio-P batch tests

For a deeper analysis of the EBPR characteristics in a membrane process, it was essential to investigate the kinetics and stoichiometry of the EBPR sludge with the use of off-line batch tests, and to compare the values with the parallel conventional system. An example of the results obtained from batch tests conducted on both mixed liquors is illustrated in Figure 3.5. The release of P into the solution was accompanied by a proportional uptake of acetate, according to the typical behavior of the EBPR sludge (Comeau *et al.*, 1987). Once nitrate was made available as an electron acceptor, the PAOs started to take up phosphate from the solution, with simultaneous utilization of NO_X which was ultimately denitrified. Under aerobic conditions, the PO_4 profile continued to decline at a rate that was dependent on the proportion of denitrifying versus non-denitrifying PAOs in the mixed liquor (Wachtmeister *et al.*, 1997).

3.3.3.1 Anaerobic P release

During the first 45 min of the anaerobic phase, the maximum specific P release and HAc uptake rates were calculated for the two parallel batch experiments. A summary of the measured anaerobic kinetic rates from all batch tests completed during the comparative study and the subsequent transition period is presented in Figure 3.6.

The highly dynamic behavior of the EBPR observed in the pilot plants was also reflected in the anaerobic batch tests, with a pattern of kinetic values showing a close relationship to the profiles of Figure 3.4a. The first six batch tests of Figure 3.6a exhibited a significantly higher maximum specific P release rate for the CEBPR than the MEBPR sludge. These experiments were conducted from the end of Period I until the completion of Period II, during which the CEBPR process demonstrated better P removal than the MEBPR counterpart (see Figure 3.2). Once the acetate content of the incoming wastewater was augmented with supplemental sodium acetate (Table 3.3), both release rates responded with a substantial increase, and similar values were measured in the two mixed liquors in batch tests 7 to 10. Despite the substantial differences introduced with membrane technology, these results demonstrated that, when carbon is non limiting, the MEBPR system developed a PAO community with comparable P-release kinetics to that of a CEBPR process.

As a result of a complete loss of bio-P activity after day 220 in the pilot plant (Figure 3.2), the measured P release kinetics demonstrated a corresponding decrease for both the MEBPR and CEBPR sludge (batch test 11 in Figure 3.6a). However, the membrane pilot plant system exhibited a faster recovery of the EBPR activity than did the CEBPR process, and this was clearly reflected in the measured kinetics of batch tests 12 and 13. The end of the comparative study was characterized by an isolated peak in the P concentration of the MEBPR effluent which was not observed in the CEBPR counterpart (Figure 3.2, day 300-320). This dissimilarity was confirmed in the batch test 14, with the CEBPR sludge exhibiting a much higher P release rate than the MEBPR sludge. Once the temporary process upset in the MEBPR system passed, the MEBPR bio-P activity returned to similar level as the CEBPR, as revealed in the last batch experiment of the comparative period.

On day 354, the secondary clarifier in the CEBPR train was replaced with a membrane module, while keeping all the operating conditions unchanged. As can be seen in Figure 3.6b, the

P-release kinetics of the PAOs in the acclimatizing sludge continued to accelerate, reaching the highest maximum P release rates ever measured in the whole investigated period (batch tests 16 to 18). These results, together with the performance of the pilot system during the transition period, indicate that the utilization of membrane solids-liquid separation has a positive impact on the established PAO community performing EBPR.

The stochiometric coefficient expressing the mass of P released per unit mass of VFA consumed in the anaerobic phase is presented in Figure 3.7. It can be noted that both the MEBPR and CEBPR sludge exhibited similar average P/VFA values of about 0.6 and 0.5 in the batch test and pilot scale systems, respectively. The slightly but significantly lower ratio in the pilot scale system is attributed to the interference of nitrates in the anaerobic compartment that were not completely utilized in the downstream anoxic zone. The majority of the P release kinetic and stoichiometric values estimated in the present study were within the ranges reported in the technical literature for EBPR plants treating municipal wastewater (Table 3.4). During periods of satisfactory performance, the maximum measured release rate was between 20 and 30 mg P/(g VSS·h), which fell above the literature range. The magnitude of this parameter is closely related to the size of the PAOs population present in the sludge. It is possible that, unlike the situation in full-scale wastewater treatment plants, the strictly controlled conditions under which this investigation was completed might have favored the development of an abundant PAO community in the two systems. The variation of the stochiometric coefficient P/VFA reported in Table 3.4, including that from the present study, could derive from a pH effect of the different wastewaters treated (Smolders *et al.*, 1994) and/or from the presence of nitrate, which would reduce the amount of acetate involved in the P release.

3.3.3.2 Anoxic and aerobic P uptake

After the anaerobic phase in the presence of HAc in each batch test, the suspension was first exposed to nitrates and subsequently, to air, to evaluate the activity of the PAOs under anoxic and aerobic conditions. The overall kinetics and stoichiometry of the P uptake are shown in Figure 3.8.

It can be observed that the ability of the MEBPR and CEBPR mixed liquors to take up P under anoxic conditions was maintained at all times, even during periods in which the pilot scale systems exhibited a net P release in the anoxic zone (see Fig. 3.4b). The maximum specific anoxic P uptake rate of the two mixed liquors was measured at between 1 and 6 mg P/(g VSS·h), and was found to be comparable in both processes for about 50% of the time, i.e. batch tests 3 to 8 (Fig. 3.8a). In these same experiments, the aerobic P uptake activity was also observed to be at similar levels, whereas significant differences were measured during the second half of the comparative study. From the reference values shown in Table 3.4, it can be noted that anoxic and aerobic P uptake rates observed in the present study were within the ranges reported in other EBPR investigations with municipal wastewater.

From the ratio of anoxic and aerobic P uptake rates, it was possible to estimate the relative proportion of the denitrifying dephosphatation activity in the MEBPR and CEBPR mixed liquors. As shown in Figure 3.8c, this proportion varied considerably during the investigated period, with values ranging from 20 to about 100%. Compared to the anoxic and aerobic P uptake rates alone, a higher degree of similarity was observed in the two mixed liquors when assessed using this ratio. It can be seen that the largest difference was measured at batch test 14, which coincided with Period V in the pilot scale systems, during which CEBPR anoxic P uptake was absent (Fig. 3.4b). Kuba *et al.* (1997) completed an assessment of anoxic P uptake in two full-scale WWTPs, with values ranging from 20 to 50% of the total P removal. In a similar study

by Brdjanovic *et al.* (2000), the fraction of denitrifying activity of PAOs was estimated to be 80%.

The ratio of removed phosphorus to nitrate-electron consumed (P/e^-) was also calculated to estimate the proportion of nitrates utilized by poly-P and non-poly-P accumulating denitrifiers (Kuba *et al.*, 1996). As can be seen in Figure 3.8d, the P/e^- ratio varied between 0.02 and 0.18, with the lowest values being observed in Periods IV and V, when the EBPR activity severely deteriorated. The measured CEBPR P/e^- ratio was significantly higher than for the MEBPR counterpart during batch tests 8 to 10, which coincided with times of higher anoxic P uptake rates (see both Fig. 3.4b and Fig. 3.8a). On the other hand, the faster recovery of the EBPR activity in the membrane system was reflected also in the P/e^- ratio, which was observed to be significantly higher in the last four MEBPR batch tests. Kuba *et al.* (1997) reported a range of P/e^- values between 0.04 and 0.18, with 0.19 stated as being the reference value for enrichment anaerobic/anoxic EBPR sludge.

3.3.4 Ammonia release rate

The long duration of the batch test represented an excellent experimental opportunity to estimate the contribution of each environmental condition to the overall MEBPR biomass decay relative to the CEBPR counterpart, through the measurement of the ammonium-nitrogen release in the solution. With the investigated sludge collected in the aerobic compartment of the pilot scale system, the presence of slowly biodegreadable substrate, which is subjected to hydrolysis, was assumed to be negligible. Therefore, the ammonia released in the batch experiments should have derived predominantly from biomass lysis. The production of ammonium-N under aerobic conditions could only be calculated from the level of nitrate generated during this phase. Figure 3.9 presents the average results of specific ammonium-N release rates under anaerobic, anoxic, and aerobic conditions.

It can be observed that the extent of ammonium-N released varied significantly in each of the three batch test phases, with the highest rate measured under aerobic conditions and the lowest under anaerobic conditions. This indicates that the endogenous decay of the biomass is more significant when exposed to oxygen, than to unaerated conditions. It should be noted that the actual production of ammonium-N during the aerobic phase might have been underestimated, since some ammonium was would have been used as a growth nutrient by the microorganisms. When comparing the release rates of the two mixed liquors, no significant differences were observed, which may indicate that the overall decay rates were similar in the MEBPR and CEBPR sludges. These results confirmed the hypothesis advanced in Chapter 2 (Monti *et al.*, 2005) that the lower CEBPR aerobic sludge mass reduced the overall biomass decay, resulting in a higher observed sludge yield compared to the MEBPR process.

The observation of different decay values under aerated and unaerated conditions is in conformity with the technical literature. McClintock *et al.* (1993) reported a significant reduction of biomass decay in a biological nutrient removal process, relative to an aerobic conventional activated sludge plant. Another two subsequent studies suggested that the overall biomass decay rate, including nitrifiers, is smaller under non-aerated than aerobic conditions (Novak *et al.*, 1994), with the greatest reduction occurring under anaerobic conditions (Siegrist *et al.*, 1999). Although the actual causes of this phenomenon are still unknown, it is imperative to estimate the relative contribution of each zone to biomass decay for modeling and design purposes.

3.4 Conclusions

The characteristics of the EBPR activity in membrane-assisted and conventional biological nutrient removal processes operated in parallel were compared by investigating the P removal performance and P release and uptake dynamics in the pilot scale systems, and the EBPR

kinetics in off-line batch tests. From the comparative study completed, under strictly controlled and identical conditions, the following conclusions were reached.

- With an adequate VFA/P ratio present in the incoming wastewater, the MEBPR and CEBPR processes featured a high degree of similarity in the observed soluble P effluent concentrations, the specific P release in the anaerobic zone, and in the maximum specific P release and VFA uptake rates. On the other hand, carbon-limiting conditions resulted in a greater P removal in the CEBPR process, which was also reflected in all the other EBPR parameters. When the pilot plant P removal performance was observed to be poor, the MEBPR train demonstrated a faster recovery of the bio-P activity than the CEBPR sludge.
- Denitrifying dephosphatation was significant during periods of satisfactory P removal, with a high degree of correlation between the two parallel pilot scale processes. When the aerobic recycle ratio was reduced to a minimum level, the CEBPR anoxic P uptake activity was significantly lower, compared to the membrane counterpart. It is possible that greater N removal in the CEBPR system, and the consequent reduced nitrate load to the anoxic zone, negatively impacted the growth of denitrifying PAOs.
- The overall biomass decay rate, estimated through the measured ammonium release rate over the course of batch experiments, was found to be comparable in the two processes. A significant reduction of the decay rate was observed under non-aerated conditions, which explained the lower observed yield previously reported for the CEBPR process.

Table 3.1 UBC Pilot Plant primary effluent wastewater characteristics.

Parameter	Mean	95% Conf. Int.	Min - Max
TSS (mg/L)	90.5	±4.7	18 – 216
CODtot (mg/L)	307.3	±9.5	182.1 – 584.6
CODsol (mg/L)	96.5	±4.3	50.5 – 265.0
Acetate (mg/L)	26.8	±1.4	6.6 – 46.4
Propionate (mg/L)	3.6	±0.2	1.0 –12.7
Tot VFA (mg COD/L)	34.1	±1.5	8.9 – 63.0
TKN (mg N/L)	33.6	±0.5	23.6 – 43.7
NH_4-N (mg N/L)	25.6	±0.3	19.5 – 34.9
NO_X-N (mg N/L)	Not detec.		
TP (mg P/L)	4.2	±0.1	2.4 – 6.9
PO_4-P (mg P/L)	2.4	±0.05	1.4 – 5.0
T (°C)	20.4	±0.3	14.0 – 23.6
pH	7.2	±0.03	6.6 – 7.9

Table 3.2 Specifications and operation of ZeeWeed® membrane module.

Module specification	Value
Total membrane surface	12 m^2 (140 ft^2)
Nominal pore size	0.04 µm
Typical operating transmembrane pressure	10-65 kPa (0.10-0.65 bar)
Duration permeation mode	9.5 min
Duration backflush mode	0.5 min
Duration intermittent air sparging	10 sec ON – 10 sec OFF
Air sparging flow rate	0.34 m^3/min (12 scfm)
Citric acid cleaning	24 hours in pH=2 solution
Hypochlorite cleaning	24 hours in 1,000 ppm
Module supplied by Zenon Environmental Inc., Oakville, Ontario, Canada.	

Table 3.3 Experimental periods and pilot plant operating conditions.

Period	Day	Temp.[1] (°C)	Operating paramters				
			HRT (h)	SRT (d)	Anx rec. ratio[2]	Aer rec. ratio[2]	HAc Addition (mg COD/L)
I	1 - 109	21.9 (0.21)	10	12	1	2	0
II	110 - 147	21.1 (0.45)	10	12	1	1	0
III	148 - 185	18.1 (0.45)	10	12	1	2	20
IV	186 - 236	16.3 (0.42)	7	12	1	2	20
V	237- 353	18.5 (0.36)	7	12	1	1	30
Transition	354 - 458	21.8 (0.26)	7	12	1	1	30

[1] Temperature expressed as average value with 95% confidence interval in parenthesis.

[2] Anx rec.: anoxic recycle; Aer rec.: aerobic recycle. The term ratio is defined as the recycle flow rate divided by the influent flow rate. The return activated sludge (RAS) from each clarifier was set to half the influent flow rate, therefore a total RAS recycle ratio of 1.

Table 3.4 Kinetics and stoichiometry of EBPR sludge acclimatized to municipal wastewater.

Study	Max Ana P Rel. Rate	P/VFA	Max Anx P Upt. Rate	Max Aer P Upt. Rate
	mg P/(g VSS·h)	g P/g COD	mg P/(g VSS·h)	mg P/(g VSS·h)
Kuba *et al.* (1997)	7 - 19	0.22 - 0.40	1.2 – 6.0	5.7 – 13.0
Brdjanovic *et al.* (2000)	6	0.29	1.7	2.2
Kerrn *et al.* (1993)			0.4 – 1.0*	0.6 – 1.3*
Petersen *et al.* (1998)	15	0.58		14
Mamais *et al.* (1992)	7 - 20	0.5		
Rabinowitz *et al.* (1986)		0.85		
Carlsson *et al.* (1996)		0.35 - 0.4		
Tykesson *et al.* (2002)	7			
MEBPR and CEBPR in this study (2005)	5 - 30	0.5 - 0.6	1.0 – 6.0	2.0 – 10.0

*measured as mg P/(g SS·h)

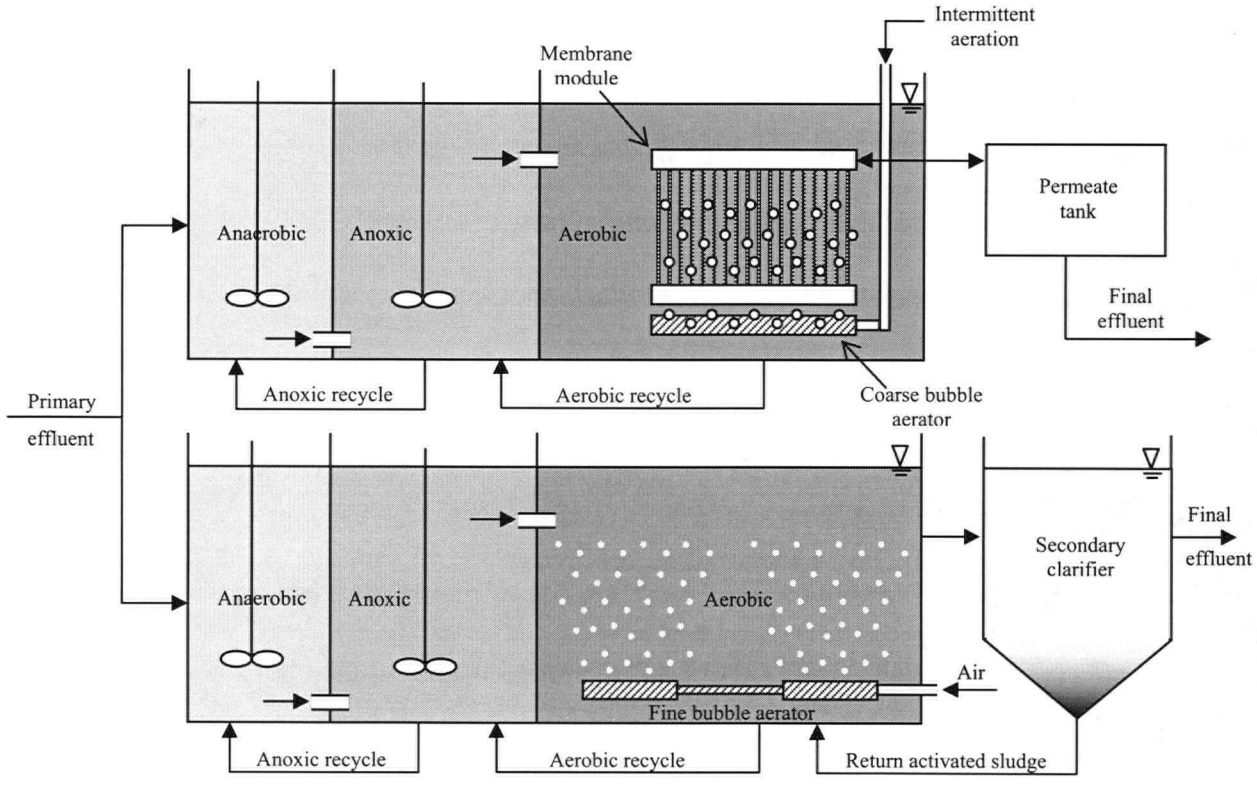

Figure 3.1 Schematic of the parallel MEBPR (above) and CEBPR (below) systems.

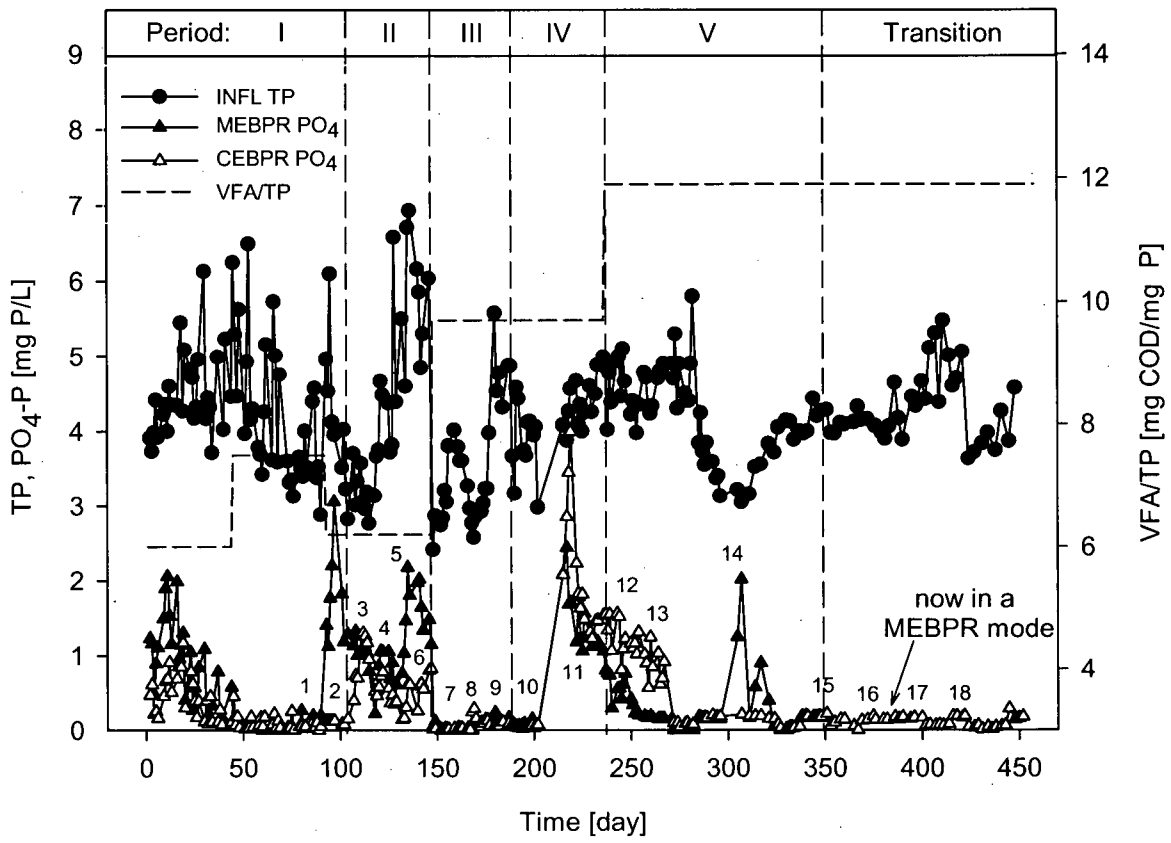

Figure 3.2 **Total phosphorus in the influent, and orthophosphate-P in the MEBPR and CEBPR effluents. The numbers from 1 to 18 represents the points in time at which each batch test was executed.**

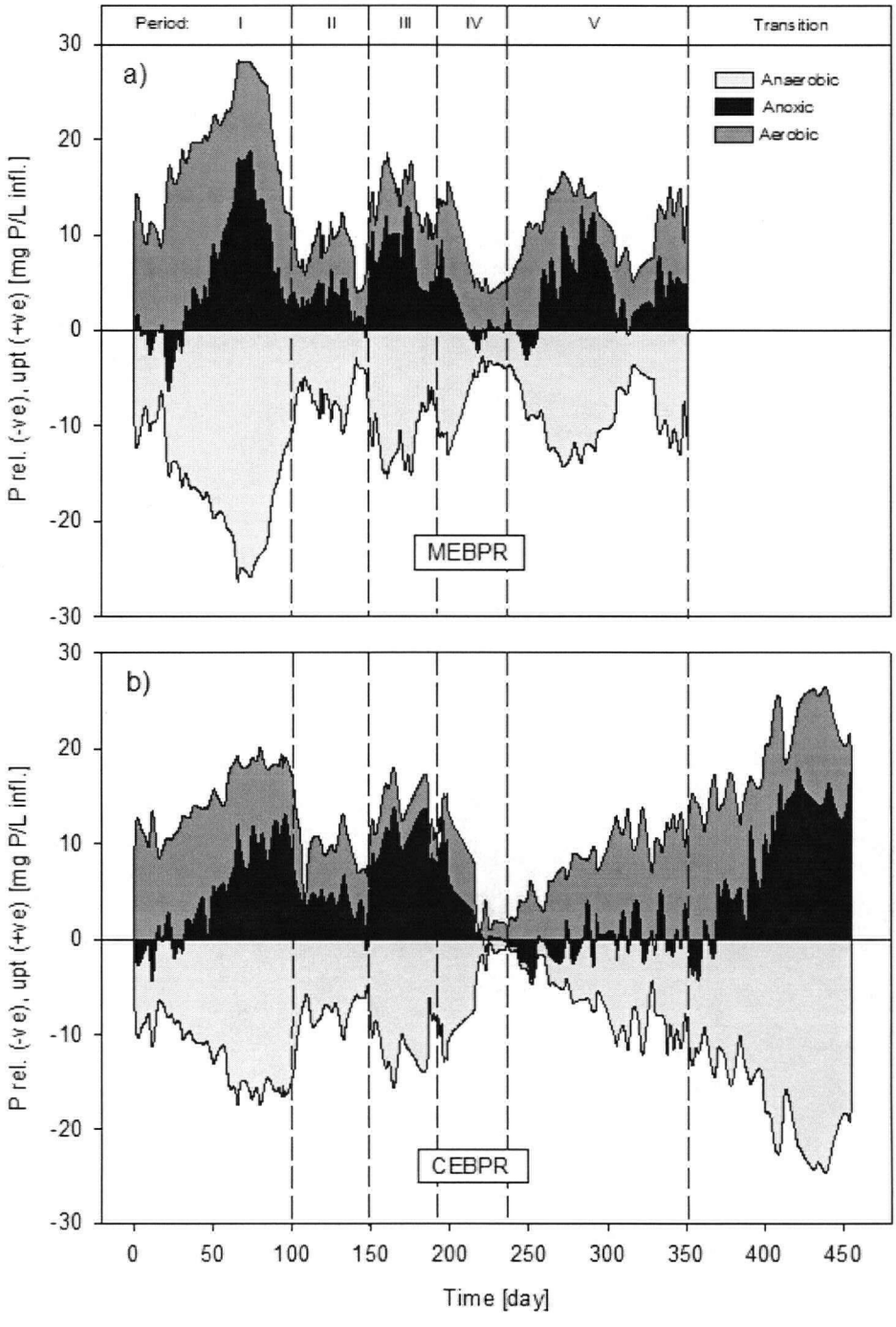

Figure 3.3 **Day to day anaerobic P release, and anoxic and aerobic P uptake in the a) MEBPR and b) CEBPR systems.**

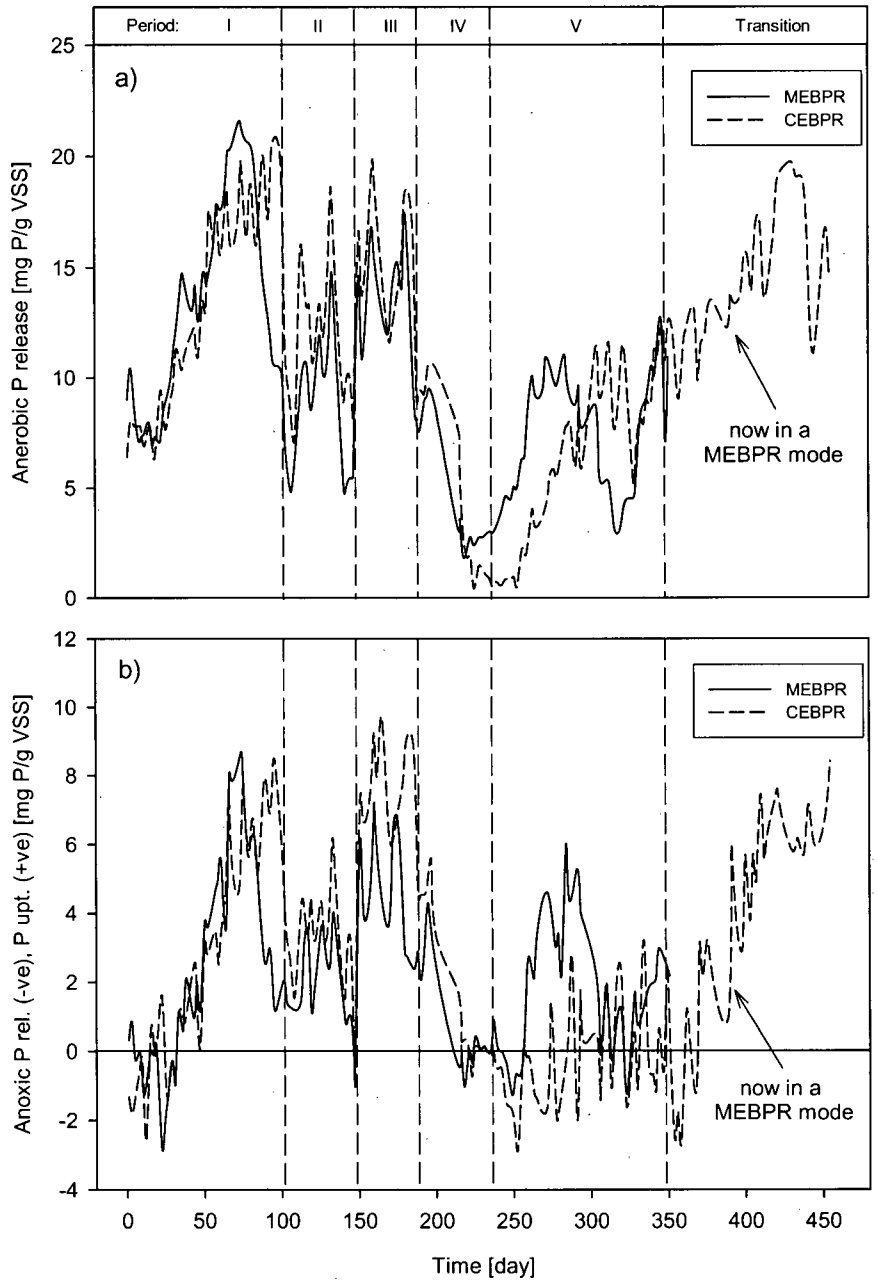

Figure 3.4 **Comparison of a) specific anaerobic P release and b) specific anoxic P uptake in the MEBPR and CEBPR processes during the entire period of investigation.**

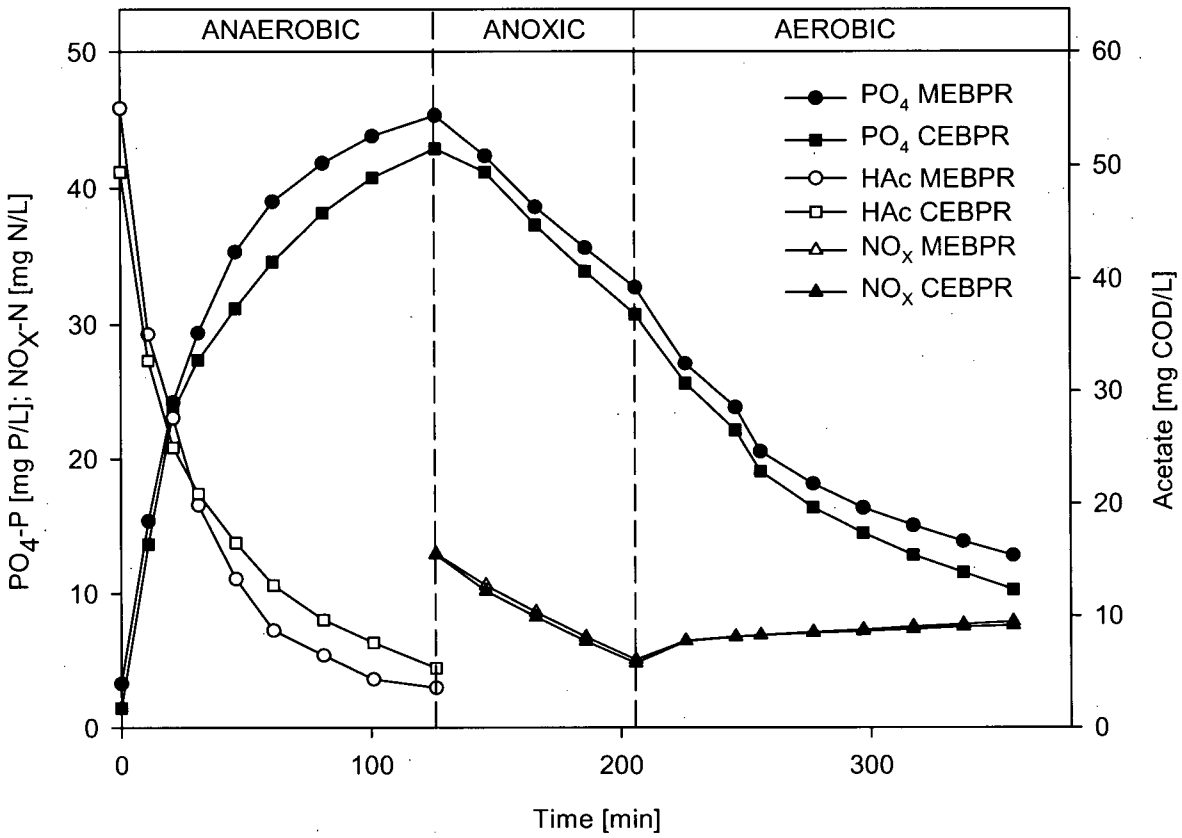

Figure 3.5 **Example of bio-P batch test (number 7) performed simultaneously on the MEBPR and CEBPR sludge.**

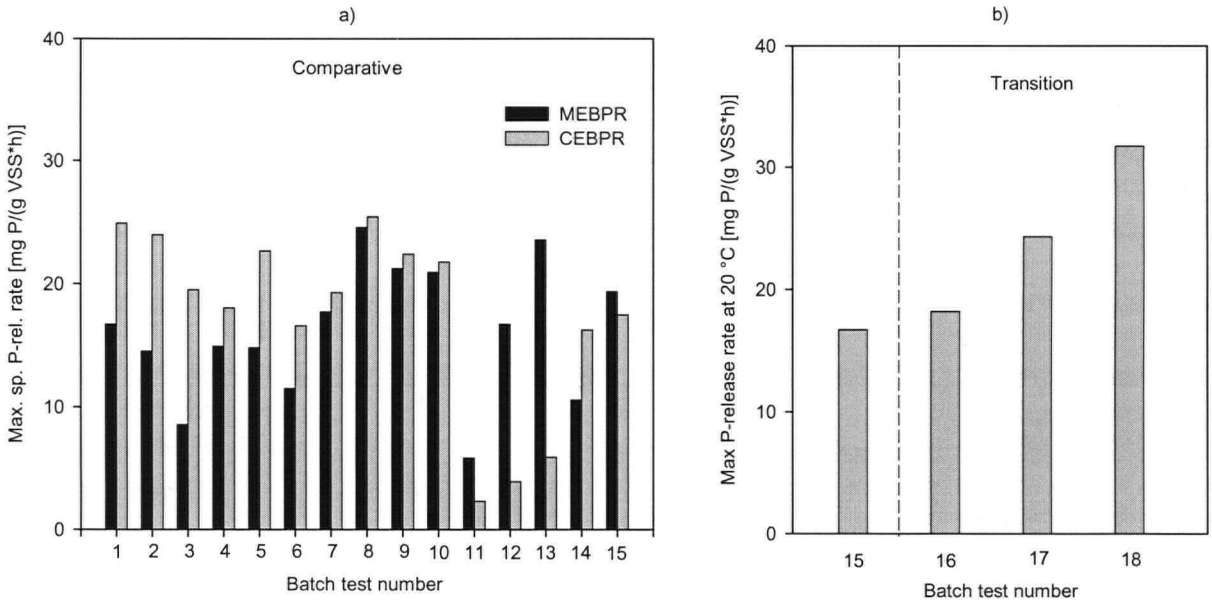

Figure 3.6 **Maximum anaerobic specific P release rate during a) the comparative study and b) the transition period.**

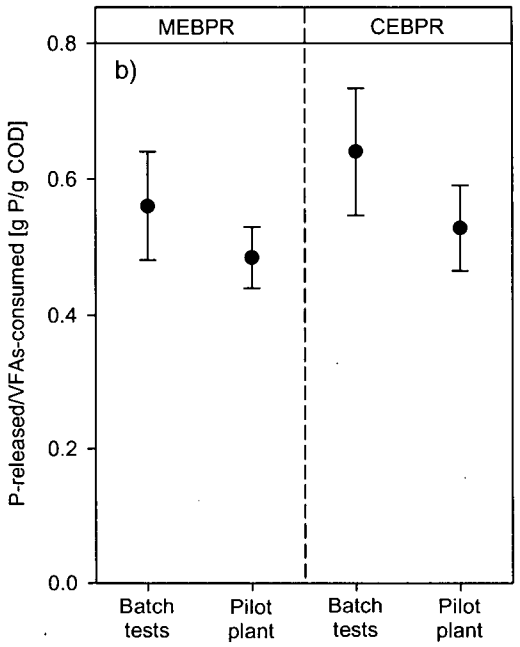

Figure 3.7 **Stoichiometric coefficient P/HAc measured in the anaerobic phase of the best tests and in the pilot plant systems (error bar: 95% confidence interval).**

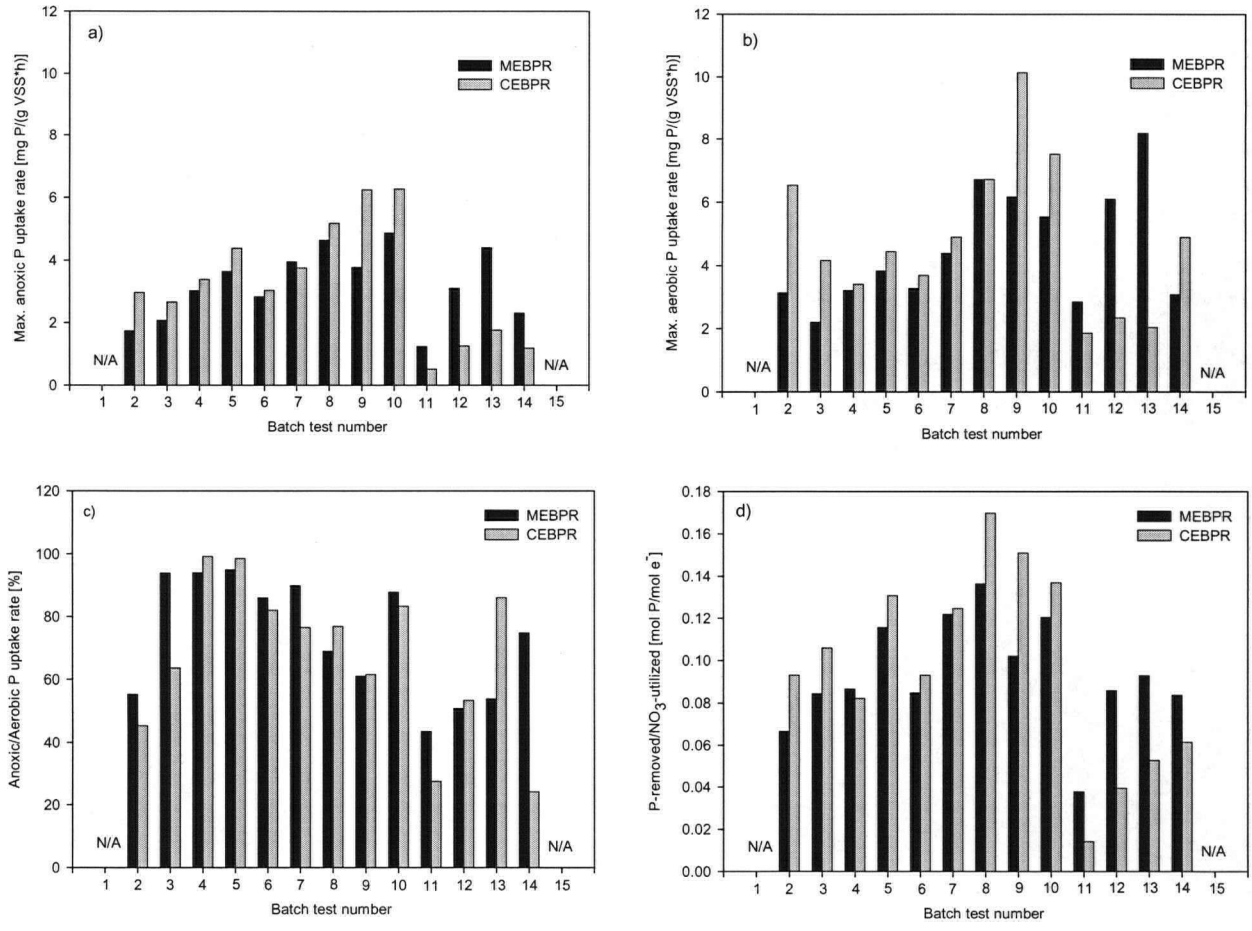

Figure 3.8 **Kinetics and stoichiometry of P uptake during the comparative period: a) maximum specific anoxic P uptake rate; b) aerobic P uptake rate; c) % of denitrifying dephosphatation activity; d) ratio of P removed per unit mass of NO_3-electron utilized. N/A: values non available.**

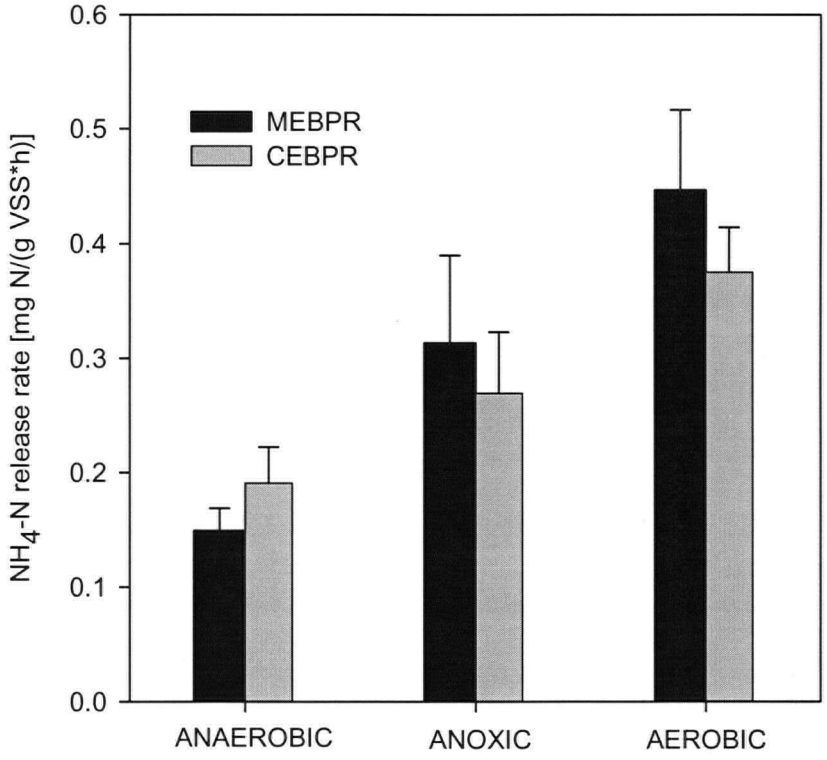

Figure 3.9 **Ammonia release rate during anaerobic, anoxic, and aerobic conditions. The release in the aerobic phase is estimated through the production of nitrate. Error bar: 95% confidence interval.**

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Chapter 4 Reaction kinetics in a membrane and conventional biological nutrient removal system. Part II: Nitrification*

4.1 Introduction

Membrane bioreactor (MBR) technology offers an innovative approach to the treatment of municipal wastewater. An aerobic MBR is a modification of an activated sludge system in which a biological reactor is coupled to a filtration membrane which replaces the secondary clarifier as the final solids-liquid separation step. The main advantages of an MBR system over the conventional activated sludge (CAS) process are: (1) the operation of the bioreactor at high volumetric loading rates (Chaize and Huyard, 1991; Côté *et al.*, 1998); (2) the establishment of a low food to microorganism (F/M) ratio in the reactor, therefore achieving a substantial reduction in the observed sludge yield (Muller *et al.*, 1995; Rosenberger *et al.*, 2002); (3) the retention of slow-growing nitrifying bacteria in the bioreactor, allowing for a complete and stable nitrification (Fan *et al.*, 1996) and; (4) the production of a suspended solids-free effluent and operation of the system independently of the settleability of the biomass (Krauth and Staab, 1993; Winnen *et al.*, 1996).

A number of nitrification and nitrogen removal processes in membrane-assisted bioreactors have been documented in the technical literature (among others, Chiemchaisri *et al.*, 1992; Muller *et al.*, 1995; Fan *et al.*, 1996; Urbain *et al.*, 1998; Rosenberger *et al.*, 2002). It appears that complete and stable nitrification is a key characteristic of MBR processes, thanks to the ability of membrane separation to retain slow-growing nitrifiers in the bioreactor at long SRTs. A few investigations have also attempted to make a comparison of nitrification activity in

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MBR and CAS processes, operated in parallel with carbon and/or nitrogen removal treatment schemes (Muller *et al.*, 1995; Zhang *et al.*, 1997; Liebig *et al.* 2001; Soriano *et al.* 2003; Gao *et al.*, 2004). However, the operating conditions and/or the configuration of the parallel processes used in these studies were far from identical, resulting in somewhat biased conclusions. Therefore, limited information has been obtained so far on the actual impact of membrane filtration itself on the nitrification process. A significant step toward parallel (although not identical) operation was made by Manser *et al.* (2004), who compared the performance and nitrification activity of a membrane and conventional nitrogen removal system. Their three-month long study indicated that minor differences existed in the nitrifying community and the same maximum nitrification activity was measured in the two microbial populations.

Recent attempts have been made to couple membrane technology to the enhanced biological phosphorus removal (EBPR) process to biologically remove both nitrogen and phosphorus from municipal wastewater (Fleischer *et al.*, 2005; Lesjean *et al.*, 2003; Adam *et al.*, 2002). Through the monitoring of the effluent, these studies confirmed that complete nitrification can also be achieved in this complex microbial community typical of biological nutrient removal (BNR) processes. However, information on the maximum nitrification activity in a membrane BNR sludge and how it compares with a corresponding conventional sludge is clearly missing. Nitrification is often regarded as the most sensitive process in conventional activate sludge plants. Therefore, it is essential to investigate the importance of the kinetic rates for the engineering design and, possibly modeling, of membrane biological nutrient removal systems.

The objectives of the present study were (1) to compare the maximum specific nitrification rate in a membrane and conventional EBPR system operated in parallel under identical conditions, and (2) to estimate the maximum specific growth rate μ_{AUT} of nitrifiers fostered in the two microbial communities. A membrane EBPR (MEBPR) pilot train was run in parallel to a conventional EBPR (CEBPR) process under identical operating conditions and with the same

municipal primary wastewater feed. The only difference permitted was the technology adopted to separate the treated water from the activated sludge: membrane-based filtration and gravity-based sedimentation. Off-line batch tests were executed on a regular basis over a period of one-year, through which the nitrification activity of membrane and conventional sludge could be simultaneously evaluated.

4.2 Materials and methods

4.2.1 Pilot plant

The long-term comparison of the nitrification activity in the MEBPR and CEBPR sludge was investigated at the UBC pilot plant which treated municipal wastewater. Detailed descriptions of the research site, process layout, and the monitoring and analytical programs can be found in Monti *et al.* (2005a) (Chapter 3).

The operating conditions of the MEBPR and CEBPR pilot scale systems in this study were similar to those described in Monti *et al.* (2005a) (Chapter 3), with the only exception being the inclusion of one additional transition period. As illustrated in Table 4.1, the comparative pilot-plant study was conducted under pseudo-steady state conditions for almost one year, at an SRT of 12 days and at two distinct HRTs of 10 and 7 hours. Following this, two transition periods were examined. As a result of a technical accident which involved a 12 hour long overflow from the MEBPR train into one of the two secondary clarifiers serving the CEBPR system, the sludge content of the membrane train was largely washed out, whereas the conventional train ended up hosting a mixture of the two mixed liquors. It was then decided to completely drain the MEBPR reactor and to re-start it with half of the biomass contained in the CEBPR side. By doing so, an excellent opportunity was created to monitor the nitrification activity of the two mixed liquors as they evolved in the two different systems (Transition I). When the two month long Transition I was completed, the CEBPR train was retrofitted to become a second MEBPR system. Without

changing the operating conditions, the new process differed only in the way the treated water was separated from the mixed liquor. By monitoring the nitrification activity of the conventional sludge over a four-month period as it evolved toward a membrane sludge, an ideal experiment was created to further explore the impact of membrane solids-liquid separation.

4.2.2 Batch test procedure

Off-line batch tests were performed in a 6 liter reactor stirred with a magnetic bar, a mechanical mixer turning at 60 rpm, and with air flushing installed for oxygen transfer. The reactor was equipped with a portable VWR probe for temperature and pH measurement and with a YSI probe for monitoring the dissolved oxygen (DO) concentration. Five liters of mixed liquor sample from the MEBPR and CEBPR process were manually transferred to two parallel batch reactors located in an adjacent working bench. The suspension was aerated for about one hour to reach the new equilibrium at the prevailing ambient temperature (not controlled), pH of 7 (through the addition of 0.1N HCl), and DO between 4 and 5 mg/L. At time zero, 380 mg of ammonium chloride were added to each reactor to establish an initial ammonium-nitrogen concentration of roughly 20 mg N/L. After 1-2 minutes from substrate addition, a sample was collected from each reactor to measure the initial concentration of ammonium (NH_4-N) and nitrite+nitrate (NO_X-N). Samples were then taken every 15-20 minutes until the completion of the reaction to measure the maximum nitrification rate of the investigated sludge. Mixed liquor samples were centrifuged for 3 minutes at 550 rcf and the supernatant filtered through a 0.45 μm pore size to obtain 10 mL of soluble sample for subsequent measurements. During the course of the experiment, drops of 0.1N NaOH were added to the reactor to counterbalance the production of acidity in the solution and to maintain pH at 7. Triplicate samples for total and volatile suspended solids (TSS and VSS, respectively) were collected from each reactor at the beginning

and at the end of the experiment and the six final values were averaged out to obtain the suspended solids concentration of the batch test.

In some batch tests, the oxygen utilization rate (OUR) during the nitrification reaction was measured as an indirect indication of the activity. Aeration was manually increased to reach a DO concentration above 6 mg/L. At this point, the aeration was stopped and the declining DO was monitored with the passage of time until a minimum level of 2 mg/L was reached. Four to six repetitions of these cycles were made during the experiment to calculate the average OUR, which was then normalized to obtain the SOUR (mg O_2/g VSS·h).

4.2.3 DNA and RNA chemical assay

Modified diphenylamine and orcinol reactions were employed to spectrophotometrically quantify total cellular DNA and RNA, respectively, in the mixed liquor sample (Gerhardt, 1994). Triplicates of 10 mL suspension were precipitated in 20 mL of 15% cold trichloroacetic acid (TCA). After spinning the sample at 15,000 × g for 10 min, the sludge pellet was re-suspended in 10 mL of 0.2 N NaOH and, if necessary, stored at -70 °C. Salmon sperm native DNA (Pharmacia Biotech) and E. coli 16 + 23S rRNA (Sigma) were used as standards. Diphenylamine reagent contained 0.5 g of diphenylamine and 1.375 mL H_2SO_4 in glacial acetic acid in a final volume of 50 mL. The solution was prepared fresh before use. The orcinol reagent contained 0.3 g of orcinol, 5 mL 95 % ethanol in 50 mL HCl with 0.02 g ferric chloride. The diphenylamine reagent was added 2:1 to dilutions of sample and DNA standard and boiled for 10 min. The reaction was stopped on ice and absorbance was measured at 600 nm. The orcinol reagent was added 1:1 to dilutions of samples and the RNA and DNA standards, incubated in boiling water for 45 min and chilled on ice. The absorbance was read at 660 nm. Because orcinol measures both RNA and DNA, the DNA absorbance measured in the diphenylamine samples was converted to an

expected DNA absorbance for the orcinol reaction, which was then subtracted from the total orcinol reading to calculate RNA concentration.

4.3 Results and discussion

Within the two distinct HRT experimental runs during the comparative study, further modifications on the aerobic recycle ratio and/or the addition of external carbon source in the anaerobic zone were necessary, to insure a satisfactory performance of the overall BNR process.

4.3.1 Pilot plant nitrogen removal performance

The nitrification activity of the MEBPR and CEBPR sludge was first compared in the pilot-scale processes, by analyzing the ammonium concentration remaining in the final effluent of the two treatment systems. The influent TKN, and the NH_4-N and NO_X-N effluent time series of the parallel pilot plants are presented in Figure 4.1. It can be noted that both the MEBPR and the CEBPR processes achieved complete and stable nitrification with measured effluent ammonium-N concentrations that were below the detection limit. Stable nitrification in MBR systems has also been reported in other studies over a wide range of operating conditions (Muller *et al.*, 1995; Fan *et al.*, 1996; Liebig *et al.*, 2001; Fleisher *et al.*, 2005). In the present study, the only significant difference in ammonium-N profiles was noticed during the coldest (13 °C) week of the study, when the MEBPR effluent ammonia exhibited a one-week spike with values ranging from 0.45 to 2.10 mg N/L. When complete nitrification is observed in parallel bioreactors, little information can be extracted on the potential differences between the two nitrifying microbial communities. For this reason, 27 batch tests were conducted on a regular basis during the duration of the research program. Figure 4.1 shows the exact point in time of these off-line experiments: 17 during the comparative study, 5 in Transition I, and 5 Transition II.

As a result of the endogenous denitrification observed in the sludge blanket of the secondary clarifier (Siegrist and Gujer, 1994), the CEBPR system produced a consistently lower NO_X-N concentration in the treated effluent over the entire comparative study. The subsequent period of Transition II indicated that the installation of a membrane module in the CEBPR system did not compromise the ability of the activated sludge to achieve complete nitrification under pseudo-steady state operating conditions. However, a sudden increase in the nitrate levels was observed in the treated effluent, confirming the inherent loss of the additional denitrification that occurred in the clarifier of the conventional system. More details on this observation are provided in Monti *et al.* (2005b) (Chapter 2).

4.3.2 Maximum nitrification rate

4.3.2.1 Comparative period

The membrane and conventional mixed liquors exhibited similar nitrification behavior in the pilot-scale systems, with the only exception of a one-week long NH_4-N peak concentration in the MEBPR effluent. Therefore, to uncover potential differences in the nitrification activity, it was necessary to take one step further and assess the maximum nitrification rate with the use of off-line batch tests. A typical profile of NH_4-N and NO_X-N concentrations during an off-line nitrification batch test is illustrated in Figure 4.2. The reaction rates were calculated using linear regression methods. Both NH_4-N consumption and NO_X-N production were observed to be linear throughout the experiment with a high regression coefficient. As in the case of Figure 4.2, the measured amounts of NO_X-N generated in the batch tests were always larger than the mass of NH_4-N injected in the reactor. This occurred because processes such as hydrolysis and ammonification of organic matter contributed to the production of additional NH_4-N, which was then converted to NO_X-N (Henze *et al.*, 1987). The volumetric rate obtained in each experiment was normalized with the measured concentration of VSS to express the maximum nitrification

rate on a specific basis (i.e. mg N/g VSS·h). The equilibrium temperature in the parallel batch tests ranged between 17 and 23 °C during the entire investigation period, depending on the time of the year. For a pair-wise comparison of the specific nitrification rates in the two systems, it was decided not to correct the rates for temperature. However, the temperature effect was included in the estimation of the maximum specific autotrophic growth rate, for which the value was expressed at a standard temperature of 20 °C (see later).

The overall summary of maximum specific nitrification rates, measured in terms of NO_X-N generated in the batch tests, during the 10 and 7 h HRT experimental runs of the comparative study is presented in Figure 4.3. It can be noted that, with the only exception of bath test 1, the CEBPR biomass exhibited maximum nitrification rates which were from 15% to 75% greater than for the MEBPR counterpart. Such observations were also reflected in the SOUR measurement in batch tests 3 to 9 (see Fig. 4.4), further supporting the observation of significant differences between the two nitrifying mixed liquors. Figure 4.5 shows the average value with the 95% confidence interval of the maximum specific nitrification rate in the MEBPR and CEBPR sludge for the HRTs of 10 and 7 hours. The significant difference in the nitrification rates between the two processes was confirmed with a paired *t*-test at a 95% confidence level. It can also be noted that the reduction of the HRT from 10 to 7 hours resulted in a slight overall increase in the specific nitrification rates in both systems, and these increases were found also to be statistically significant. The specific nitrification rates in the membrane and conventional sludge were observed to be between 3 and 5 mg N/(g VSS·h), falling in the high end of the typical range reported in the technical literature (among others, Fan *et al.*, 1996; Mueller *et al.*, 1995; Harremoes and Sinkjaer, 1995). In a comparative nitrogen removal study performed by Soriano *et al.* (2003) at similar HRT and SRT conditions, specific nitrification rates between 4 and 7 mg N/(g VSS·h) were measured, and these were observed to be similar in both membrane and conventional systems. Manser *et al.* (2004) reported that membrane and conventional

nitrogen removal processes operated in parallel exhibited comparable nitrification rates, with values averaging about 2 mg N/(g VSS·h).

For the present study, it was initially hypothesized that the utilization of membrane filtration would result in the accumulation of a significant amount of organic and inorganic inert material in the MEBPR sludge compared to the CEBPR counterpart. Therefore, it was believed that the biomass viability in the MEBPR system might have been somewhat lower, causing a reduced specific biological activity of the mixed liquor. This hypothesis was tested by comparing the VSS/TSS ratio in the two mixed liquors during the 18 batch tests. Even though this ratio does not specifically measure the viable biomass, it can be used as an indicator of the organic content of the sludge which contains the overall microbial mass. Average values of 82.9 % and 83.7 % (±0.48 % and ±0.56%, 95% confidence interval) were estimated for the MEBPR and CEBPR mixed liquors, respectively. This difference was not found to be statistically significant with a paired *t*-test at 95% confidence level, suggesting a comparable level of organic matter in the two systems. It was also speculated that the significant reduction in the MEBPR nitrification rates might have been derived from substantial stripping of CO_2 (i.e. the primary substrate for autotrophic nitrifying organisms) due to extensive coarse bubble aeration in the aerobic zone. As a matter of facts the 12 scfm (0.34 m^3/min) coarse bubble air flow rate in the membrane system was much higher than the 3 scfm (0.085 m^3/min) fine bubble air flow rate in the conventional counterpart. The paired pH data in the aerobic zone were found to be highly correlated in the two systems (see Fig. 4.6), and they were concluded to be not significantly different. Comparable pH values in the aerobic mixed liquor do not necessarily indicate a similar bicarbonate equilibrium or similar CO_2 concentrations in the solutions. The pH in the aerobic compartment was likely determined by other factors, most notably the ammonium-ammonia and phosphorus equilibria (Stumm and Morgan, 1996), and the aeration rate (Tchobanoglous *et al.*, 2003). In addition, the retention of biomass debris and colloidal material by the membrane likely altered the water

chemistry of the aerobic zone. Direct measurement of CO_2 or total inorganic carbon concentration in the solution would be helpful to quantitatively assess the contribution of vigorous aeration on gas stripping.

4.3.2.2 Transition period

The observation of significant differences in the maximum specific nitrification rates during the comparative period, was further tested in two transition experiments. It was expected that, by starting both processes with the same inoculum (i.e. Transition I), the nitrification activity initially would be comparable in the two systems and, subsequently, the activities would diverge as demonstrated in the comparative period. In addition, by replacing the clarifier with a membrane filtration module (i.e. Transition II), it was hypothesized that the maximum specific nitrification rate of the evolving sludge would fall in a similar range as previously observed for the membrane sludge. Figure 4.7 illustrates the overall trend of the nitrification rate measured in the off-line batch tests during the two transition periods.

The MEBPR and the CEBPR treatment systems were re-started with the same inoculum on day 330. The nitrification activity measured at this time in batch test 18 (Fig. 4.7a) was similar in the two mixed liquors. Within two weeks, the maximum specific nitrification rate in the CEBPR sludge was at significantly higher level than for the MEBPR counterpart, and this difference remained until the end of Transition I (batch tests from 19 to 22). When the CEBPR system was then converted to a second MEBPR train (MEBPR-B), the maximum nitrification activity of the evolving sludge progressively declined over the following three months, eventually decreasing to the same range as that previously observed for the MEBPR-A sludge (Fig. 4.7b). It should be noted that the nitrification rates in Transition II were normalized to 20 °C using a temperature dependency coefficient, θ, of 0.9 (Henze *et al.*, 2000). This allowed for an accurate comparison of the results obtained from the batch tests, each performed at a different

ambient temperature. The two transition experiments provided an additional demonstration of the impact of membrane solid-liquid separation in lowering the specific nitrification activity. Even though the MEBPR and CEBPR systems exhibited stable and complete nitrification throughout the study, the off-line batch tests revealed that the intrinsic nitrification potential of the two mixed liquors was clearly different. It is worth noting that the incident of NH_4 breakthrough during the coldest period of the research was measured only in the MEBPR effluent (see Fig. 4.1), possibly caused by the reduced nitrification capacity of the MEBPR sludge.

During Transition I, the biomass viability hypothesis previously discussed was further tested with DNA and RNA chemical assays. It is generally accepted that the amount of RNA in a cell is primarily dependent on the growth rate and, therefore, on its microbial activity. On the other hand, the cellular DNA content can be exploited to estimate the biomass in a sample (Muttray, 2001). The results of the chemical assay are presented in Figure 4.8 for batch tests 19, 20, and 22. The data for DNA and RNA were normalized with the VSS concentration of the sludge to estimate the content on a specific basis. According to Figure 4.8, the RNA/VSS was not found to be significantly different in the two mixed liquors, indicating that the overall microbial activity was indeed comparable in the two systems. In contrast, the DNA/VSS ratio of the MEBPR sludge was observed to be 12 to 15 % lower than that of the CEBPR sludge, and the averaged values were significantly different at a 95% confidence level. These results may suggest that the MEBPR was hosting a microbial community with lower overall viability, even though a direct correlation with the nitrification activity remains difficult to propose. Molecular techniques targeting microorganisms at the gene level may be helpful to uncover differences in the two nitrifying microbial communities.

4.3.3 Nitrifiers maximum spericif growth rate

The assessment of the nitrification process with the use of maximum nitrification rates, normalized to the VSS of the sample, is somewhat flawed because it does not consider the actual concentration of nitrifiers present in the mixed liquor. As a matter of fact, the observation of slower nitrification rates in the MEBPR sludge may have been the result of a nitrifying community with slower growth kinetics or, alternatively, of a mixed liquor with similar type of nitrifiers, but in reduced numbers. Kinetic parameters such as the maximum growth rate are better suited to deliver a more accurate representation of the nitrification activity. For this reason, a technique combining mathematical modeling and batch testing was employed to estimate the maximum specific growth rate of autotrophic biomass, μ_{AUT}, in the MEBPR and CEBPR processes (Novak *et al.*, 1994a; Wanner *et al.*, 1992). Each nitrification batch test was simulated in AQUASIM (Reichert, 1998) and μ_{AUT}, normalized to 20 °C, was estimated by best fitting the measured NH_4-N and NO_X-N profiles. The TUDP model (Meijer *et al.*, 2001) was adopted for the simulation of the experiments, with only the most significant processes affecting nitrification activated for parameter estimation. These included: aerobic growth and decay of autotrophs, decay of heterotrophs, aerobic hydrolysis of slowly biodegradable organic matter, and aerobic growth of heterotrophs on soluble fermentable carbonaceous matter. The initial values of the sludges used for the batch experiments were calculated in a previous steady-state simulation of the pilot-scale processes during each of five investigated periods. Since nitrification in the pilot plants was always complete, the initial concentration of nitrifiers had to be hand-calculated, according to the approach described below.

4.3.3.1 Initial concentration of nitrifiers

A modified version of the steady-state model proposed by WERF (2003) was used to calculate the nitrifiers concentration in the mixed liquor during each experimental period:

$$X_{AUT} = \frac{(TKN_{INFL} - TKN_{EFFL} - TKN_{SLUDGE}) \cdot SRT}{(1 + b_{AUT,WA} \cdot SRT) \cdot V} \cdot Y_{AUT}$$

where:

X_{AUT} = initial concentration of autotrophs; TKN_{INFL} = influent TKN load [mg N/d]; TKN_{EFFL} = effluent TKN load [mg N/d]; TKN_{SLUDGE} = waste sludge TKN load [mg N/d]; SRT = total sludge retention time [d]; Y_{AUT} = autotrophs growth yield coefficient [g COD/g N]; $b_{AUT,WA}$ = weighted average autotrophs decay rate [g/(g·d)]; V = system volume [L].

X_{AUT} was calculated in each of the five periods during the comparative study. The batch test simulations falling in the same experimental periods (see Fig. 4.1) were completed with the X_{AUT} initialized to the same steady state value.

Y_{AUT} was set equal to 0.24, which is the default value suggested for activated sludge models (Henze *et al.*, 2000). This stoichiometric coefficient value was proposed following thermodynamic consideration of the bacterial cell (Rittmann and McCarty, 2001) and therefore, is not subject to significant variation from one biological process to the other. If the volume of the MEBPR system can be assumed to be equivalent to the bioreactor volume, the same is not true for the CEBPR train. Here, the bioreactor volume had to be added to the volume occupied by the sludge blanket in the clarifier to obtain the total biological process volume. The sludge blanket volume was estimated from manual measurement of its height and was also calibrated with the steady state model, to match the NO_X concentration in the return activated sludge recycle flow.

A direct measurement of the autotrophic decay rate, b_{AUT}, was not available in this study. Dold *et al.* (2004) pointed out that b_{AUT} has a significant impact on the estimation of the maximum specific growth rate, μ_{AUT}. Therefore, it was initially assumed that the nitrifying populations in the MEBPR and CEBPR systems were subject to the same decay rate coefficient of 0.15 g/(g·d), the default value in the activated sludge model (Henze *et al.*, 2000). Nowak *et al.*

(1994b) and, later, Siegrist *et al.* (1999) suggested that the overall biomass decay rate, including that of nitrifiers, is smaller under unaerated than aerobic conditions. The same phenomenon was also observed in this study, when the sludge from both systems was subjected to batch tests under a sequence of anaerobic, anoxic and aerobic conditions to evaluate the EBPR kinetics (Monti *et al.*, 2005a, Chapter 3). By monitoring the ammonia release in the first two unaerated phases and the nitrate production in the subsequent aerobic phase, it was determined that the overall biomass decay rate under anaerobic and anoxic conditions was about 40% and 70% respectively, of that under aerobic conditions. Although in the present study the estimation of the decay reduction was obtained for the overall biomass, it seemed reasonable to apply the same reduction factor also for the nitrifier decay rate. Therefore, when setting b_{AUT} for the X_{AUT} equation, the following weighted average decay value was used :

$$b_{AUT,WA} = \pi_{ANA} \cdot \eta_{ANA} \cdot b_{AUT} + \pi_{ANX} \cdot \eta_{ANX} \cdot b_{AUT} + \pi_{AER} \cdot b_{AUT}$$

where:

$b_{AUT,WA}$ = weighted average autotrophs decay rate [g/(g·d)]; π = sludge mass fraction in the anaerobic (ana), anoxic (anx) and aerobic (aer) zone; η = decay rate reduction factor; b_{AUT} = autotrophs decay rate [g/(g·d)].

4.3.3.2 Estimation of μ_{AUT}

Figure 4.9 presents an example of curve fitting for nitrification batch test 8 performed with the MEBPR sludge. The initial concentrations of NH_4 and NO_X in the batch experiment, together with μ_{AUT}, were left available for parameter estimation to obtain the best fit of the measured profiles. The averaged estimated maximum specific growth rate μ_{AUT} of the MEBPR and the CEBPR nitrifying communities are illustrated in Figure 4.10 for the experimental HRTs of 10 and 7 hours. As can be seen, the estimated μ_{AUT} of the CEBPR sludge (0.84 – 1.11 1/d) was significantly higher than for the MEBPR counterpart (0.64-0.78 1/d). The μ_{AUT} values

calculated in this investigation fell in the typical range reported in the literature (Copp *et al.*, 1995). It can then be concluded, by assuming the same prevailing decay rate b_{AUT}, that the membrane solids-liquid separation favors the growth of a distinct nitrifying microbial community with reduced maximum specific growth rates.

Jiang *et al.* (2004), on the other hand, measured a significantly higher nitrifier decay rate in a side-stream MBR system, compared to the default values in ASM1, pointing to the extended shear stress in the pressurized external membrane as the possible explanation. Even though in the present study a submerged membrane was employed, significant shear stress on the MEBPR sludge was created by the vigorous coarse bubble aeration applied for fouling control. Therefore, it seemed reasonable to challenge the initial assumption of equivalent b_{AUT} in both systems and to hypothesize a larger decay rate of the MEBPR nitrifying community, thereby reducing the concentration of nitrifiers in the system relative to the CEBPR process. In other words, the lower maximum specific nitrification rates measured in the MEBPR batch tests could be the result of a nitrifying sludge containing similar organisms to those in the CEBPR process (i.e. similar μ_{AUT}), but which may have been present at reduced concentrations. A new decay rate for the MEBPR nitrifiers was estimated, so that their maximum specific growth rate would be equal to that in the CEBPR process. For each MEBPR batch test simulation, the initial concentration of nitrifiers was gradually decreased by raising the value of the decay rate, and the new μ_{AUT} estimated. This was repeated until the estimated MEBPR μ_{AUT} was equivalent to that measured with the CEBPR biomass during the same batch test. The averaged estimated MEBPR nitrifier decay rate, b_{AUT}, in all the experiments during the comparative study is presented in Figure 4.11 and compared with the initial default value. According to this elaboration, the MEBPR b_{AUT} was estimated to be 0.25 g/(g·d), significantly greater than the default value of 0.15 g/(g·d). Dedicated measurements of the nitrifier decay rate (e.g. Dold *et al.*, 2004), together with the analysis of the nitrifying population at the genetic level (e.g. Wagner *et al.*, 1996), are two critical tasks that may help to

elucidate the causes of the reduced maximum specific nitrification activity in the MEBEPR sludge.

4.4 Conclusions

A one-year long comparative study was conducted with membrane and conventional EBPR processes in parallel under identical operating conditions to assess the impact of membrane solid-liquid separation on the nitrification kinetics. Both systems exhibited complete and stable nitrification throughout of the investigation. However, when the maximum specific nitrification activity was measured in off-line batch tests, significant differences were observed in the two systems, with the CEBPR sludge exhibiting 15 to 75% greater nitrification rates. Additional evidence of this dissimilarity was gathered through SOUR measurements in the batch experiments and by monitoring the nitrification activity of the conventional sludge over a period of four months, as it evolved toward a membrane sludge.

With the help of mathematical modeling, the maximum specific growth rate of autotrophs, μ_{AUT}, was estimated in each batch test by fitting the NH_4-N and NO_X-N profiles. By assuming the same nitrifier decay rate, b_{AUT}, at the default value of 0.15 g/(g·d), it was found that the μ_{AUT} of the membrane sludge was 30% lower than that of the conventional counterpart, which implied distinct nitrifying microbial communities in the two systems. Due to the vigorous coarse bubble aeration employed in the membrane system, it was hypothesized that lower specific nitrification activities might be the result of a reduced number of nitrifying organisms due to more extensive decay occurring in the MEBPR. It was estimated that, the decay rate, b_{AUT}, under MEBPR conditions could be increased from the default value of 0.15 to 0.25 g/(g·d).

Table 4.1 Experimental periods and relative operating conditions.

Period	Day	Temp.[1] (°C)	Operating parameters				
			HRT [h]	SRT [d]	Anx rec. ratio[2]	Aer rec. ratio[2]	HAc Addition [mg COD/L]
I	1 - 109	21.9 (0.21)	10	12	1	2	0
II	110 - 147	21.1 (0.45)	10	12	1	1	0
III	148 - 185	18.1 (0.45)	10	12	1	2	20
IV	186 - 236	16.3 (0.42)	7	12	1	2	20
V	237- 329	18.5 (0.36)	7	12	1	1	30
Trans. I	330 - 384	19.2 (0.23)	7	12	1	1	30
Trans. II	385 - 485	21.8 (0.26)	7	12	1	1	30

[1] Temperature expressed as average value with 95% confidence interval in parenthesis.

[2] Anx rec.: anoxic recycle; Aer rec.: aerobic recycle. The term ratio is defined as the recycle flow rate divided by the influent flow rate. The return activated sludge (RAS) from each clarifier was set to half the influent flow rate, therefore a total RAS recycle ratio of 1.

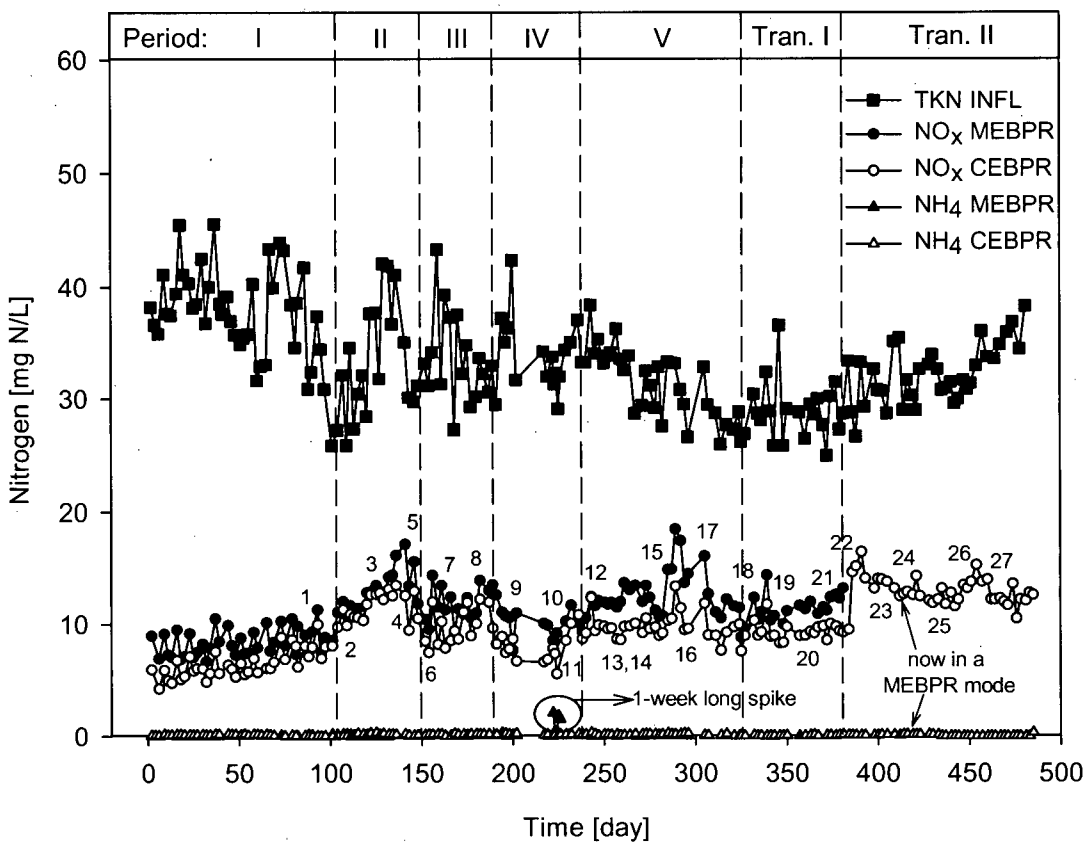

Figure 4.1 **Total nitrogen in the influent, and ammonium and nitrite+nitrate in the MEBPR and CEBPR effluent. The numbers from 1 to 27 represent the points in time at which each batch test was executed.**

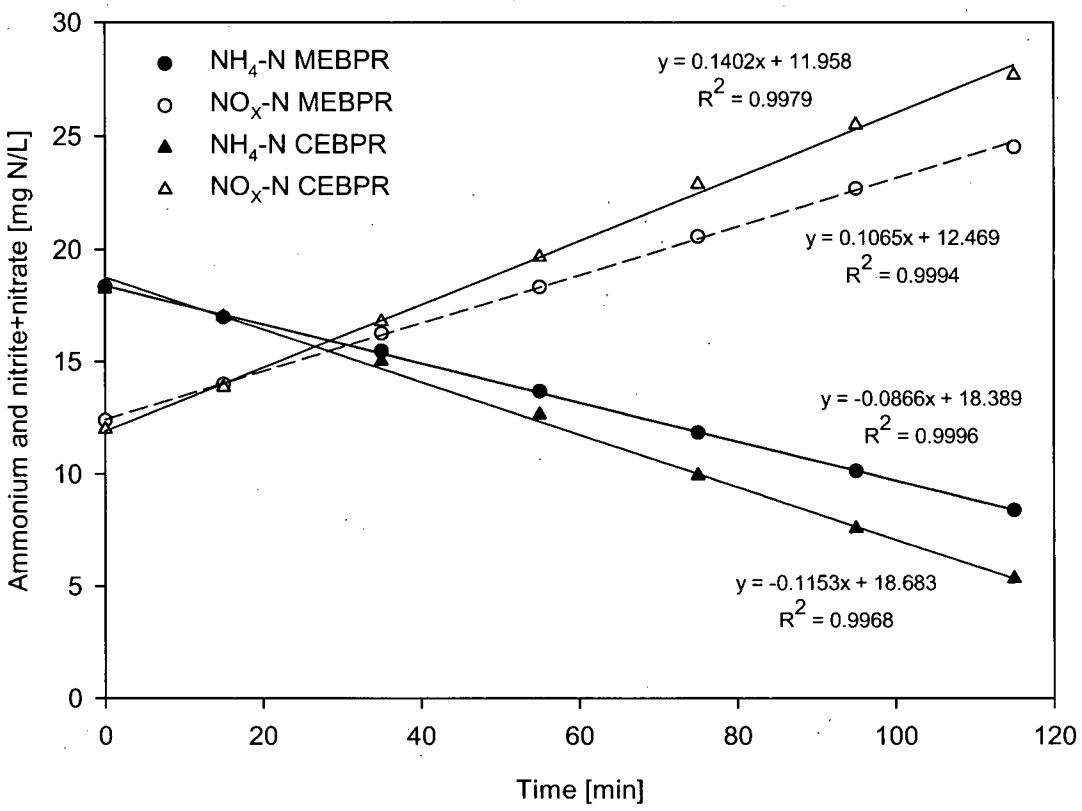

Figure 4.2 **Example of a nitrification batch test with MEBPR and CEBPR mixed liquors. The volumetric nitrification rates together with the linear regression coefficient are also shown.**

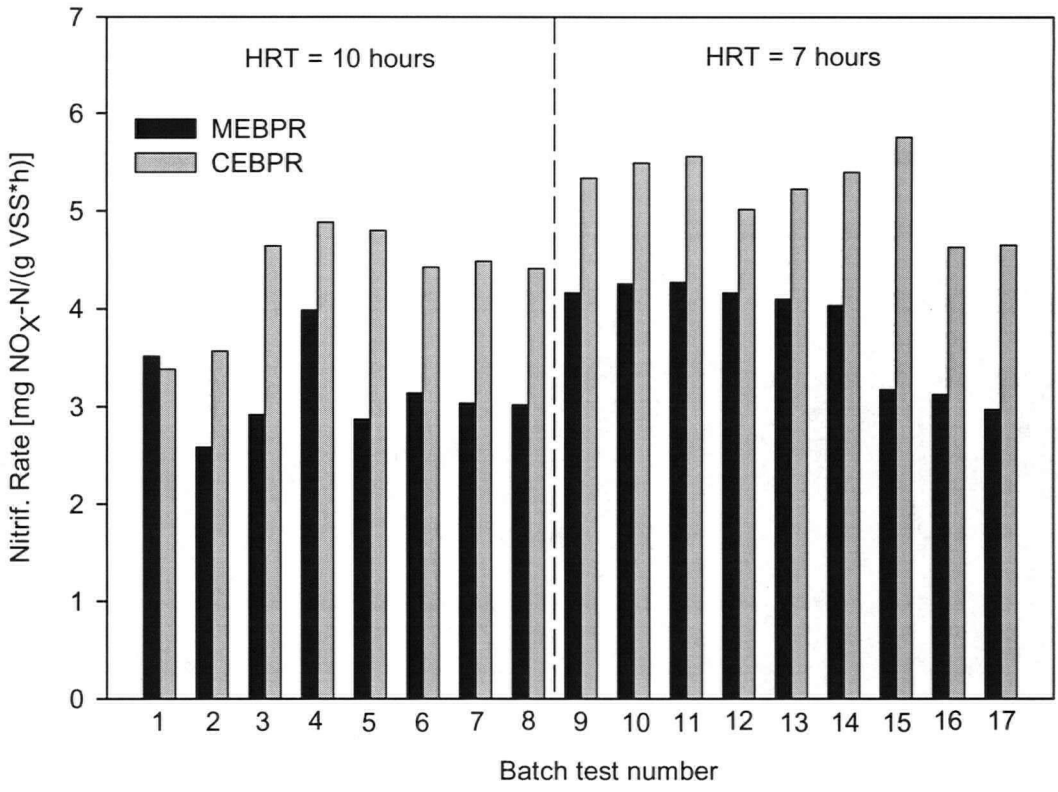

Figure 4.3 **Comparison of maximum specific nitrification rates in the MEBPR and CEBPR processes during the comparative study.**

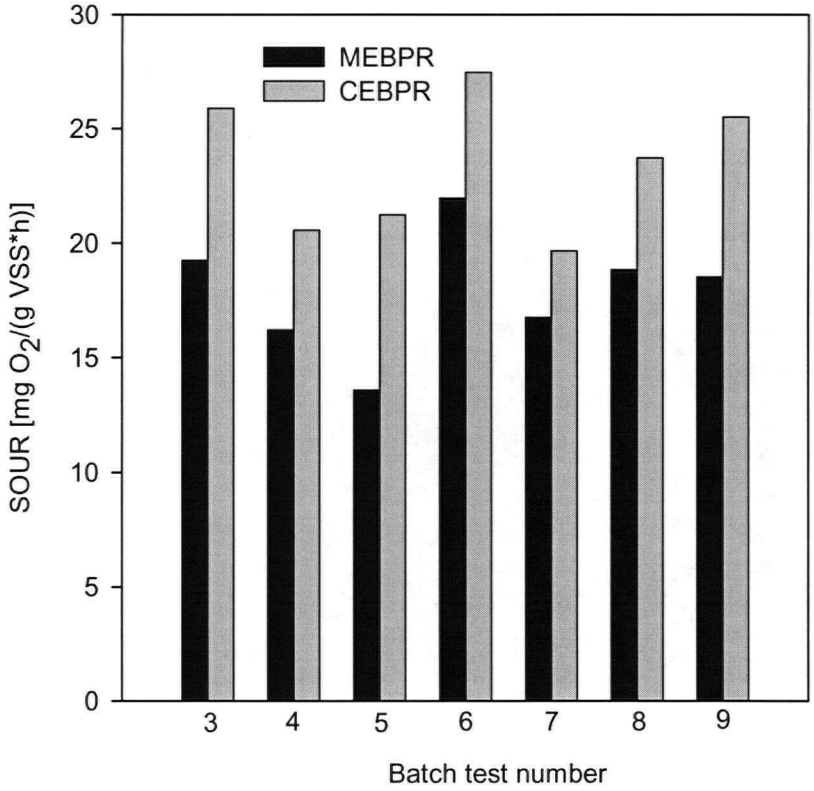

Figure 4.4 **Comparison of the specific oxygen utilization rate (SOUR) during the course of several batch experiment with MEBPR and CEBPR mixed liquors.**

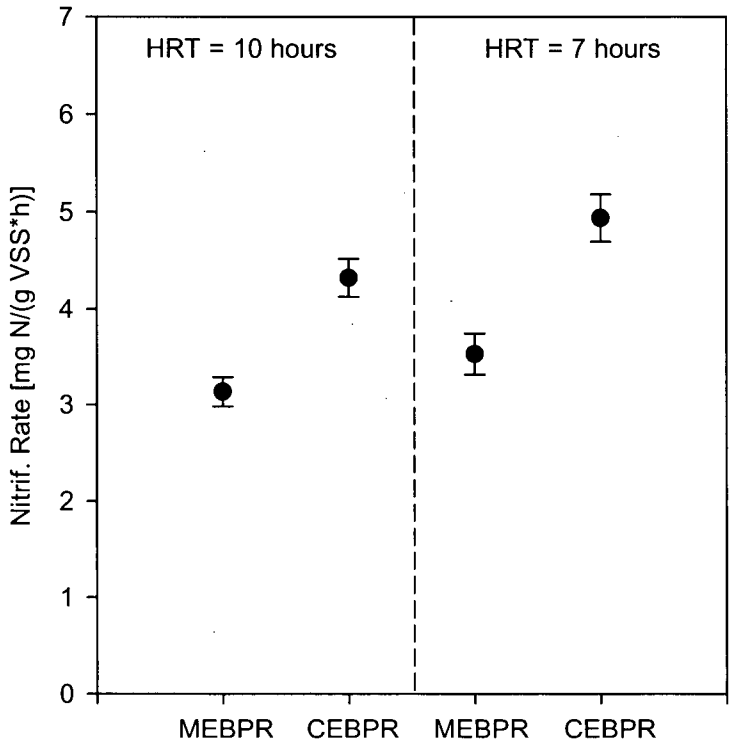

Figure 4.5 **Average maximum specific nitrification rate in the MEBPR and CEBPR process at the HRTs of 10 and 7 hours. Error bar: 95% confidence interval.**

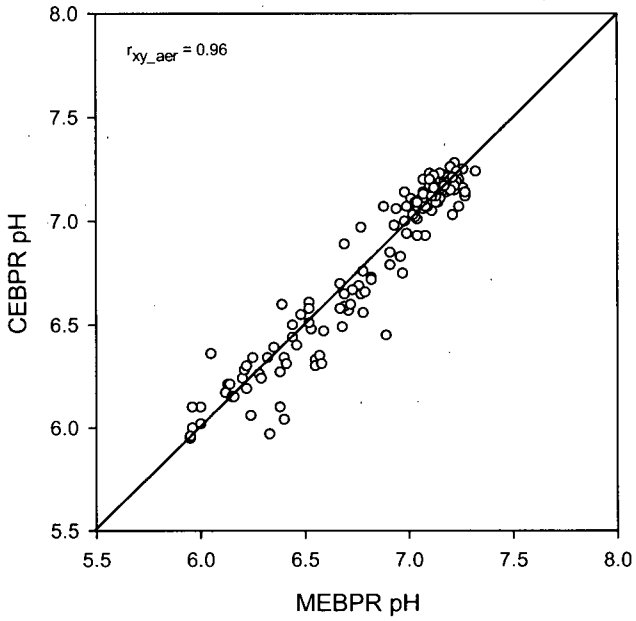

Figure 4.6 **Correlation between the aerobic pH data in the two systems during the comparative study.**

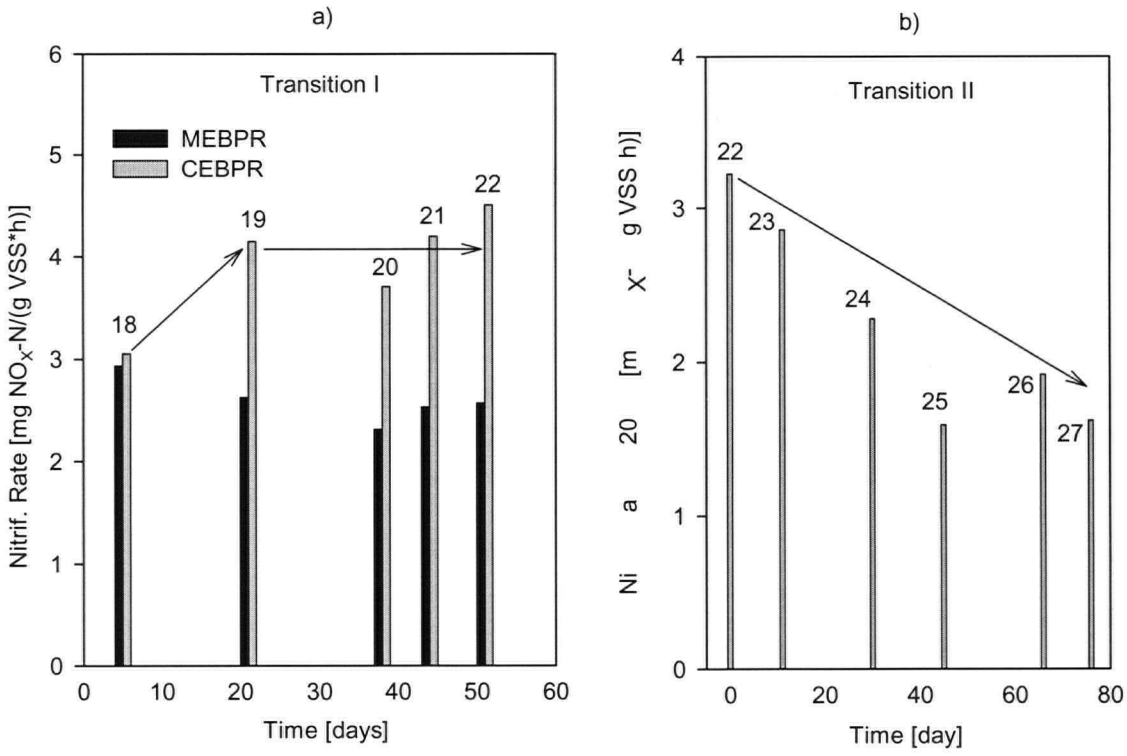

Figure 4.7 **(a) Comparison of maximum specific nitrification rate in the MEBPR and CEBPR processes initially inoculated with the same sludge (transition I). (b) Evolution of the nitrification activity in the conventional sludge as it moved toward a membrane sludge (transition II). The number of the batch test is reported on each column.**

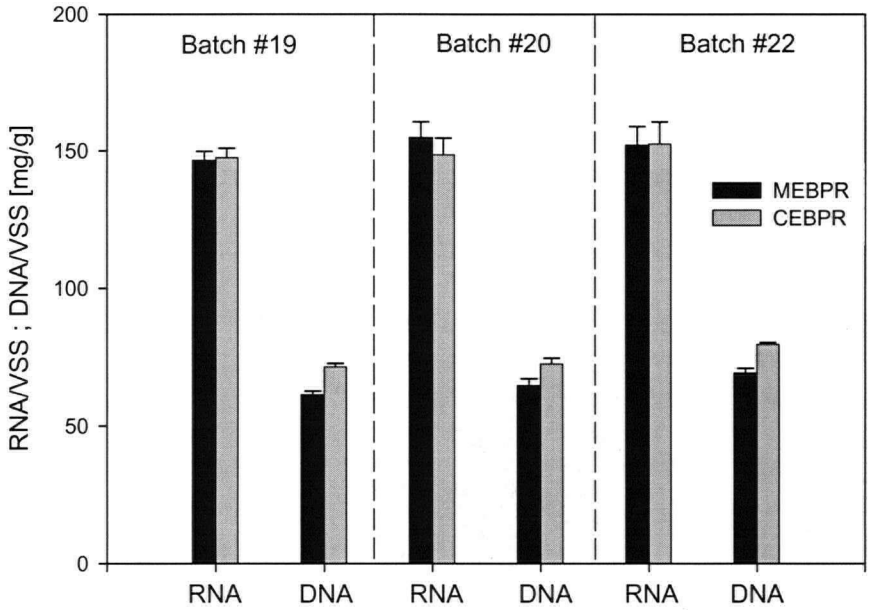

Figure 4.8 **Specific RNA and DNA content of the MEBPR and CEBPR sludge during the transition I. Error bar: 95% confidence interval.**

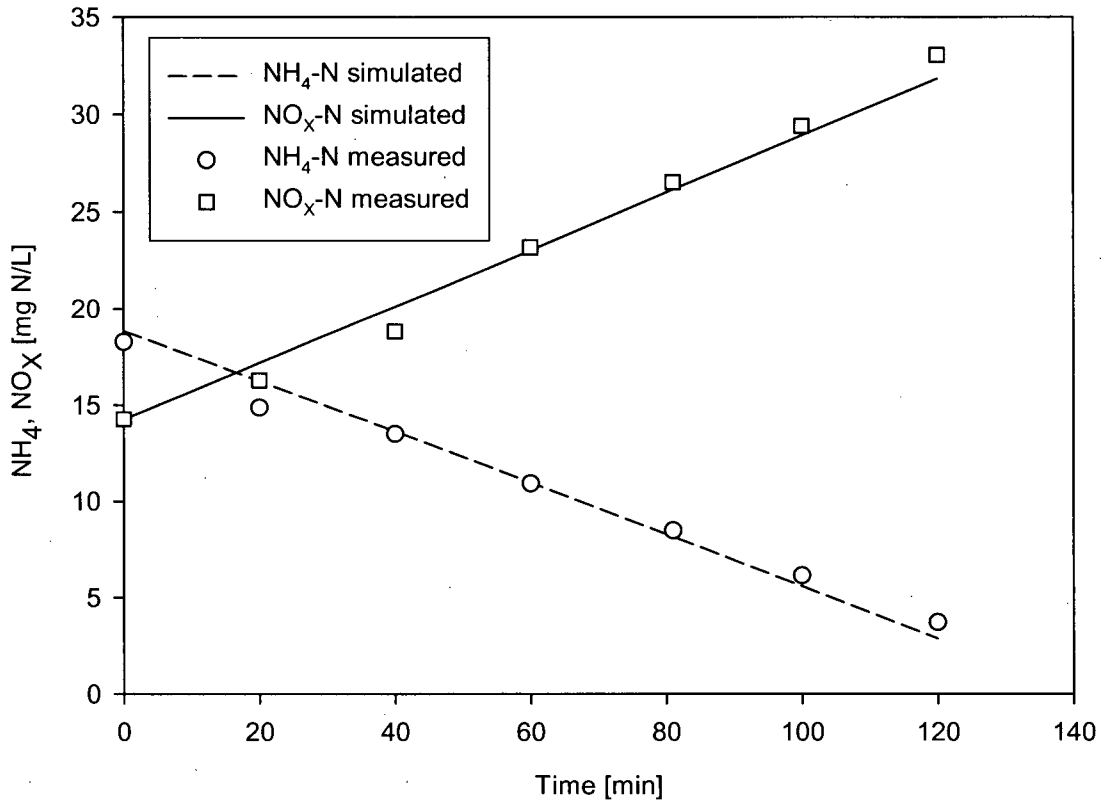

Figure 4.9 **Example of curve fitting to estimate the maximum specific growth rate of autotrophs.**

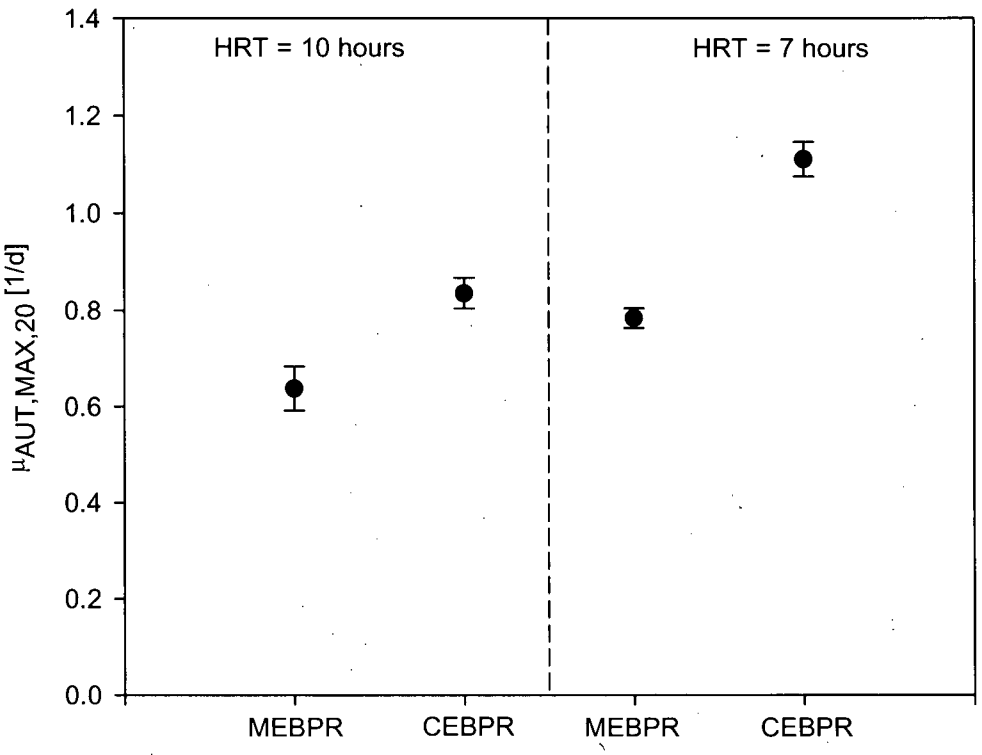

Figure 4.10 **Estimated maximum specific growth rate of autotrophs μ_{AUT} in the MEBPR and CEBPR nitrifying sludge, at the HRT of 10 and 7 hours. Error bar: 95% confidence interval.**

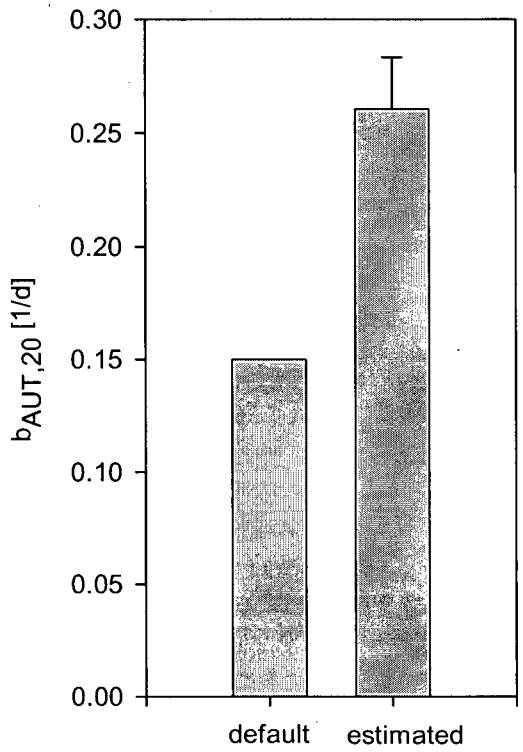

Figure 4.11 Comparison of the nitrifier decay rate b_{AUT} default value (Henze *et al.*, 2000) and the estimated value for the MEBPR sludge.

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Chapter 5 A comparative study of bacterial community dynamics in an enhanced biological phosphorus removal process with membrane and gravity solids-liquid separation*

5.1 Introduction

With the implementation of more stringent requirements on the quality of municipal wastewater discharges in the industrialized world, the biological nutrient removal (BNR) process coupled with membrane solids-liquid separation is increasingly considered to be a cost-effective and sustainable treatment solution. Bioreactors performing BNR harbor a complex community of microorganisms that, when circulated through anaerobic and aerobic phases, simultaneously remove carbon, nitrogen and phosphorus from the influent wastewater. In the past two decades, significant progresses in cultivation-independent techniques have allowed a more detailed characterization of microbial populations fostered in conventional BNR bioreactors with settling tanks. For example, the use of molecular tools for the analysis of BNR systems demonstrated that the relative abundance of *Acinetobacter,* long indicated as the key organism in the enhanced biological phosphorus removal (EBPR) mechanism (Fuhs and Chen, 1975), was dramatically overestimated, and a broader range of organisms are in fact capable of EBPR (Wagner *et al.*, 1994). Similarly, model organisms involved in the nitrification process, such as *Nitrosomonas europaea*, have been questioned by several researchers using fluorescence in-situ hybridization techniques, pointing to other ammonia-oxidizers as more relevant players (Wagner *et al.*, 2002).

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The advent of membrane filtration in wastewater treatment processes has brought several improvements, which include the production of a solids-free effluent and the operation of the system at higher loading rates with minimum sludge wasting (Manem and Sanderson, 1996). When the secondary clarifier is replaced with membrane filtration for the separation of microorganisms from the treated water, the following changes are expected to occur: (1) all the microbial species are retained in the bioreactor irrespective of their ability to settle, therefore 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 no longer equivalent to the hydraulic retention time (HRT) but, rather, to the solids retention time (SRT) (Ben Aim and Semmens, 2003); (3) the formation of flocs will be hindered by the strong course-bubble aeration or the high shear employed to reduce the membrane fouling, resulting in a biomass particle distribution that is finer than that in the conventional system (Cicek *et al.*, 1999), and; (4) the sludge blanket normally present at the bottom of secondary clarifiers is eliminated, as is the anoxic environment associated with the sludge blanket. All these modifications that accompany the replacement of the secondary clarifier with a membrane filtration module are hypothesized to impact the microbial community composition of a biological treatment system.

This comparative study, to assess the impact of membrane separation on the performance and removal kinetics of the process, revealed that significant changes do occur. The elimination of the anoxic sludge blanket at the bottom of the secondary clarifier caused a reduction of the denitrification capacity of the system and an overall higher biomass decay (Monti *et al.*, 2005ab, Chapter 2 and 3). In addition, the maximum specific nitrification rate in the membrane process was observed to be significantly lower than that of the conventional counterpart, likely due the high shear conditions of the coarse-bubble aeration that increased the nitrifier decay rate (Monti *et al.*, 2005c, Chapter 4). Finally, the membrane process exhibited a propensity to accumulate

large quantities of foam at the surface of the anoxic zone (Monti *et al.*, 2005d, Chapter 7). These fundamental differences further support the hypothesis that the presence of a membrane filtration module favored the establishment of a microbial community with a distinct composition relative to that of the parallel conventional system. Whereas considerable research effort has been devoted to the membrane process operation, the microbiological aspects of this innovative biotechnology and how they compare to the conventional activated sludge process remain still scarcely investigated. A few recent studies attempted a comparison of the microbial community composition in two such 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 two parallel systems in these studies were far from identical. Although these studies contributed indisputably to the advancement of the understanding of the microbiology of membrane bioreactors, only limited information was obtained on the true impact of membrane filtration on the microbial population. In particular, it is not certain whether the main differences in the performance between conventional and membrane systems (Monti *et al.*, 2005a, Chapter 2) are due to differences in the community composition selected in the respective system. Further, the long-term community composition dynamics have not been characterized in either type of system in well controlled studies.

In the present study, we analyzed the bacterial community composition in parallel membrane and conventional processes operated under comparable conditions and with the same municipal wastewater feed. The objectives of the study were (1) to quantify the similarity of the bacterial communities supported in the two systems over time, (2) to determine if distinctive populations are associated with the MEBPR and CEBPR processes, and (3) to relate the dynamics of the community composition with the process treatment performance. The composition of the bacterial population was investigated with a DNA-based fingerprinting method called ribosomal intergenic spacer analysis, or RISA (Gürtler and Stanisich, 1996). This

molecular tool differentiates microorganisms present in a population by utilizing the length heterogeneity of the ribosomal intergenic spacer region between the small (16S) subunit and large (23S) subunit rRNA genes. The polymerase chain reaction (PCR) amplicons are then separated in acrylamide gels, resulting in a fingerprint band pattern. Compared to the more commonly used PCR-DGGE method, which targets a fragment of the 16S rRNA gene (Muyzer *et al.*, 1993), RISA has been shown to better distinguish closely related strains due to the greater variability of the ribosomal intergenic spacer region (Garcia-Martinez *et al.*, 1999; Toth *et al.*, 2001). In addition, RISA amplification can include ca. 500 base pairs of the 3' end of the 16S rRNA gene that provide enough sequence information to conduct a phylogenetic analysis (Yu and Mohn, 2001).

5.2 Materials and methods

5.2.1 Pilot plant operation and sampling

The long-term comparison of the microbial community composition in the membrane enhanced biological phosphorus removal (MEBPR) and conventional enhanced biological phosphorus removal (CEBPR) processes was investigated at the UBC pilot plant which treated municipal wastewater. Detailed descriptions of the research site, process layout, and the monitoring and analytical programs can be found in Monti *et al.* (2005a) (Chapter 2). The operating conditions of the MEBPR and CEBPR pilot scale systems in this study were similar to those described in Monti *et al.*, 2005c (Chapter 4): a nine-month-long comparative period, followed by two transition periods. The set up of the CEBPR treatment train commenced only after the retrofitting of the MEBPR system was completed. For this reason, the CEBPR process was started up about two months later, using the MEBPR biomass as inoculum.

Mixed liquor samples were collected every two to four weeks from the aerobic recycle line of the two systems which returned the activated sludge from the aerobic zone to the anoxic

compartment. The samples were transferred to the laboratory on ice, and the biomass was harvested within 2 hours of sampling. Triplicates of 1.80 mL sample were centrifuged at 4 °C for 5 min at 14,000 x g and stored at -20 °C until further DNA extraction. A complete overview of the experimental design and sample collection is given in Table 5.1.

5.2.2 DNA extraction

The total DNA was extracted from the sample according to the method of Yu and Mohn (1999). The bead beating procedure was modified by replacing the previously used apparatus (BioSpec Products, Inc., Okla.) with the FastPrep® Instrument and processed at 4.5 m/s for 45 sec, after several optimization trails. The extracted DNA was quantified by running 3 μL of DNA extract on a 0.9 % agarose gel for 50 min at 100 V. The gel was then stained with ethidium bromide and the absorbance was measured at 260 nm.

The optimization study on the new bead beating process was conducted by comparing the original method with five combinations of velocity and duration in the FastPrep, from less to more vigorous conditions: (1) original bead beating; (2) 4.5 m/s and 30 sec; (3) 4.5 m/s and 45 sec; (4) 5.5 m/s and 30 sec; (5) 5.5 m/s and 45 sec; (6) 6.5 m/s and 30 sec. Each condition was tested in triplicate, with sludge samples coming from both pilot processes. The original method consisted of two-minute-long bead beating, repeated twice.

5.2.3 PCR-RISA

The resultant DNA sample was diluted to 10 ng/μL and used in the PCR amplification with primers S92f (5'-CTYAAAKGAATTGACGG-3') and L189r (5'-TACTGAGATGYTTMARTTC-3') which anneal to position 910 to 926 of the 16S rRNA gene and 189 to 207 of the 23S rRNA gene (*Escherichia coli* numbering). The resultant PCR products contain the length-variable ribosomal intergenic spacer (RIS) region, and they are referred to as rDNA-RIS amplicons. PCR amplification was carried out in a total volume of 50 μL containing

39.15 µL dH_2O, 1x PCR buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), 2.0 mM $MgCl_2$, 672 µL of bovine serum albumin per mL, 200 µM concentrations of each deoxynucleoside triphosphate, 30 pmol of each primer, 1.25 U of *Taq* DNA polymerase (Invitrogen™, CA), and 50 ng of template DNA. To reduce risks of contamination, all pipettes, cotton-plugged pipette tips, eppendorf tubes and PCR tubes were uv-irradiated for 30 min before the PCR set-up. Cycling parameters on the Robocycler Gradient 96 (Stratagene®, CA) were 95 °C for 5 min, 30 cycles of 94 °C denaturation for 30 sec, 47 °C annealing for 30 sec and 72 °C extension for 2 min and ending with a final extension at 72 °C for 5 min. Negative and positive control containing no DNA template and *E. coli* DNA template, respectively, were included in parallel. The outcome of the PCR step was verified by running 5 µL of rDNA-RIS amplicons on 1.2 % agarose gel. The rDNA-RIS amplicons were purified with a QIAquick PCR purification kit (Qiagen, CA) and eluted with 30 µL of EB (10 nM Tris-Cl, pH 8.5) prior to electrophoresis.

To facilitate handling of the acrylamide gels during staining and imaging, the long glass plates used for the electrophoresis were treated with Sigmacote® (SIGMA CHEMICALS CO., MO) to prevent the gel from sticking after electrophoresis, and the short glass plates had a Bind Silane (Promega, WI) treatment to make the gel stick during staining and imaging. The samples were loaded on the gel in a randomized order, and rDNA-RIS amplicons were electrophoretically separated on a 3.5% Duracryl (30% Duracryl 30T, 2.C; Genomic Solutions, MI) gel at 70 V for 16 hours. Subsequently, the gel was stained with 3 µL of Gelstar (Bio-Whittaker Molecular Applications, Rockland, ME) in 200 mL 1x TAE (40 mM Tris Acetate, 2 mM EDTA) for 1.25 hours, then double-stained with 5 µL of SYBR Green I (Molecular Probes, OR) in 200 mL 1x TAE for 1.25 hours. Fingerprint patterns were imaged on a Typhoon Imager (Amersham Biosciences, CA) using a 526 SP/Green (532 nm) laser, 600 photo multiplier tube for detection and quantification of the emitted light, medium sensitivity and a pixel size of 50 µm. Fingerprint banding patterns were analyzed with GelCompar II™ (Applied Maths, Belgium) and normalized

using a 100 base pair ladder (Invitrogen™, CA), which was loaded in at least 3 positions on every gel. For the band selection step, minimum profiling was set at 7.5% relative to the maximum value to adjust for fingerprint pattern with uneven intensity. This value maximized for the capture of real bands while excluding apparently false bands. The band report was exported from GelCompar to a spreadsheet and band classes (operational taxonomic units – OTUs) were established from the band mobility and intensity data. Starting from the smallest value of band mobility, equal ranges (band classes) of band mobility were identified, maximizing the band classes containing a single band. Inevitably, some band classes were empty, while others contained the sum of two bands.

5.2.4 Numerical analysis of RISA bands

The band diversity of microbial community fingerprints were examined with the richness, Shannon-Wiener, and evenness indices. Richness is defined as the number of bands, in this case, the number of bands classes that contained at least one band. The Shannon-Wiener index ($H' = -\sum_{i=1}^{S} p_i \cdot \log p_i$) was calculated based on the number of bands (S) and the relative intensity of those bands (p_i), and it measures the species composition of the fingerprint (Legendre and Legendre, 1998). Evenness, the relative equal abundance of different bands in a fingerprint, was computed according to Pielou ($E = H'/H_{max}$), where H_{max} is the maximum diversity value when all the bands are equally represented (Pielou, 1966). All these three indices were calculated in PC-ORD software (McCune *et al.*, 2002). Analysis of variance was conducted on the Shannon diversity indices, and the results were considered significant at $p < 0.05$.

The similarity between samples was computed with the Pearson product moment correlation coefficient (Pearson, 1926), which directly compares densiometric curve data generated by band patterns. The Pearson correlation coefficient has been shown to be an

appropriate method for the analysis of complex bacterial community fingerprints (Haene *et al.*, 1993). Cluster analysis was performed in GelCompar II™ by constructing dendograms relating band pattern similarities with the unweighted pair group method with arithmetic mean (UPGMA) algorithm.

Principal Component Analysis (PCA), a multivariate statistical analysis technique, was used to plot the maximum variance of the bacterial community band classes data in two dimensions (i.e. axis 1 and axis 2). In a PCA ordination plot, samples with similar fingerprints are placed close to one another, and those that are dissimilar are placed far apart. In addition, this plot allows for an easy visualization of how the microbial community changes with time. The main matrix was constructed as rows of samples and columns of band classes and was used as an input file in PC-ORD software.

Indicator Species Analysis, based on the Dufrene and Legendre's (1997) method, was used in PCR-ORD software to select those bands significantly different between groups of samples. The method combines information on the concentration of band abundance in a particular group of fingerprints and the faithfulness of occurrence of a band in that group. It produces indicator values for each species in each group. These were tested for statistical significance using a Monte Carlo technique, and bands with p values smaller than 0.1 were selected.

Multi-response permutation procedures (MRPP) were performed (Mielke and Berry, 2001) in PC-ORD software to test the null hypothesis of no difference in average within-group ranked distances (δ). A Ranked Sørenson non-metric distance measure was used, and groups were defined according to the two systems. T is the test statistic which describes the separation between groups. The more negative the T value, the stronger the separation. A is the agreement statistic which describes the within-group similarity. When all items are identical, A=1. When the heterogeneity within a group equals expectation by chance, then A=0, and when there is less

agreement within groups, A<0. Last, the *p*-value evaluates the likelihood of getting a δ as extreme or more extreme than the observed δ. Results were considered significant if $p < 0.05$.

5.3 Results and discussion

Before addressing the central research question on the comparison between the membrane process and conventional process microbial communities, two preliminary but essential experiments were conducted. The first one revolved around the improvement in the bead beating step as a part of the DNA extraction methodology. The second one dealt with the critical question of which location in the bioreactor to sample from in order to obtain a representative sample of the microbial community composition.

5.3.1 Revising the bead beating procedure

The DNA extraction method developed by Yu and Mohn (1999) included a bead beating step of 2 min, repeated twice, and it was performed in an apparatus that could only accommodate one sample at a time. In order to expedite the extraction process, the FastPrep® Instrument was considered as a potential replacement. It was critical to insure that a similar fingerprinting pattern was obtained compared to the original and validated method.

A visual comparison of the RISA gels generated in the bead beating experiments is presented in Figure 5.1 for both membrane and conventional sludge. The FastPrep-based bead beating generated an overall fingerprinting pattern highly similar to that of the original apparatus, with the only exception of few bands on the right end side of the gel being more intense with the original method. In addition, a visual analysis of results from the five FastPrep treatment conditions (2 to 6) suggest that a small variability is produced from more gentle to more vigorous conditions. A quantitative assessment of these band patterns was performed with Shannon diversity index. As illustrated in Figure 5.2, the bead beating treatment applied to the MEBPR sludge resulted in an initial increase of the diversity followed by a slight but significant reduction

under more vigorous conditions. On the other hand, a more stable diversity was observed for the CEBPR sludge when subjected to the same treatment conditions. Since Treatment 3 appeared to maximize the diversity of the microbial community extracted from the two types of sludge, the combination of 4.5 m/s and 45 sec was selected as the new standard for all the subsequent samples.

The results from the present bead beating experiment indicated that replacement of the original apparatus with FastPrep was warranted. Not only could the new bead beater apparatus reduce drastically the duration of the bead beating (from 4 to less than 1 min), but it could process up to 12 samples at a time, increasing the overall efficiency significantly. The application of FastPrep for the extraction of DNA from activated sludge samples is spreading, particularly with the availability of the a FastDNA kit (Dionisi *et al.*, 2003).

5.3.2 Comparison among bioreactor zones

The biomass performing EBPR at the UBC pilot plant was continuously cycled through three different compartments: an anaerobic zone (i.e. no oxygen nor nitrate), an anoxic zone (i.e. presence of nitrates) and an aerobic zone (i.e. presence of nitrates and oxygen). For an accurate characterization of the overall system microbial community at a given time, it was essential to determine whether or not each zone harbored a bacterial community with similar compositions. To this end, triplicate samples were collected from each zone of the two pilot scale EBPR systems and their similarities were examined.

A visual illustration of the band patterns generated by RISA in the present experiment is given in Figure 5.3. The three bioreactor zones featured remarkably similar bacterial community compositions in both pilot scale systems, whereas slight differences exist in the clarifier sludge blanket. A quantitative comparison of the different zones within each bioreactor was performed with the MRPP test. In Table 5.2, it can be noted that the overall MRPP (Ranked Sørensen) with

RISA fingerprints data resulted in weak difference among the MEBPR system zones ($p > 0.05$) and in strong differences among the CEBPR system zones ($p < 0.001$). Multiple comparisons between MEBPR zones indicated that no significant differences ($p > 0.05$) were observed, supporting the overall weak differences noted above. Similarly, the multiple comparisons between CEBPR bioreactor zones suggested that differences were not significant ($p > 0.01$ or 0.05). However, when the multiple comparisons included the clarifier, significant dissimilarities were measured ($p < 0.001$), which explains the overall strong CEBPR difference noted earlier. The small value of the T statistic ($T < -2.0$) indicates that fingerprint patterns were placed in close proximity in the ordination species space, whereas T values larger than 3.0 suggests a stronger separation.

The high similarity of the microbial community composition within the three zones of both bioreactor is consistent with the results of several tracer studies performed in the two treatment trains (data not shown). These results demonstrated that the bioreactor behaved as a series of three completely mixed tanks, with an overall strong back mixing due to the presence of two recycle lines. Similarly, Mudaly *et al.* (2001) reported that the bacterial predominance measured with FISH, in the three stages of a Phoredox process for biological phosphorus removal was comparable. On the other hand, Yu and Mohn (2001) showed that the spatial distribution of the microbial community composition varied along an aerated lagoon behaving like a plug flow reactor without biomass recycle. The outcome of this preliminary experiment led to the conclusion that one sample collected from the aerobic zone would be representative of the composition of the overall bacterial communities in the MEBPR and CEBPR systems.

As noted above, the bacterial population located in the CEBPR bioreactor was significantly different from that of the sludge blanket in the clarifier. Two bands in Figure 5.3b were found to be more abundant in the clarifier sludge blanket than in the corresponding bioreactor mixed liquor, suggesting an enrichment of some microbial populations from passage

through the clarifier. Unlike in the three bioreactor zones, the biomass entering the clarifier is subjected to a plug-flow type of movement toward the bottom, where it accumulates as a sludge blanket before leaving with the return activated sludge recycle. As was demonstrated in Chapter 2 (Monti *et al.*, 2005a) and also reported in other studies (Siegrist and Gujer, 1994), the biomass in the sludge blanket is exposed to anoxic conditions and to a gradient of nitrate concentration, leading to endogenous dentrification. It was speculated these specific hydraulic and environmental conditions peculiar to the clarifier favored the growth of specific organisms, which were then enriched compared to the upstream bioreactor.

A visual comparison of the RISA profiles in Figure 5.3 revealed a different composition of the bacterial community in the MEBPR and CEBPR systems. A cluster analysis with samples representing each zone from both processes was performed to quantify this observation. As illustrated in Figure 5.4, the MEBPR and CEBPR microbial communities clustered in two different groups, with approximately 70% similarity. This was a first demonstration that a membrane and conventional process, operated in a EBPR mode, selected for distinct bacterial populations. The Shannon index indicated that the MEBPR bacterial community fingerprint (H' = 2.537) was significantly less diverse than that of the CEBPR system fingerprint (H' = 2.744). Even though the two systems treated the same wastewater and were operated under identical conditions, the presence of a submerged membrane solids-liquid separator was sufficient to select for a microbial population with a distinct and less diverse fingerprint. From the dendogram of Figure 5.4, it can also be noted that the two clarifiers community fingerprints clustered separately from those of the corresponding bioreactor with a 90% similarity, confirming the differences already mentioned earlier.

5.3.3 Comparison of the MEBPR and CEBPR system

A three stage investigation was conducted on the bacterial community composition of the MEBPR and CEBPR systems. Initially, the bacterial population was examined over a period of nine months with the two systems operated under comparable conditions. Subsequently, the two sludge populations were purposely mixed and the development of the communities was monitored in the two systems over a period of two months (Transition I). Lastly, the CEBPR process was retrofitted to become a second MEBPR, while maintaining the operating conditions unchanged (Transition II), and the bacterial population was subsequently studied for almost four months. It was believed that a multi-stage approach over an extensive period of time would allow for a robust characterization of the two bacterial populations and their correlation with the process performance.

5.3.3.1 Comparative period

To determine the relationship between the sludge bacterial communities in the two systems during the comparative study, PCA analysis was performed on bacterial fingerprint data. As illustrated in Figure 5.5, RISA fingerprints of the two sources of sludge formed two distinct groups, with few overlapping points. It can also be noted that the CEBPR data tended to vary more with axis 1, i.e. principal component (PC) 1, whereas the MEBPR data, with the axis 2, PC 2. The overall community differences between the two systems were found to be significant with the MRPP test (T = -2.556, A = 0.018; p = 0.026). At each sampling event, the similarity of the two bacterial communities varied from 63 to 86%, with an average of around 71%. These results clearly indicate that, although the MEBPR and CEBPR processes were operated under comparable conditions, they developed two different bacterial communities in the bioreactor. Other researchers have also reported significant differences in the microbial composition of two such systems with the use of DGGE and FISH techniques (Luxmy *et al.*, 2000; Witzig *et al.*,

2002). However, in these studies, the membrane bioreactors were operated without sludge wasting, resulting in an SRT that was significantly longer than that of the parallel conventional systems. It is reasonable to expect that the SRT difference itself contributed to the establishment of distinct microbial communities, therefore confounding the effect of the membrane. In the present study, it was demonstrated that the presence of a membrane solids-liquid separation device in a bioreactor, per se, was sufficient to select a significantly dissimilar bacterial community.

The PCA analysis also shed light on the community change within each system over time. From Figure 5.5, it can be observed that both communities were characterized by a clear dynamic pattern, with the CEBPR bacterial population exhibiting a more pronounced shift over time. Several studies have reported continuous changing of the microbial population in lab-scale biological systems at steady state, both with the use of molecular techniques (Kaewpipat and Grady, 2002) and with fatty acid profiling (Son and Hall, 2003). The dynamics of the microbial population in this study also derived from the natural changing of the characteristics of the municipal wastewater feed, as discussed below.

In order to objectively spot those bands most responsible for distinguishing the MEBPR community from that of the CEBPR, the species indicator analysis was performed on the fingerprint data collected during the comparative study. A total of five (C1, C2, C3, C4, and C5) and three bands (M1, M2, and M3) were significantly correlated ($p < 0.1$) with the CEBPR and MEBPR sludge, respectively (Fig. 5.6). The higher number of unique bands in the conventional sludge is consistent with the greater diversity of the bacterial population noted earlier. The identification of specific organisms associated with these bands will represent a further step toward a better understanding of the impact of membrane separation on the microbial community.

RISA fingerprints generated from the CEBPR system over the entire duration of the comparative study were found to be more diverse than those fingerprints from the MEBPR process. As shown in Table 5.3, this difference was supported by all three indices. The Shannon diversity index of the CEBPR fingerprints was statistically higher (p = 0.045) than that of the MEBPR. Although each band does not necessarily mean one specific species (Buchholz-Cleven *et al.*, 1997; Nubel *et al.*, 1996), it can be reasonably assume that the fingerprint bands diversity represent the actual community diversity. Microbial diversity is an important characteristics of an ecological system (Martin, 2002). It has been suggested that the diversity within an activated sludge process has an important impact on its stability (Rowan *et al.*, 2003). The higher diversity of the CEBPR system may indicate that this process was more functionally stable than the MEBPR process. Alternatively, it might reflect additional niches created by the presence of clarifiers, which was shown to select some unique populations.

In this regard, the relationship between the microbial community composition and the system performance is of interest. The present molecular study was conducted in parallel with a comprehensive analysis of the system treatment performance, which was presented in detail in Chapter 2 (Monti *et al.*, 2005a). The removal of phosphorus (P) exhibited the most significant variation compared to the removal of carbonaceous or nitrogenous matter, with the P effluent profiles in the two processes being highly correlated (Fig. 5.7a). During the comparative experimental period, the pair-wise similarity between the two bacterial communities at each sampling time (Fig. 5.7b) was observed to fluctuate, with the most pronounced variation occurring in the first 60 days of operation. A similar pattern was noticed also in the changing of the population within each system, as measured by the similarity between a given bacterial community sample and the one at time zero (Fig. 5.7c). Interestingly, the process performance instability observed from day 40 was anticipated and followed by a major shift in the bacterial populations supported in the two systems. As explained in Chapter 2 (Monti *et al.*, 2005a), this

event of P removal decline was caused by a shortage of volatile fatty acids (VFA) in the influent, which are known to be essential for successful operation of the EBPR process (Rabinowitz and Oldham, 1986). It follows that a sudden reduction of a key substrate, such as VFAs in the feed, may have triggered a significant change of the microbial composition responsible for the biological treatment. The order of events suggest that the community change may have been the immediate cause of the lapse in the system performance.

The superior P removal of the CEBPR system during the first event of process failure was partly attributed to the shorter bioreactor SRT, compared to that of the MEBPR process (Monti *et al.*, 2005a, Chapter 2). However, the higher microbial diversity could have also played a positive role in maintaining a more functionally stable process. Saikaly *et al.* (2005) indicated that activated sludge systems with short SRTs will have higher diversity than those operated at long SRT. Nevertheless, it still remains unclear whether the more diverse microbial community supported by a CEBPR system was derived from the somewhat shorter SRT or from the utilization of a gravity solids-liquid separator.

The second instance of P removal deterioration (Fig. 5.7a, day 160), hypothesized to be related to a sudden temperature drop (Monti *et al.*, 2005a, Chapter 2), was not accompanied by any major shift in the community composition, and this observation is true for both systems. Temperature has a strong impact on microbial reaction kinetics, including those of EBPR (Brdjanovic *et al.*, 1997). It is therefore conceivable that short-term temperature shocks do not impact the composition of the microbial community, but may have reduced the activities of phosphorus-accumulating organisms.

5.3.3.2 Transition periods

An additional step toward the objective of evaluating the impact of membrane separation in the activated sludge community was the inclusion of two experimental transition periods.

Transition I involved the monitoring of the bacterial community in the membrane and conventional system after both had been inoculated with the same biomass. Transition II characterized the community of the CEBPR system as it adapted to an MEBPR system.

The RISA fingerprint data from both transition periods were analyzed with PCA to determine their correlation. As expected, at the onset of Transition I, the two communities were positioned in close proximity, indicating a high degree of similarity (Fig. 5.8). With the passage of time, the distance between the two systems progressively increased, with the last point of Transition I (i.e. sample 5) exhibiting the largest departure. This experiment further validated previous conclusions that the presence of a different solids-liquid separation device in a bioreactor results in distinct bacterial communities. From Figure 5.8, it is worth noting that, during the initial stage of Transition I, the CEBPR system exhibited a more chaotic behavior than the MEBPR counterpart. In addition, when the clarifiers employed in the CEBPR system were replaced with a membrane module, the microbial community continued to change although in a smaller space (samples 5 to 9, Fig. 5.8). These observations together with those made during the comparative experimental period indicate that the presence of membrane filtration reduced the dynamics of the bacterial community.

A visual comparison of the RISA fingerprint images from the two transition periods is presented in Figure 5.9. Bands that appeared to be clearly different in the pair-wise comparison, either in abundance or in presence/absence, are marked with a triangle. This simple analysis permitted an assessment of whether the identified bands from the comparative study (see Fig. 5.6) exhibited any significant role during the two transition periods. The following observations further supported the correlation of bands M1 and M4 to the MEBPR system, and C2 and C4 to the CEBPR system (Fig. 5.9). (a) Band M1 was absent throughout Transition I. Eventually, this band appeared in the last sample of the MEBPR system, when the community became significantly different from the parallel CEBPR. (b) Band M2 was present in both systems at the

onset of Transition I. However, this band quickly disappeared from the CEBPR community, whereas it was detected intermittently in the MEBPR system. More interestingly, band M2 returned to the CEBPR system, at varying intensities, once it was transformed in a second MEBPR process (Transition II). (c) Band C2 and C4 were constantly present in the CEBPR community during Transition I. However, band C2 was not detected in the last sample collected in Transition II, whereas band C4 quickly faded off and was absent in sample 7.

The P removal profiles during the two transition periods, together with the change of the microbial community are reported in Figure 5.10. Once the two systems were started up with the same inoculum (Transition I), they exhibited a remarkably similar instability in P-removal and the subsequent recovery of the treatment performance. In this period, the similarity between the two communities dropped from nearly 100% to 70% (Fig. 5.10b), which coincided with the average similarity observed during the long-term comparative period. It is interesting to note that the change of the community composition within each system from the onset of Transition I was rapid, and the transition occurred at comparable rates (Fig. 5.10c). Once the CEBPR had been transformed to a new MEBPR system, the bacterial community continued to change, although at reduced rates. However, the moderate fluctuation of the population was accompanied by stable and complete removal of P from the system.

5.4 Conclusions

A multi-stage experimental design, consisting of one long-term comparative period and two transition periods, was used to investigate the composition of the microbial communities supported by activated sludge systems with membrane and gravity solids-liquid separation.

The MEBPR and CEBPR systems, running at comparable operating conditions, developed microbial communities that were found to be statistically different, with an average calculated similarity of 70%. The CEBPR bacterial population appeared to have higher diversity,

and this may have been the primary reason why the CEBPR treatment train was more functionally stable that the MEBPR counterpart. Moreover, the more diverse bacterial population established in the CEBPR system was observed to be more dynamic than that of the MEBPR process. Several bands were found to be characteristic of either the membrane or conventional biological system, potentially indicating that the different solids-liquid separation device selected for different microorganisms.

For the first time, the present study showed that a simple replacement of a secondary clarifier with membrane solids-liquid separation is sufficient to shift the microbial community significantly. It is expected that the use of membrane filtration, in combination with the extreme operating conditions that are feasible in membrane processes (e.g. long SRTs and short HRTs), will dramatically alter the microbial community normally observed in conventional activated sludge systems.

Table 5.1 **Experimental design followed for the microbial community analysis, and prevailing plant operating conditions.**

Experimental period					
Comparative			Transition		
Day	Sample #	SRT (d); HRT (h)	Day	Sample #	SRT (d); HRT (h)
15	1	12; 10	Transition I		
30	2	12; 10	0	1	12; 7
52	3	12; 10	11	2	12; 7
73	4	12; 10	29	3	12; 7
84	5	12; 10	50	4	12; 7
91	6	12; 10	59	5	12; 7
100	7	12; 10	Transition II		
110	8	12; 10	70	6	12; 7
131	9	12; 10	90	7	12; 7
144	10	12; 7	104	8	12; 7
175	11	12; 7	126	9	12; 7
211	12	12; 7			
251	13	12; 7			
275	14	12; 7			

Table 5.2 **Comparison of the differences in RISA community fingerprintings among zones with nonparametric multi-response permutation procedures (MRPP), based on a ranked Sorensen distance measure (Bray-Curtis method); T = description of the separation between the groups; A = description of the effect size.**

	MEBPR (n = 14)		CEBPR (n = 18)	
	T	A	T	A
Ranked Sørensen	-1.590^{++}	0.092	-4.033^{**}	0.235
Multiple comparisons:				
Anaerobic & Anoxic	-1.250^{++}	0.097	-0.446^{+}	0.025
Anaerobic & Aerobic	-1.513^{++}	0.113	-1.710^{+}	0.116
Anoxic & Aerobic	0.322^{++}	-0.022	-0.871^{++}	0.040
Clarifier & Anaerobic			-3.204^{**}	0.206
Clarifier & Anoxic			-3.066^{**}	0.181
Clarifier & Aerobic			-3.662^{**}	0.384

* $p < 0.01$, ** $p < 0.001$
$^{+}$ $p > 0.01$, $^{++}$ $p > 0.05$

Table 5.3 **Average values of richness, evenness and Shannon diversity indices, together with the Pearson correlation coefficient of the two microbial communities in the MEBPR and CEBPR system during the entire comparative study (n = 14).**

	RICHNESS	EVENESS	SHANNON	PEARSON CORREL.
MEBPR	14	0.966	2.58	71.22
CEBPR	17	0.974	2.72	

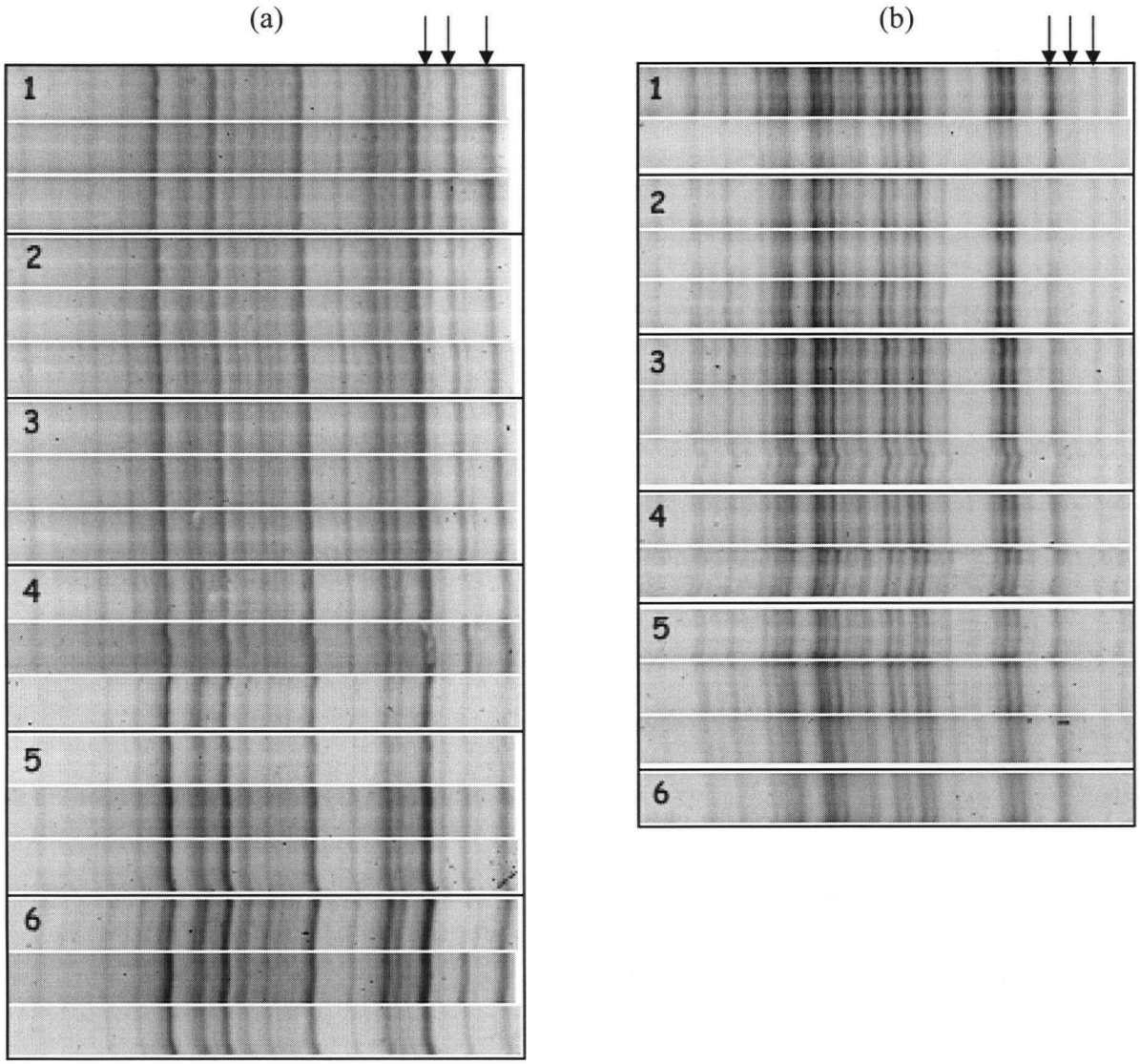


Figure 5.1 **RISA gel image with membrane (a) and conventional (b) sludge for the evaluation of the bead beating step in FastPrep. Replicates samples shown for 1: original method; 2: 4.5 m/s and 30 sec; 3: 4.5 m/s and 45 sec; 4: 5.5 m/s and 30 sec; 5: 5.5 m/s and 45 sec; 6: 6.5 m/s and 30 sec. Arrows indicates those bands whose intensity appeared to vary among treatments.**

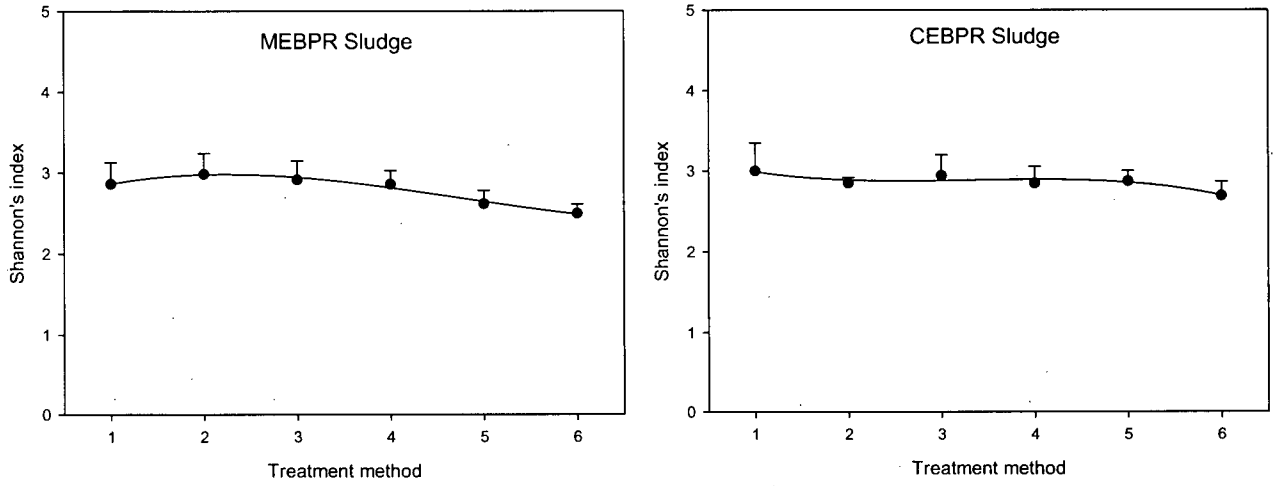


Figure 5.2 **Shannon diversity index with 95 % confidence interval of the bacterial community fingerprints generated by RISA under different bead beating treatment methods.**

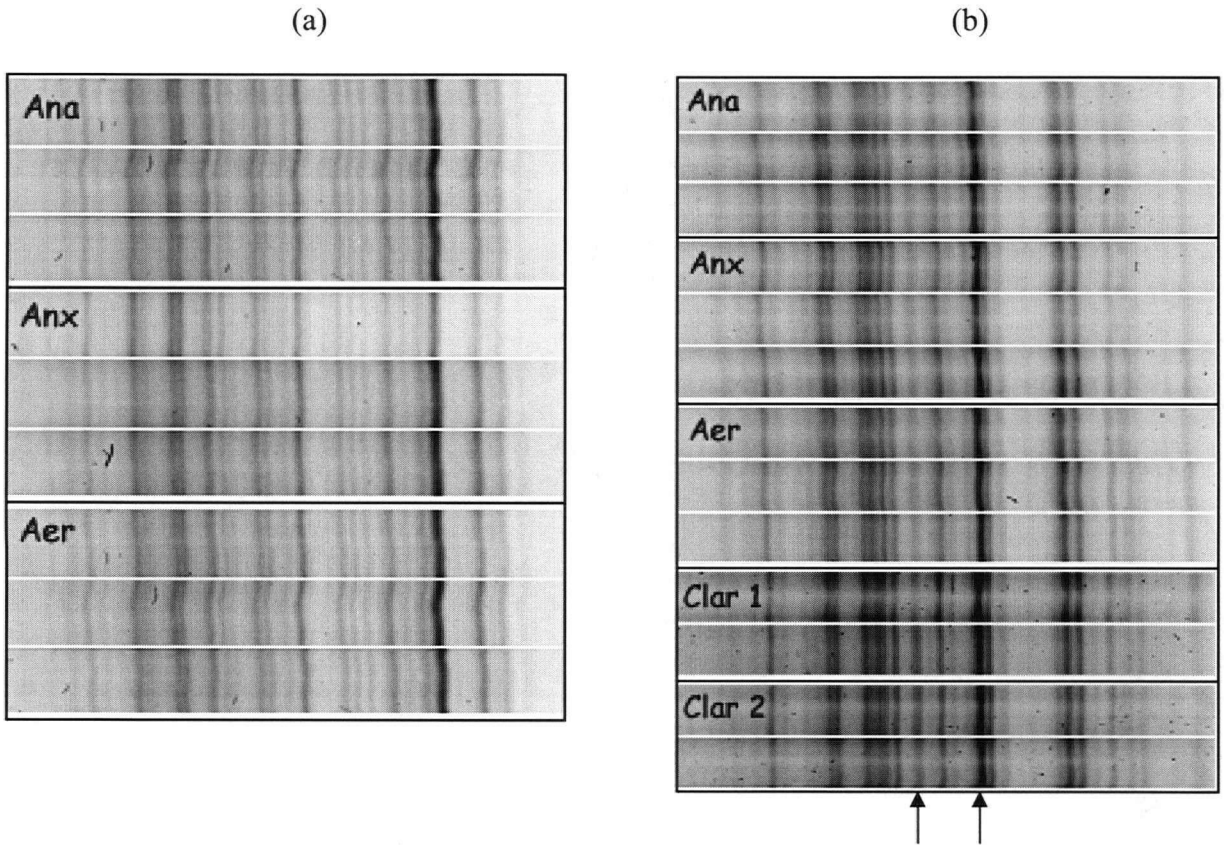


Figure 5.3 **Comparison of the microbial community compositions in each zone in the (a) MEBPR and (b) CEBPR system. Replicate samples shown for Ana: anaerobic; Anx: Anoxic; Aer: Aerobic; Clar 1&2: Clarifier 1 and 2 working in parallel. Arrows indicates bands that appeared enriched in the clarifier relative to the bioreactor.**

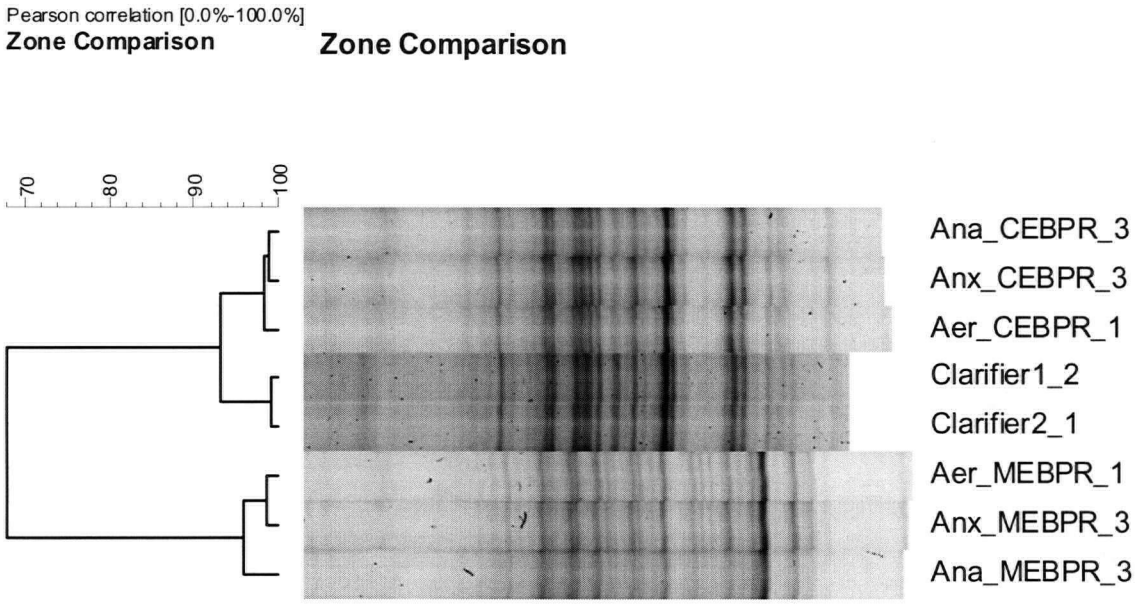


Figure 5.4 **Cluster diagram of samples collected from each bioreactor zone from the two treatment systems.**

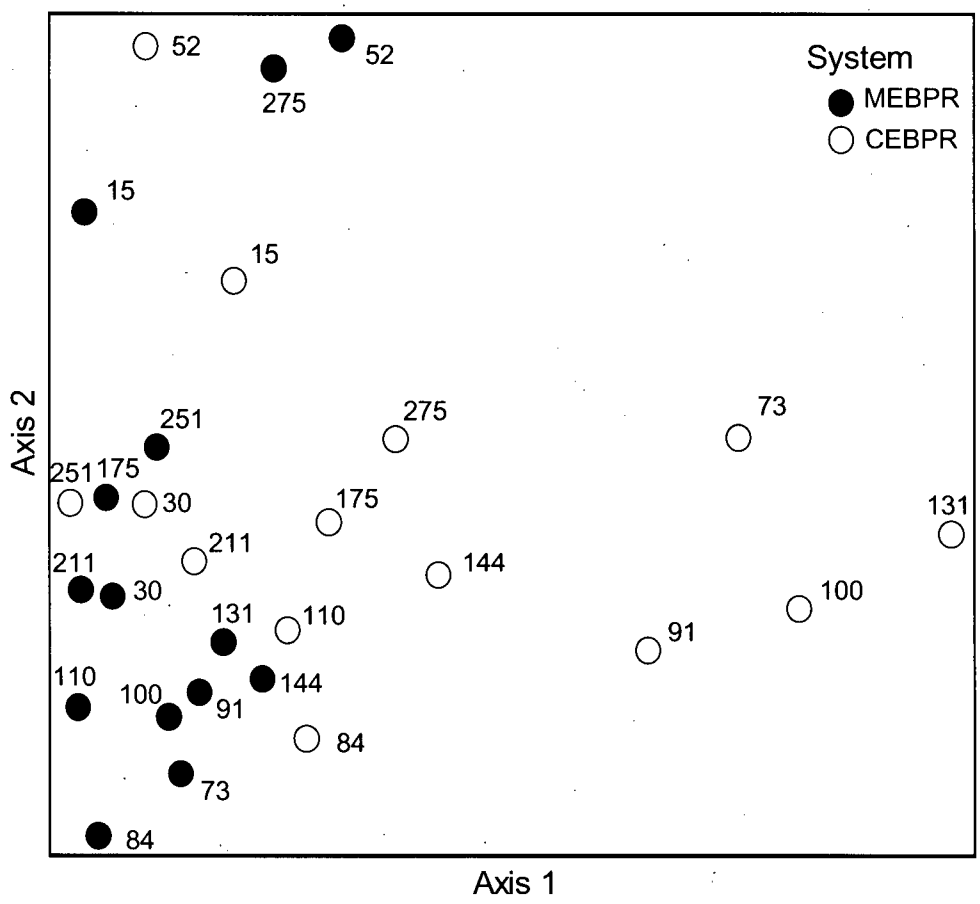


Figure 5.5 **PCA of the RISA fingerprints obtained from MEBPR and CEBPR samples during the comparative study (n = 28). A total of 25% of the total variance in the data set was explained by the first two axes (axis 1 = 13%; axis 2 = 12 %). Numbers indicate the day when each sample was collected, as reported in Table 5.1.**

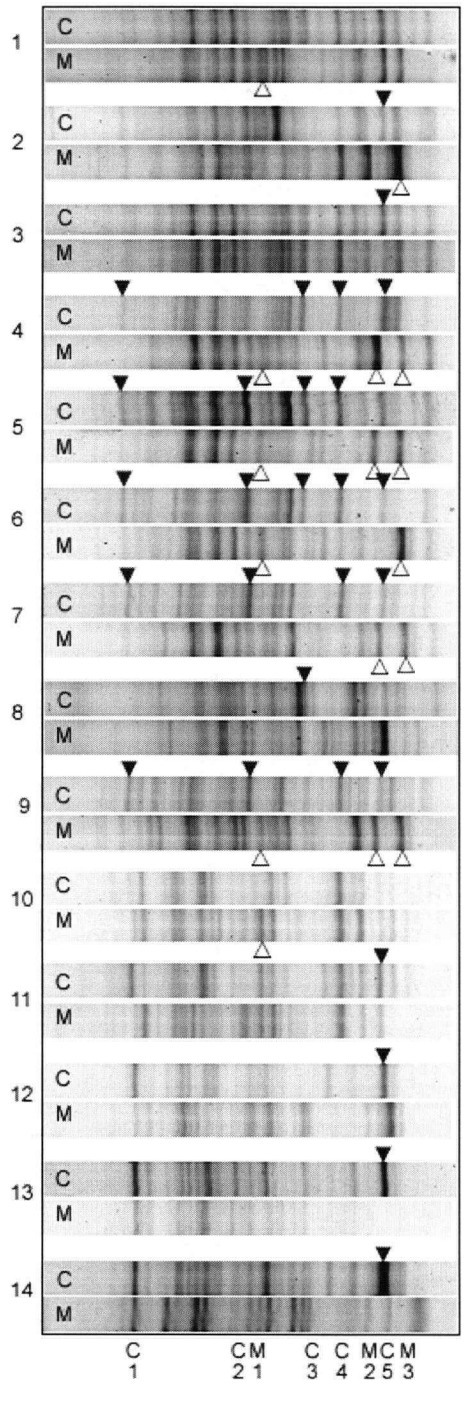


Figure 5.6 **RISA fingerprints of the 14 CEBPR (C) and MEBPR (M) samples collected during the comparative study. Characteristic bands, based on indicator species analysis, for the MEBPR (△) and CEBPR (▼) system are indicated.**

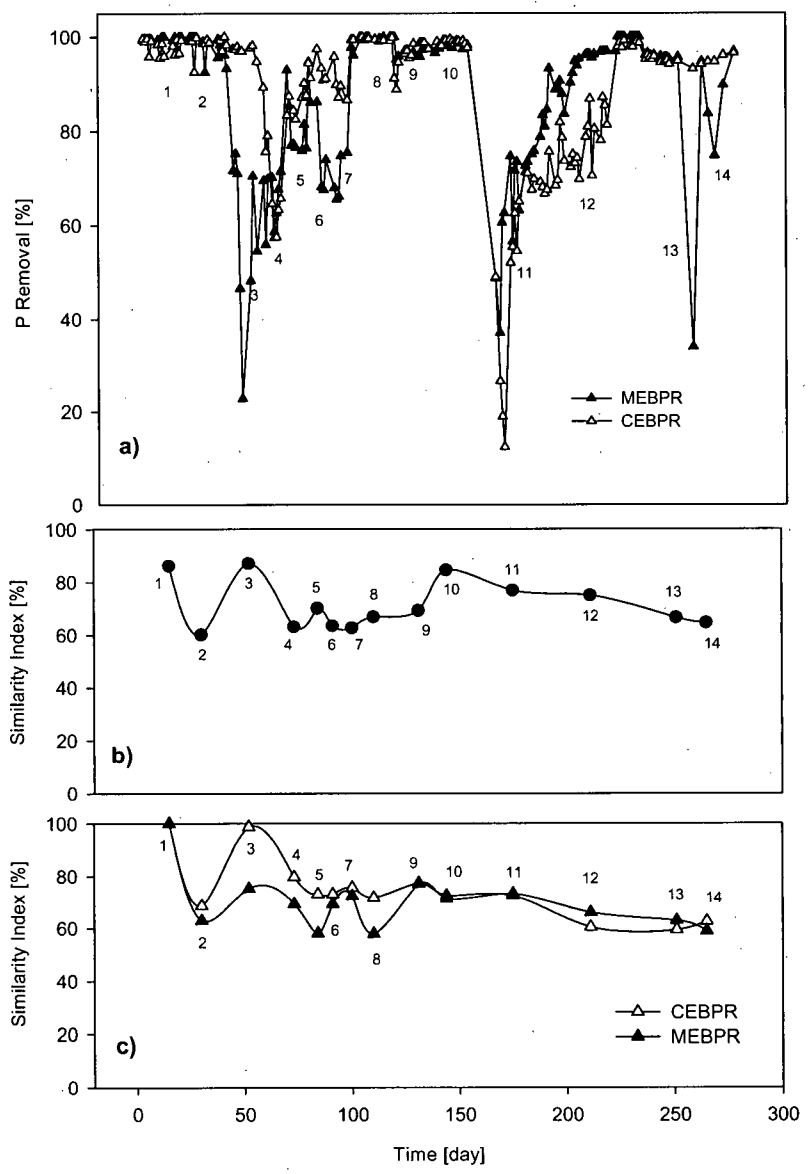


Figure 5.7 **P removal and bacterial community dynamics in treatment systems during comparative phase of study. (a) P removal from the two systems calculated by using the effluent soluble P concentration. (b) Similarity index between the two communities at each given time during the comparative study. (c) Similarity index of the community within the system by comparing each sample with that collected at time zero.**

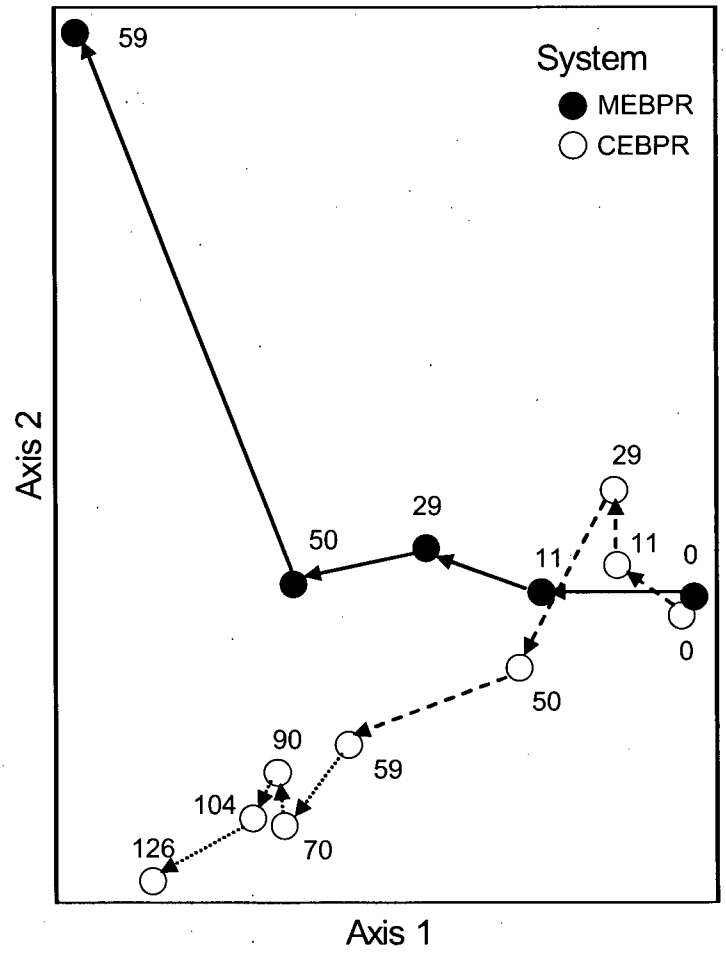
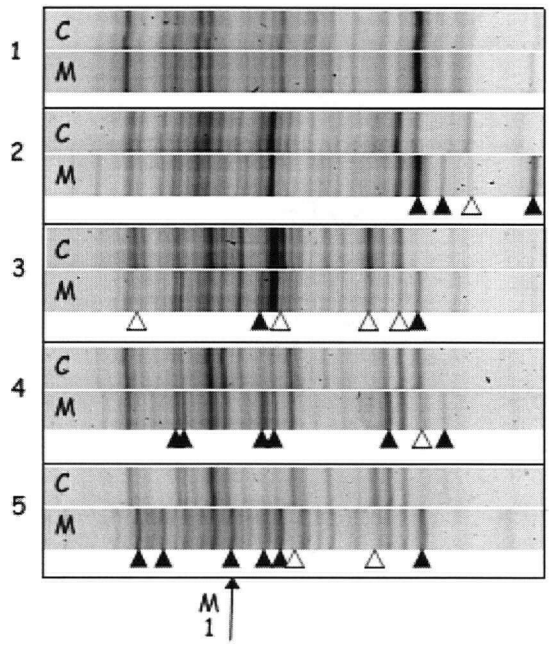


Figure 5.8 **PCA of the RISA fingerprints obtained from MEBPR and CEBPR samples during Transition I (sample 1 to 5) and Transition II (sample 5 to 9). A total of 39 % of the total variance in the data set was explained by the first two axes (axis 1 = 20%; axis 2 = 19 %). Numbers indicate the day when each sample was collected, as reported in Table 5.1.**

(a)



(b)

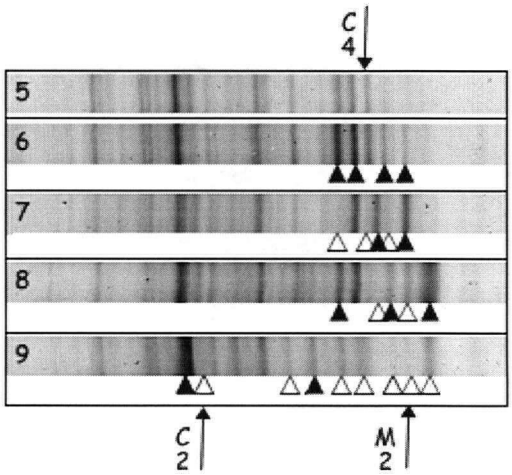


Figure 5.9 **RISA fingerprints of the CEBPR (C) and MEBPR (M) samples collected during (a) Transition I and (b) Transition II. MEBPR bands increasing (▲) and decreasing (△) in the sludge are marked. Band designations correspond to those in Figure 5.6.**

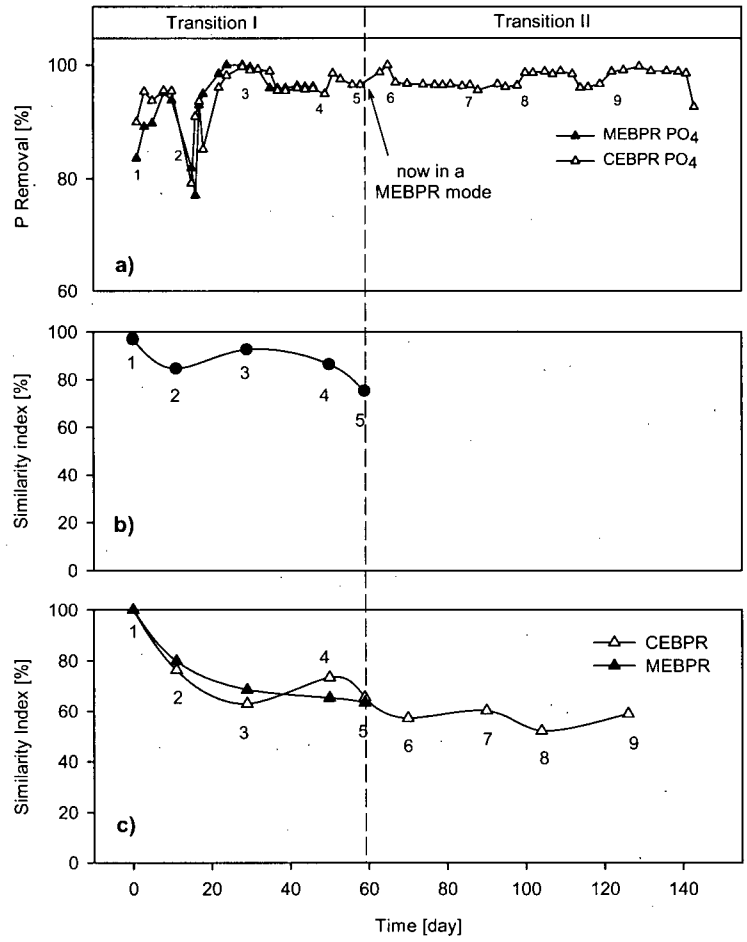


Figure 5.10 **P removal and bacterial community dynamics in treatment systems during the two transition periods. (a) P removal from the two systems calculated by using the effluent soluble P concentration. (b) Similarity index between the two communities at each time during Transition I. (c) Similarity index of the communities within the systems calculated by comparing each sample with that from the same system collected at time zero.**

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Chapter 6 Toward a high rate EBPR process in a membrane-assisted bioreactor*

6.1 Introduction

Biological nutrient removal (BNR) processes offer a cost-effective and environmentally sound wastewater treatment solution to control the enrichment of nutrients in receiving water bodies. For this reason, in the last twenty years, a considerable number of municipalities in western Canada has adopted BNR technology to protect recipient water quality from algal blooms (Oldham and Rabinowitz, 2001). Recently, the increasing pressure to expand existing BNR wastewater treatment plants (WWTPs) has prompted municipalities to consider membrane bioreactor (MBR) technology as an innovative solution to the engineering problem. MBR processes replace the secondary clarifier with membrane filtration to separate suspended solids and microorganisms responsible for biodegradation, from the treated water. In the context of BNR systems, membrane-based separation offers three distinct advantages over conventional gravity sedimentation: (1) the possibility to maintain high concentrations of mixed liquor suspended solids (MLSS) in the bioreactor (Buisson *et al.*, 1998), resulting in a small treatment plant footprint, (2) the complete uncoupling of solids retention time (SRT) and hydraulic retention time (HRT), allowing for unusually high rate operating conditions, and (3) the production of a suspended solids-free effluent (Krauth and Staab, 1993), thereby eliminating the tertiary treatment (e.g. sand filtration and disinfection) normally employed to polish the secondary effluent.

High concentrations of MLSS in the bioreactor can be achieved by extending the SRT or by lowering the HRT, i.e. by manipulating the solids-concentration ratio (Rittmann and McCarty,

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2001). The majority of reported MBR studies attempting carbon and/or nitrogen removal have explored the option of long/infinite SRT as a way to increase the biomass in the bioreactor and, at the same time, to drastically reduce the observed sludge yield (Muller *et al.*, 1995; Rosenberger *et al.*, 2002; Pollice *et al.*, 2004). However, full-scale experience with conventional BNR processes suggests that short SRTs (7-15 days) are generally required to achieve efficient biological phosphorus removal from wastewater (Oldham and Rabinowitz, 2001; Mulkerrins *et al.*, 2004). Factors that determine the selection of short SRTs include: (1) the significant portion of phosphorus (P) used as a growth nutrient by the fast growing mixed microbial population, therefore reducing the amount of P to be removed by the enhanced biological phosphorus removal (EBPR) process (Smolders *et al.*, 1996), and (2) the more efficient use of volatile fatty acids (VFA) per unit of P removed from the phosphorus-accumulating organisms (PAOs) (Rodrigo *et al.*, 1996). Consequently, when exploring the combination of BNR processes with membrane technology, it appears more appropriate to choose the HRT, rather than the SRT, as a variable to exploit the benefits of high suspended solids in an MBR system.

The first attempt to couple EBPR with membrane solid-liquid separation was reported by a group of researchers in Berlin (Adam *et al.* 2002; Lesjean *et al.*, 2003). Following the general approach of long SRTs for MBR systems, they challenged the EBPR mechanism at sludge ages between 15 and 25 days while keeping the HRT between 14 and 18 hours. The two-year long study showed that satisfactory removal of P in MBR systems is possible under such conditions, although a significant portion of P (up to 45%) was sequestered through natural chemical precipitation. Further, in the Berlin study, a high VFA to PO_4-P (> 20) ratio was present in the incoming influent. More recently, Fleisher *et al.* (2005) reported a pilot-scale study in Virginia on the coupling of MBR with the EBPR process at an SRT of 23 days and a HRT of 9 hours. In this case, only the combination of biological and chemical precipitation, through alum addition, could guarantee a consistent effluent total P as low as 0.1 mg P/L. Even though the above studies

have constituted important preliminary investigations on the feasibility of BNR in MBR systems, data are still limited on the influence of operating parameters such as SRT and HRT on the EBPR mechanism, when membrane filtration solid-liquid separation is employed. In addition, the response of the EBPR process when subjected to high rate hydraulic conditions typical of low HRT systems remains still unknown.

The objectives of this study were (1) to demonstrate the feasibility of a high rate EBPR process in a membrane-assisted bioreactor and (2) to assess the impact of HRT and SRT on the system performance and EBPR mechanism. To this end, a membrane EBPR (MEBPR) process was studied under four distinct operating conditions, starting from current design conditions and moving toward unexplored design frontiers (Fig. 6.1).

6.2 Materials and methods

The present research study was conducted at the University of British Columbia (UBC) wastewater treatment pilot plant facility between May 2003 and December 2004. Municipal primary effluent was used to feed the MEBPR process to best relate the outcome of the study with real-scale applications.

6.2.1 Description of the test site

The UBC pilot plant is a dual train facility in which two 2500 liter activated sludge trains (Side A and Side B) can be operated in parallel for comparison of different experimental treatments or process designs. The feed to both trains was primary effluent produced on site by clarification of municipal wastewater pumped continuously from two 15,000 liter external storage tanks. The wastewater was collected two to four times a day from the trunk sewer that runs nearby and was temporarily stored in the external storage tanks. The primary effluent, always in excess of the required process flow rate, was collected in a 20 liter holding tank that provided a constant head to the process feed pumps in order to assure minimum variation of

flow. The characteristics of the wastewater used for this research study are reported in Table 6.1. The relatively low pH and the limited amount of calcium, iron and aluminum in the incoming wastewater (data not shown) assured that the P removal mechanism would be predominantly due to biological activity, with a negligible contribution from chemical precipitation.

6.2.2 MEBPR process configuration

A University of Cape Town (UCT) type configuration (Tchobanoglous *et al.*, 2003) was adopted for the MEBPR study. This configuration included a series of three separated compartments: 10% of the total bioreactor volume was operated under anaerobic conditions, followed by a 30% volume under anoxic conditions and the remaining 60% under aerobic conditions. The membrane filtration module was directly immersed in the aerobic zone where suspended solids-free effluent was continuously generated. Figure 6.2 illustrates the final configuration of the MEBPR process selected for the present pilot-scale study. All four experimental runs (Fig. 6.1) were completed in the MEBPR process side-A, whereas side-B was initially used for a comparative study against a conventional EBPR system (Monti *et al.*, 2005a, Chapter 2) and later upgraded to a second MEBPR system, to replicate two of the four operating conditions (Run II and III).

One ZeeWeed® hollow fiber membrane module, provided by Zenon Environmental Inc. (Oakville, Ontario, Canada), was used in this investigation. The membrane fiber had a nominal pore size of 0.04 μm and the module featured 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 of 0.5 min, using the clean effluent stored in a permeate tank. It follows that the membrane module was extracting slightly a higher flow rate of permeate than that of the influent to compensate for the backflush operation. Excess permeate was recycled back to the aerobic compartment under the control of a float valve that maintained a constant water level at all times. Intermittent coarse-

bubble aeration (10 sec ON and 10 sec OFF) was provided to the module, at an air flow of 0.34 m^3/min (12 scfm), to reduce the cake of solids on the membrane surfaces and to prevent the packing of solids between the membrane fibers. A back-up, fine-bubble aerator system was necessary to meet the biochemical oxygen demand, particularly when the system was subjected to high volumetric loads. When the transmembrane pressure exceeded the recommended value of 65 kPa (0.65 bar), the service module was replaced with another module that had been restored, by soaking in solutions of concentrated citric acid and sodium hypochlorite.

6.2.3 Selected operating conditions

The MEBPR process was started at an HRT of 10 hours and an SRT fixed at 12 days. This first experimental set point represented the current design practice for conventional BNR processes in Western Canada during winter conditions (Fig. 6.1, Table 6.2). Subsequently, the first move toward high rate hydraulic conditions was made with the reduction of the HRT to 7 hours at the same SRT. The following third operating set point featured the extension of the SRT to 20 days with unchanged flow rate to explore the response of the MEBPR process in the high end region of recommended sludge ages. Finally, the treatment system was subjected to the highest flow regime, corresponding to a HRT of 5 hours, while keeping the previous SRT of 20 days. The structure of the research program aimed to initially evaluate the MEBPR process under the same conditions used in a parallel conventional system with gravity settling and, subsequently, to launch the EBPR toward previously unexplored operating conditions achievable in a membrane-assisted treatment systems. The anoxic recycle ratio was fixed at 1 throughout the entire research program. On the other hand, the aerobic recycle ratio was allowed to vary between 1 and 2, depending on the denitrification potential of the anoxic compartment.

6.2.4 Monitoring and analytical procedures

The following operating data were monitored daily: influent and effluent temperature, pH in the influent and in each reactor zone, wasting volumes, and dissolved oxygen (DO) levels in the aerobic zones. Temperature and pH were measured using a portable VWR probe, whereas dissolved oxygen concentration was monitored by a dedicated *in situ* YSI probe. Approximately 1.5 kg of sodium bicarbonate was added once a day to each external storage tank to prevent the aerobic pH from dropping as a result of the nitrification activity and related alkalinity consumption. In addition, all flow rates on each process were checked at least every 10 days.

A systematic sampling and analytical program was developed for long-term monitoring of process performance. Grab samples of the influent and effluent were collected five days per week and analyzed for total and volatile suspended solids (TSS and VSS), total chemical oxygen demand (COD), soluble COD, volatile fatty acids (VFA) comprising acetate and propionate (only for influent), total Kjeldahl nitrogen (TKN), ammonium nitrogen (NH_4-N), nitrite plus nitrate nitrogen (NO_X-N), total phosphorus (TP), and orthophosphate phosphorus (PO_4-P). Mixed liquor samples were collected three times a week from each compartment for the entire investigation period and analyzed for TSS, VFAs (only anaerobic zone), NH_4-N, NO_X-N, and PO_4-P. During selected periods, the measurement of sludge total COD, TKN and TP was carried out for mass balance calculations. All analyses were performed according to Standard Methods (APHA *et al.*, 1998).

With the exception of the MEBPR effluent, all other samples required filtration for the measurement of soluble parameters. The filtration method employed a two liter reactor in which a ZeeWeed® membrane module (ZW-1, nominal pore size of 0.04 μm) was immersed in the sample with a vacuum applied by means of a peristaltic pump. Air or nitrogen gas was bubbled through the sample to keep the suspension mixed and to control the formation of cake solids on the membranes. Before collecting a representative sample, a filtrate volume of 500 mL was

discarded to flush away the old sample entrapped in the membrane fibers, module header, and pump tube. This filtration method required between 5 to 10 minutes per sample and was found to be economically advantageous over the use of filter papers with pore size of 0.45 μm or less.

6.3 Results and discussion

The present pilot-scale study was conducted at four distinct operating conditions. The overall experimental program for both MEBPR Side A and Side B is illustrated in Table 6.2.

At the onset of the study, the MEBPR process Side A relied on the VFA naturally present in the incoming wastewater and was operated with an aerobic recycle ratio fixed at 2 to take full advantage of the denitrification potential (Period Ia). At the second instance of phosphorus breakthrough, it was decided to insure complete removal of nitrates in the anoxic zone by reducing the aerobic recycle ratio from 2 to 1 (Period Ib). As a result of a period during which the system failed to remove phosphorus satisfactorily, the ratio of VFA to total phosphorus in the incoming wastewater was raised from 5 to 10 mg COD/mg P, by adding external sodium acetate corresponding to a concentration of 20 mg COD/L (Period Ic). During this period, the aerobic recycle ratio was re-set back to 2. After 186 days, the research program entered the 7-hour HRT phase with all other operating parameters kept constant (Period IIa). Following a third instance of EBPR failure, it was decided to further raise the external acetate addition to 30 mg COD/L and to bring the aerobic recycle ratio back to 1, to protect the EBPR mechanism from the deleterious effect of anoxic zone nitrates (Period IIb). On day 354, the suspended solids in the bioreactor were allowed to increase to reach a new SRT set point of 20 days without changing any other operational parameter (Period III). In the last 45 days, the HRT was reduced to 5 hours, while maintaining everything else constant (Period IV). The MEBPR process Side B was started up at 7 hours HRT and 12 days SRT (Period II), the same conditions applied on Side A during

Period IIb. Following this, Side B replicated the SRT change from 12 to 20 days (Period III) that had been implemented on Side A during Period III.

The average results are presented for each main period (I to IV), with Period II and III representing the combined performance of Side A and Side B.

6.3.1 Suspended solids profile and sludge inventory

The average suspended solids concentrations established in the three reactor zones, when both internal recycles were set at 1, are illustrated in Figure 6.3a. In any experimental period, the highest level of TSS was measured in the aerobic zone and the lowest in the anaerobic zone, as predicted by solids mass balance calculations. This was because the process effluent was extracted from the membrane in the aerobic zone, which concentrated the sludge in this zone relative to the upstream zones. As the solids-concentration ratio (i.e. SRT/HRT) increased from Period I to IV, the TSS in each zone accumulated accordingly in each compartment, with the concentration in the aerobic zone increasing from 3 to 9 g/L.

A complete sludge inventory was estimated during each experimental period from the average suspended solids concentration in each reactor zone (Fig. 6.3b). The graph clearly illustrates that, of the total mass in the bioreactor, about 70% was present in the aerobic zone, 18% in the anoxic zone, 4% in the anaerobic zone, and the remaining 8% as foam in the anoxic zone. The sludge mass fractions for the present study and the corresponding zone SRTs were skewed toward high aerobic values, which allows stable nitrification even at low temperature. As reported by Grady *et al.* (1999), an anaerobic SRT of about 0.5 days at 20 °C represents the minimum value for EBPR processes that receive an influent wastewater with sufficient concentrations of VFAs and readily biodegradable organic matter. In addition, a total anaerobic plus anoxic SRT of 2 to 3 days should favor the hydrolysis of slowly biodegradable substrate, which is beneficial to the denitrification process. If though, the total anaerobic plus anoxic SRT

for the present study was within the recommended range, the anaerobic SRT was close to the minimum value of 0.5 days, which can be problematic when the VFA/P ratio in the influent is low. The foam sitting on the surface of the anoxic zone appeared as a concentrated floating sludge, with TSS concentrations reaching values as high as 60 g/L. This dense mass accounted for about 8% of the overall biomass present in the MEBPR system, and it was estimated by measuring the TSS in the three compartments before and after re-suspending the foam in the anoxic zone (Monti *et al.*, 2005b, Chapter 7).

6.3.2 COD removal

6.3.2.1 Mass balances

Mass balances of the material flowing in and out of an activated sludge process are essential to check the reliability of the data at hand. Wentzel *et al.* (1989) and, later, Barker and Dold (1995) indicated that mass balance recoveries above 85-90 % are necessary to reach conclusive data elaborations. In addition, mass balances represent a useful tool to highlight the impact of different operating conditions on the process performance.

In a COD mass balance around an activated sludge system, the COD entering via the influent should be equal to the summation of the COD leaving with the effluent, the COD associated with the wasted sludge, the COD oxidized through denitrification, and the COD oxidized with oxygen consumption in the aerobic zone. The latter was estimated from the measured total oxygen utilization rate (OUR), after deducting the oxygen required for nitrification. The total OUR was calculated from measurement of the dissolved oxygen decline over time after stopping the air supply in the aerobic zone. The average results of the COD mass balances, as a percentage of the total incoming COD, over the four investigated periods are presented in Figure 6.4. The calculated COD recovery ranged between 85 and 90%, with the lower values being associated with the 10 h HRT period. Similar recoveries have been

documented in the literature for BNR processes (Barker and Dold, 1995, Hu *et al.*, 2003) with the "missing" COD being attributed to fermentation processes occurring in the anaerobic zone. The portion of COD consumed for denitrification represented a small 12%, since nitrogen removal was not optimized in this study to safeguard the bio-P process. The change in the SRT from 12 to 20 days (from Period II to III) caused the COD associated with the sludge wasting to decrease at the expense of the oxygen utilization, which exhibited a proportional increase. The COD leaving with the effluent was found to be slightly lower at longer SRT (Period III and IV), indicating that a lower food-to-microorganism ratio contributed to improved removal of the soluble COD.

6.3.2.2 Performance

Figure 6.5 presents the influent and effluent COD data for the MEBPR process over the entire research program. The significant decrease in the influent COD on day 100 resulted from a change in the filling frequency of the external storage tanks. During Period I, fresh sewage was pumped into the tanks twice a day, at 7 am and at 7 pm. In anticipation of the planned lower HRT operation, it was necessary to increase the filling frequency to four times a day to provide sufficient feed to the pilot plant. The new late night and early afternoon filling cycles served to dilute the wastewater and this negatively affected the COD/TKN and COD/TP ratios. From the COD profiles shown in Figure 6.5, it was estimated that the COD removal efficiency remained above 90% at all times, with the exception of the second half of Period II when the effluent COD was unusually high. It can also be noted that the increase in the hydraulic load (Period I to II, and III to IV) did not affect the ability of the process to satisfactorily remove the soluble carbonaceous matter. On the other hand, two weeks following the SRT step increase (Period II to III), the MEBPR effluent COD stabilized at low levels and the process was able to consistently maintain greater than 95% COD removal for the rest of the study.

A summary of the influent and effluent COD data distribution for each experimental run is illustrated in Figure 6.6. The top box plot shows that the soluble COD portion made up about 30% of the total influent COD, with the exception of Period IV where this portion was reduced to 25%. The effluent COD data (bottom graph) were distributed over a wide range in Periods I and II, resulting in COD removal efficiencies of 93 and 89%, respectively. When the system SRT was increased from 12 to 20 days, the effluent COD data indicated a significantly smaller variation and an average effluent COD that was lower than previously observed. A *t*-test (Gilbert *et al.*, 1987) confirmed that the difference between the effluent COD of Period II and III was statistically significant at the 95% confidence level. On the other hand, the two paired data sets within the same SRT were found to be comparable, confirming that the change of HRT (i.e. from 10 to 7 h, and from 7 to 5 h) did not affect the COD removal efficiency. No influence of HRT on the COD removal was reported by Rosenberger *et al.* (2002) for a membrane bioreactor operated with nitrogen removal. Similarly to the present study, Rosenberger *et al.* observed higher COD removal efficiency with increasing sludge concentration (i.e. system SRT). This phenomenon could be attributed to the establishment of a greater bacterial diversity, also favored by the ability of the membrane to retain all microorganisms, including non-floc forming and free-swimming bacteria. Removal efficiencies in the range of 90-95% have been documented by several other authors (Stephenson *et al.*, 2000), confirming the potential of membrane–assisted processes, operated at long SRT, to consistently achieve good COD removal. However, in the present study, high COD removal efficiencies were demonstrated in an MEBPR process with an HRT as low as 5 hours, corresponding to a volumetric carbon loading and F/M ratio of 1.5 kg COD/(m^3·d) and 0.24 kg COD/(kg VSS·d), respectively.

6.3.2.3 Sludge yield

From the extensive data set available for each experimental run, it was possible to calculate the observed sludge yield using a cumulative approach. The total mass of suspended solids generated, and corrected for the accumulation in the bioreactor, was divided by the total mass of COD removed over the duration of each experimental period. The results of the calculated sludge yield in this study are illustrated in Figure 6.7. It can be seen that the MEBPR process exhibited values around 0.26 and 0.20 g VSS/g $COD_{removed}$ at the SRT of 12 and 20 days, respectively. Not surprisingly, the impact of the HRT change within each SRT set point was negligible. The advantage of membrane processes to reduce excess sludge production is well documented in the literature, with some studies even reporting zero sludge yield (Muller *et al.*, 1995; Rosenberger *et al.*, 2002; Pollice *et al.*, 2004). However, in the context of biological phosphorus removal, long SRT operation and the consequent reduced sludge yield negatively impacted the performance of the process with carbon-limited wastewater as noted below.

6.3.3 Nitrogen removal

6.3.3.1 Mass balances

In a N mass balance, the total N entering an activated sludge system via the influent as TKN should be equal to the total N leaving with the effluent as TKN and NO_X-N, the NO_X-N denitrified to N_2 gas, and the N in the waste sludge stream. The average results, as a percentage of the influent total N, over the four experimental periods are presented in Figure 6.8. A very high recovery was obtained for the N balances around the system, with values ranging from 95 to 98%.

Period I exhibited the highest percentage of N denitrified due to extended operation with the aerobic recycle ratio set at 2. Consequently, the smallest amount of N was contained in the final effluent compared to the other experimental runs. Similar to the COD mass balances, the

first two periods during which the system was operated at a 12 day SRT were characterized by relatively large amounts of N associated with the sludge wasting, and this is directly correlated with the higher observed sludge yields. When the SRT was increased from 12 to 20 days, a proportional reduction of "waste N" was measured in the mass balance, with the N remaining partitioned between the effluent and the gas production through denitrification.

6.3.3.2 Performance

Influent TKN together with effluent nitrate and ammonia concentrations are presented in Figure 6.9. It can be noted that the MEBPR process achieved complete nitrification with measured effluent NH_4-N concentrations that were below the detection limit. The large fraction of aerobic sludge favored the establishment of a robust nitrifying community at all times, even during periods of colder temperatures (13-14 °C). Stable nitrification in MBR systems has also been reported in other studies over a wide range of operating conditions (Cicek *et al.*, 2001; Fan *et al.*, 1996; Urbain *et al.*, 1998; Liebig *et al.*, 2001; Fleisher *et al.*, 2005). The impact of SRT on nitrification in membrane-assisted processes has been thoroughly evaluated in the technical literature, and the clear advantage of these systems to limit the "wash-out" of nitrifiers and avoid incomplete nitrification has been noted. On the other hand, little evidence is available on nitrification in membrane activated sludge systems operated at short HRTs. In the present study, it was demonstrated that the MEBPR process could deliver complete nitrification at HRTs as low as 5 hours, corresponding to a volumetric nitrogen loading of 0.17 kg N/(m^3·d). In addition, by systematically monitoring the effluent quality during the transition period from one HRT set-point to the next, it was possible to show that a step increase in the influent flow rate did not affect nitrification efficiencies, which continued to be complete. Côté *et al.* (1998) and Trussel *et al.* (2005) were among the first to demonstrate complete nitrification in high rate carbon and/or nitrogen removal processes, with HRTs as low as 4.5 and 1.5 hours, respectively. More recently,

Gao *et al.* (2004) reported the successful operation of a submerged membrane bioreactor treating ammonia-rich inorganic wastewater, with volumetric nitrogen loadings between 0.18 and 1.30 kg NH_4^+-N/(m^3·d).

A summary of the influent TKN data together with effluent nitrate and total nitrogen (TN) concentrations during each experimental run is illustrated in Figure 6.10. It is worth noting that, because of the effluent polishing of the membrane unit, the MEBPR system generated a TN concentration which was only slightly above the nitrate concentration. The TKN concentration in the membrane permeate averaged around 1 mg N/L and it likely included refractory soluble nitrogenous matter. As reported earlier, denitrification was not optimized in this study to protect the anaerobic zone from the confounding effect of nitrates, resulting in N removal efficiencies of between 52 and 70% from the incoming wastewater.

6.3.4 Phosphorus removal

6.3.4.1 Performance evaluation

In contrast to the consistent removal of COD and N that was observed, the removal of P was characterized by periods of good performance, followed by breakthroughs. Figure 6.11a presents the time series of influent TP and the effluent PO_4-P concentration of the MEBPR process over the four experimental periods. The nature of the first four P-removal "failure" incidents (labeled as 1, 2, 3, and 4 in the graph) was already discussed in Chapter 2 (Monti *et al.*, 2005a), in which the MEBPR was compared to a parallel conventional EBPR system. In the following discussion, emphasis will be placed on the effect of HRT and SRT on the MEBPR process performance.

Impact of system HRT. The MEBPR process was assessed at HRTs of 10, 7 and 5 hours to determine the potential of this novel biotechnology to function at increasingly higher loading rates. From Figure 6.11a, it can be observed that the transition from 10 to 7 hour HRT (i.e. from

Period I to II) was smooth, and the system was able to entirely absorb the extra hydraulic load. When the recovery from "failure 3" was complete (caused by a sudden temperature decrease), the MEBPR process exhibited satisfactory performance at an HRT of 7 hours, with the exception of a short unstable period recorded between 300 and 320 days. To further assess the bio-P process under these operating conditions, it was decided to replicate Period II (i.e. HRT of 7 h and SRT of 12 d) on Side B with the performance results shown in Figure 6.11b. Here, it was demonstrated that an MEBPR process can reliably generate low PO_4-P concentrations in the permeate, when a VFA to TP ratio of 12 is maintained in the influent. The HRT change from 7 to 5 hours (i.e. from Period III to IV, Fig. 6.12a) was initially accompanied by continuing satisfactory bio-P performance. However, one week later, this was followed by an effluent phosphorus concentration spike ("failure 6") that was related to a mechanical problem in the acetate dosing pump. Once the addition of sodium acetate was resumed, the MEBPR process quickly recovered and continued to deliver excellent effluent quality with PO_4-P concentrations as low as 0.1 mg P/L.

Impact of system SRT. The MEBPR process was evaluated at two distinct SRTs of 12 and 20 days, with the objective of assessing the impact of high suspended solids concentrations on the bio-P removal mechanism. When the MEBPR system on side A had completed a pseudo-steady state Period II, the sludge wasting line was closed for 8 days to accumulate solids in the bioreactor and to quickly approach the new equilibrium at a 20 day SRT. From Figure 6.11a, it can be noted that this transition (Period II to III) was followed by a week of continuing good performance and, thereafter, by a significant peak in effluent phosphorus concentrations (i.e. "failure 5"). Without any further system modification, it took about 30 days for the EBPR mechanism to return to satisfactory P removal. The same experimental conditions were replicated on Side B once the MEBPR process had attained stable performance at 12 days SRT. On day 114 (see Fig. 6.11b), sludge wasting was discontinued for 4 days and a peak in effluent

phosphorus appeared following the transition, as before. This second episode of process upset passed much faster than the corresponding "failure 5" previously observed on MEBPR Side A, likely due to the shorter duration of the period without sludge wasting. The reproducibility of this type of process failure suggested that the EBPR mechanism was negatively affected by prolonged periods of operation without sludge wasting. In the anoxic and aerobic zone, PAOs grow and sequester phosphate from the wastewater to store it in the cells in the form of poly-P (Comeau *et al.*, 1986). Owing to a net growth of the cells, the accumulated phosphate can be removed from the system as excess sludge. By discontinuing the sludge wasting, the P-removing mechanism was interrupted, which resulted in the accumulation of P in the bioreactor and eventually, in the final effluent. Therefore, when moving the EBPR process from low to high SRTs, it is recommended to avoid any rapid sludge accumulation in the system (i.e. discontinuing the sludge wasting) but, rather, to gradually achieve the new equilibrium by continuously wasting the corresponding daily required volume.

Even though the MEBPR process was characterized by periods of P breakthrough, it also exhibited long stretches optimal P removal over the four experimental runs. To illustrate the potential technological benefits of the MEBPR process, a summary of the performance during these satisfactory P removal periods is given in Figure 6.12. When sufficient carbon was present in the wastewater (a VFA to TP ratio of 10 to 12, in this study), the MEBPR process was able to sustain excellent soluble P removal over a wide range of operating conditions, including high rate operation at a 5 hour HRT. In addition, the TP of the MEBPR effluent was only 0.05 to 0.1 above the PO_4-P concentration. These results are particularly significant, as they confirm the potential for successful operation of MEBPR systems at operating conditions that are far beyond those achievable with conventional EBPR technology. Fleisher *et al.* (2005) were among the first to investigate the potential of a membrane-assisted EBPR process at relatively high flow rates, at an overall HRT of 8.8 hours. However, only by incorporating chemical precipitation through

alum addition was it possible to achieve a consistent effluent total P as low as 0.1 mg P/L. Successful operation of MEBPR processes were also reported by Adam *et al.* (2002) and Patel *et al.* (2005) at HRTs of 14 and 12 hours, respectively. The above three studies attempted to operate the EBPR process at relatively long SRTs of about 20 days. As confirmed in the present research, satisfactory P removal can be attained at these conditions, although additional details on the P release and P uptake are needed to understand the impact of SRT on the bio-P mechanism.

6.3.4.2 P release and P uptake

To evaluate the EBPR behavior during periods of satisfactory performance, mass balance calculations were performed to determine the amount of soluble P (measured as PO_4-P) released in the anaerobic zone and the amounts taken up in the subsequent anoxic and aerobic zones. The calculated P-release and P-uptake masses per day were divided by the influent flow rate, to express the release and uptake in milligrams of P per liter of influent flow. The average results for each experimental condition are illustrated in Figure 6.13. The soluble P removed from the influent can be obtained by subtracting the P released in the anaerobic zone from the total P taken up in the system. It can be seen that anoxic P uptake contributed significantly to the total uptake, with contributions ranging from 30% in Period III up to 60% in Period I. Hu *et al.* (2002) indicated that the nitrate load to the anoxic zone is positively correlated with the ability of PAOs to utilize nitrate as an electron acceptor for P uptake. This observation was confirmed in the present research from the large portion of anoxic P uptake that was measured during Period I and II, when the aerobic recycle ratio was set at 2 (see Table 6.2). From Figure 6.13, it can also be observed that the mass of P released in the anaerobic zone, and the corresponding amount subsequently taken up, increased as more biomass was accumulated in the system (lowest in Period I and highest in Period IV). The mass of P released per unit mass of VFA utilized was

calculated through a mass balance around the anaerobic zone. As shown in Figure 6.14a, the PO_4-P to VFA ratio was relatively consistent for each of the four investigated periods at around 0.6, with the exception of Period II for which the observed value was 0.5. Smolders *et al.* (1994) reported that the PO_4-P/VFA ratio can not be considered to be a metabolic constant, as was initially proposed by other researchers (Wentzel *et al.*, 1986), because of the strong influence of the pH in the solution. The range of PO_4-P/VFA values observed in the four experimental periods in the present study is in close conformity with other EBPR systems reported in the literature (e.g. Wentzel *et al.*, 1989).

The impact of the amount of P released in the anaerobic zone should be evaluated together with the mass of VFA utilized in the influent per unit mass of PO_4-P removed. This ratio was calculated from the data set available in each experimental period, and the results are shown in Figure 6.14b. As can be seen, the VFA/PO_4-P ratio increased as the system progressed from Period I to IV. The amount of PO_4-P removed from the wastewater remained approximately the same in all four investigated periods. However, as the system accommodated more biomass, the same removal was accomplished with increasingly larger amounts of PO_4-P released in the anaerobic zone (see Fig. 6.13), which required proportionately larger quantities of VFA for uptake. From Figure 6.14b, it can also be seen that the impact of the SRT change (from Period II to III) on the VFA/PO_4-P ratio was more significant than the HRT reduction (Period I to II and Period III to IV). Three main factors help to explain this observation. First, the operation at longer SRT resulted in a decline of the observed sludge yield (see Fig. 6.7) and, consequently, in reduced P utilization for cell growth. This extra P remaining in solution had to be removed by the PAOs in the form of poly-P pool, which required additional VFA consumption. Secondly, because of the conservative nature of P and the declining yield of the microorganisms at longer SRTs, the accumulation of P in the bioreactor, relative to the overall biomass, increased at longer SRT. This results in a higher % P in the sludge which, together with the increased level of

suspended solids, contributes to a larger mass of P being released in the anaerobic zone. Finally, the impact of the maintenance process within PAOs becomes more significant at longer SRT, which causes P to be released to the solution without any carbon utilization (also known as secondary release).

From an engineering point of view, it is preferable to operate a BNR plant under a set of conditions which require the lowest possible VFA/PO_4-P ratio, essentially because municipal wastewaters are often limited in VFA content. Not surprisingly, the design of current BNR processes covers a range of conditions that target short SRTs and relatively low levels of suspended solids in the bioreactor. Therefore, the application of membrane-assisted EBPR processes in a high rate mode and/or long SRTs may be hampered, when limited VFA are present in the feed. In these cases, addition of extra carbon sources to the anaerobic zone, or side-stream P-recovery units, may be essential design elements for a successful operation of a MEBPR system.

6.4 Conclusions

An MEBPR process was evaluated under four different operating conditions which led to the following conclusions.

- In the absence of carbon-limiting conditions, the MEBPR process was shown to be capable of achieving excellent bio-P performance at increasingly higher hydraulic loads, with the lowest applied HRT being 5 hours. Operating at these high rate conditions is considered to be a significant technological advance beyond conventional BNR technology.
- Satisfactory P removal was demonstrated at 12 and 20 day SRTs. The successful performance of the MEBPR process with high mixed liquor suspended solids concentrations resulted in increased VFA utilization per unit mass of P removed, with the SRT having a significantly larger impact than the HRT. The reduced observed sludge yield, the large mass

of P released in the anaerobic zone, and the significant contribution of PAO maintenance were thought to be critical factors in the variation of the VFA/P ratio.

- Stable and complete nitrification was found to be independent of the system operating conditions, as the MEBPR process was able to nitrify at volumetric nitrogen loading rates as high as 0.17 kg $N/(m^3 \cdot d)$.
- A consistent 90-95% removal of total COD was achieved throughout the research program at volumetric COD loading rates in the range of 0.7-1.5 kg $COD/(m^3 \cdot d)$.
- A step increase in the flow rate that accompanied the passage from one HRT set point to the next was absorbed by the process, without any disturbances in COD, N and P removal. On the other hand, the cessation of sludge wasting to quickly reach the new suspended solids equilibrium for a 20 day SRT resulted in P breakthrough, which was subsequently re-absorbed.
- The complete solid-liquid separation afforded by the membrane permitted the generation of a treated effluent of superior quality. Successful performance of the MEBPR process produced effluent total P concentrations that were only slightly above the PO_4-P concentrations.

Table 6.1 UBC Pilot Plant primary effluent wastewater. CI: confidence interval.

Parameter	Mean	95% CI	Min - Max
TSS [mg/L]	91.5	±4.6	18 – 216
CODtot [mg/L]	306.3	±8.9	182.0 – 584.6
CODsol [mg/L]	97.3	±4.2	50.5 – 265.0
Acetate [mg/L]	25.0	±1.3	6.6 – 46.4
Propionate [mg/L]	3.4	±0.2	1.0 –12.7
Tot VFAs [mg COD/L]	32.2	±1.4	6.9 – 62.4
TKN [mg N/L]	34.0	±0.5	23.6 – 45.5
NH_4-N [mg N/L]	25.3	±0.3	17.2 – 34.9
NO_3-N [mg N/L]	Not detec.		
TP [mg P/L]	4.2	±0.1	2.4 – 6.9
PO_4-P [mg P/L]	2.4	±0.04	1.4 – 5.0
T [°C]	20.3	±0.3	14.0 – 23.6
pH	7.2	±0.03	6.4 – 7.8

Table 6.2 Experimental program for the MEBPR process Side A (top) and Side B (bottom).

Period	Date	Day	HRT [h]	SRT [d]	Aer rec. ratio[1]	HAc[2] Addition [mg COD/L]
Ia	May 26-Sep 11	1 - 109	10	12	2	0
Ib	Sep 15-Oct 22	110 - 147	10	12	1	0
Ic	Oct 31-Dec 7	148 - 185	10	12	2	***20***
IIa	Dec 8–Jan 28	186 - 236	*7*	12	2	20
IIb	Jan 28–Jun 21	237 - 353	7	12	***1***	***30***
III	Jun 22 – Nov 1	354 - 480	7	***20***	1	30
IV	Nov 1 – Dec 17	481 - 526	***5***	20	1	30

Period	Date	Day	HRT [h]	SRT [d]	Aer rec. ratio[1]	HAc[2] Addition [mg COD/L]
II	Jun 22 – Oct 11	1 - 113	7	12	1	30
III	Oct 12 – Nov 26	114 - 159	7	***20***	1	30

[1] Aerobic recycle ratio, defined as the recycle flow rate divided by the influent flow rate

[2] HAc: Acetate

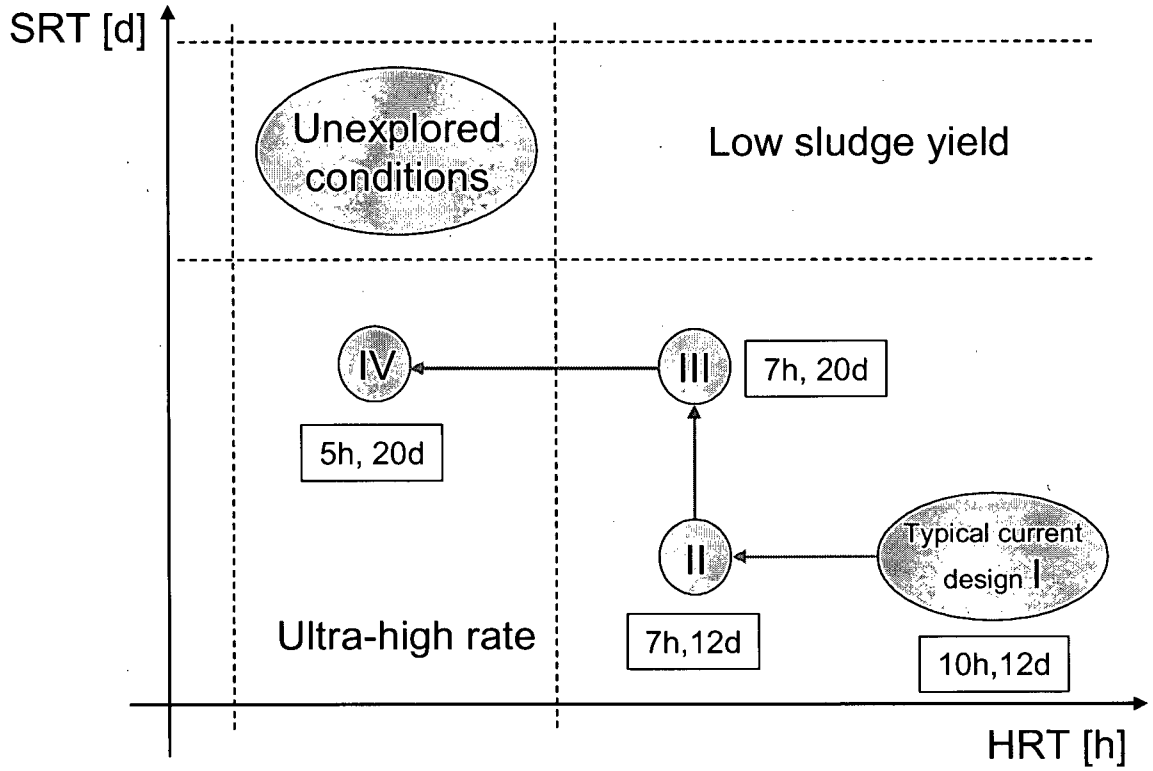

Figure 6.1 **Experimental design followed for operating Periods I-IV.**

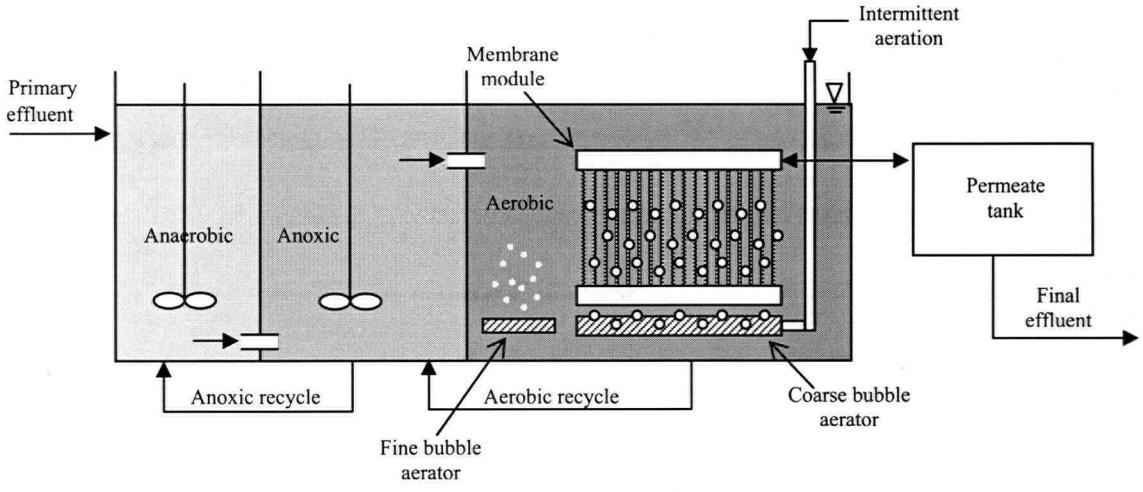

Figure 6.2 Schematic of the MEBPR process used in this study.

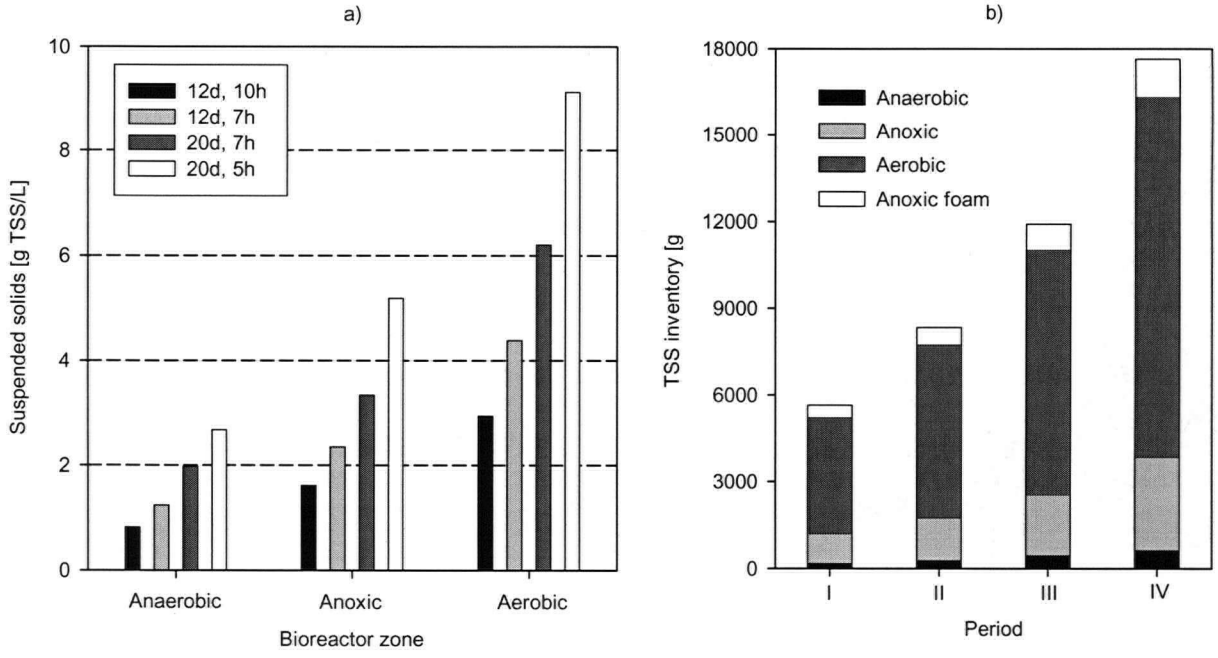

Figure 6.3 **(a) TSS profiles in the reactor and (b) TSS inventory in each compartment over the investigated periods.**

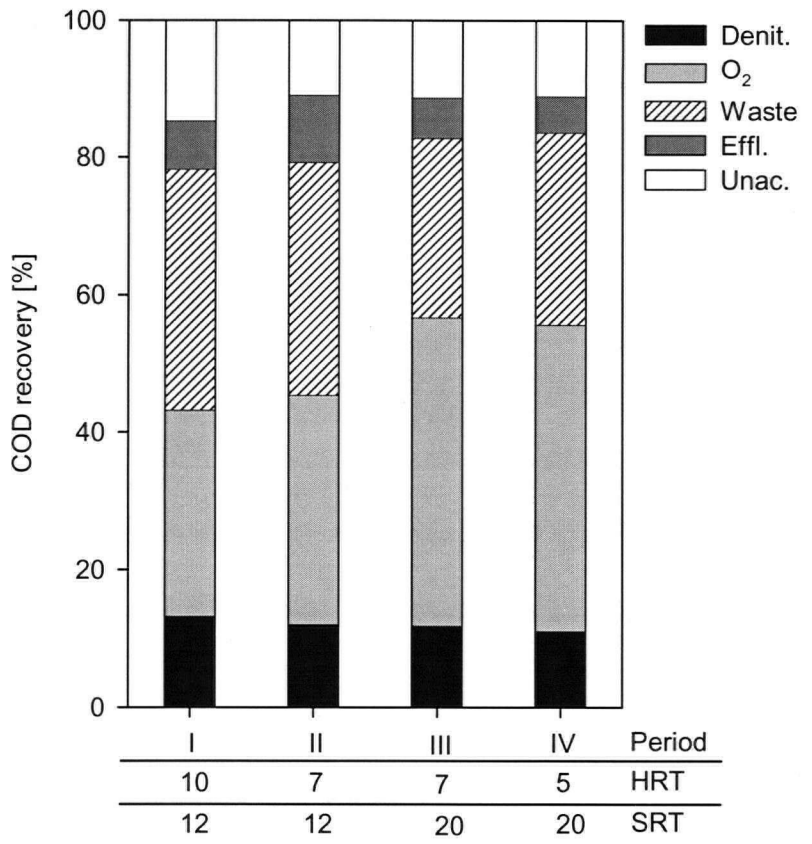

Figure 6.4 **COD mass balances for the MEBPR systems over the four periods. Denit: denitrification; O_2: oxygen utilization; Waste: waste activated sludge; Effl: effluent flow; Unac: unaccounted for.**

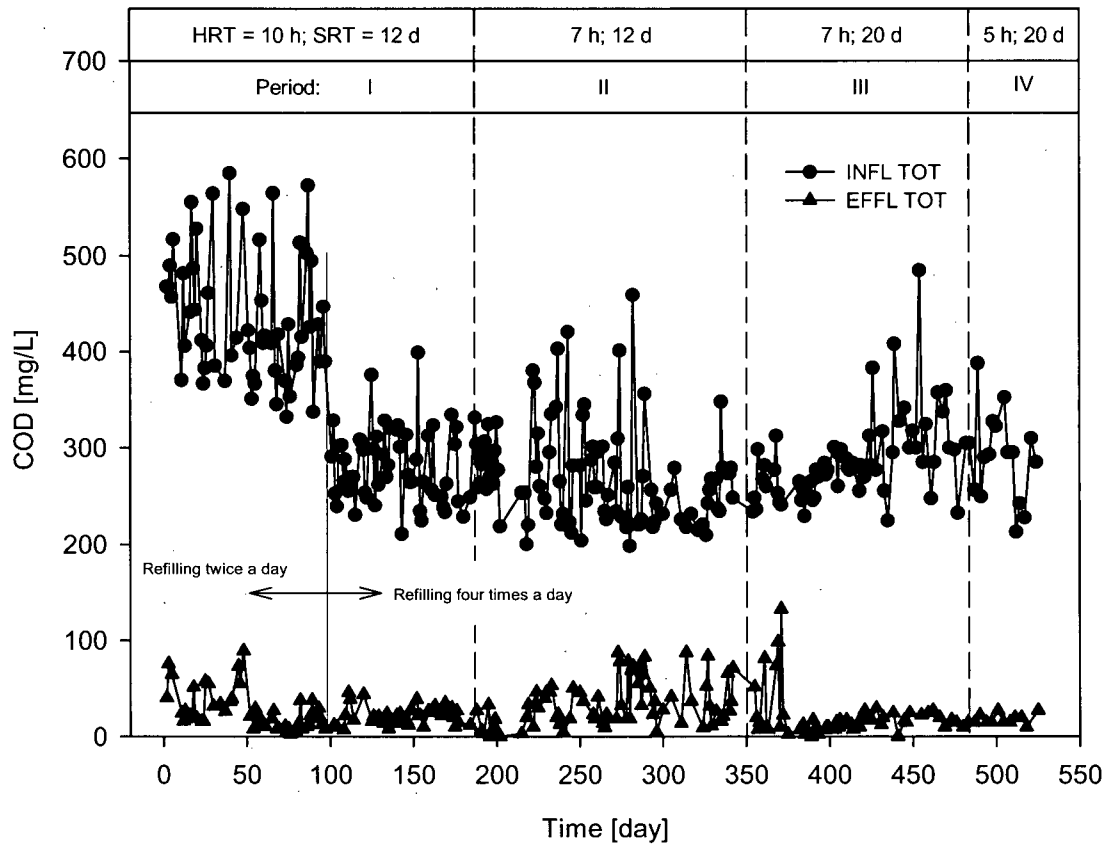

Figure 6.5 **Time series of influent and effluent total COD over the entire duration of the study.**

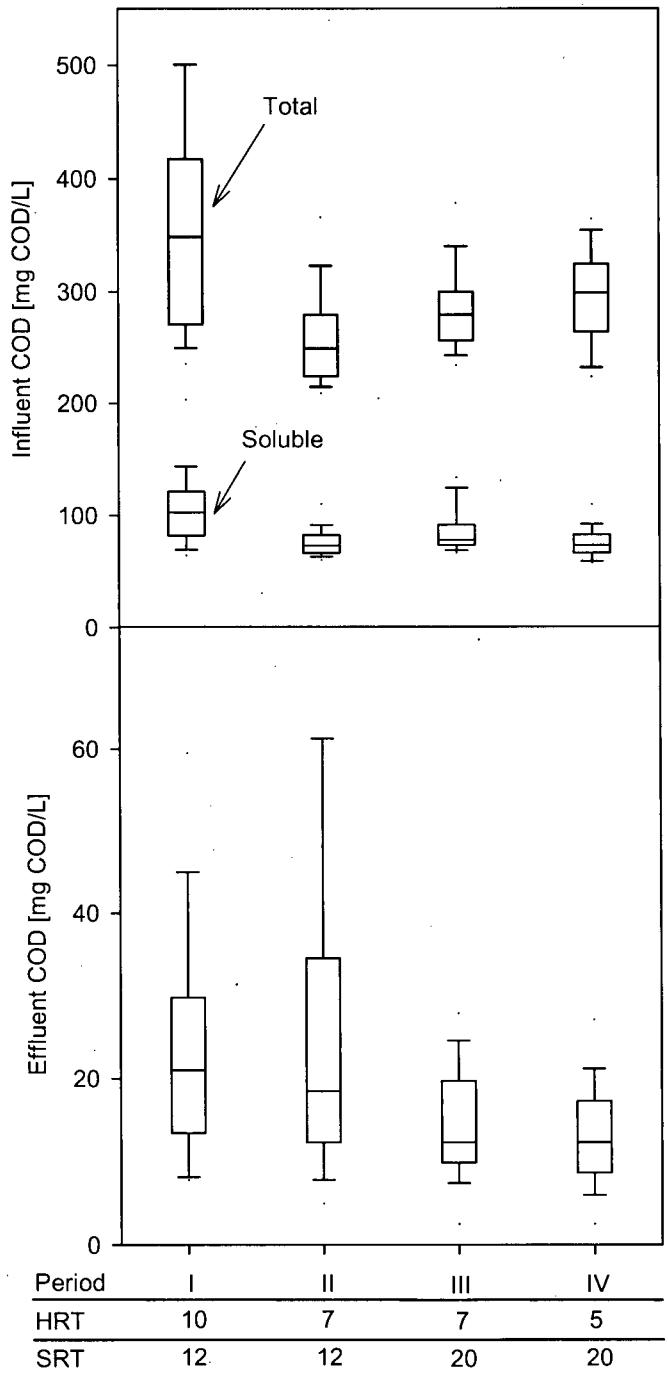

Figure 6.6 **Distribution of total and soluble influent COD (above) and effluent COD data (bottom) during each experimental period.**

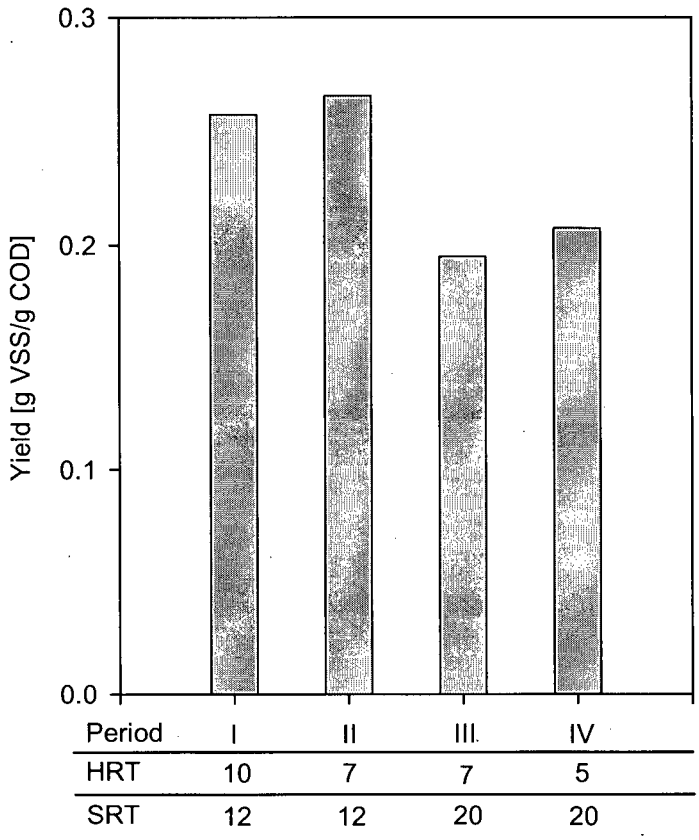

Figure 6.7 **Observed sludge yield in the MEBPR process during each experimental period.**

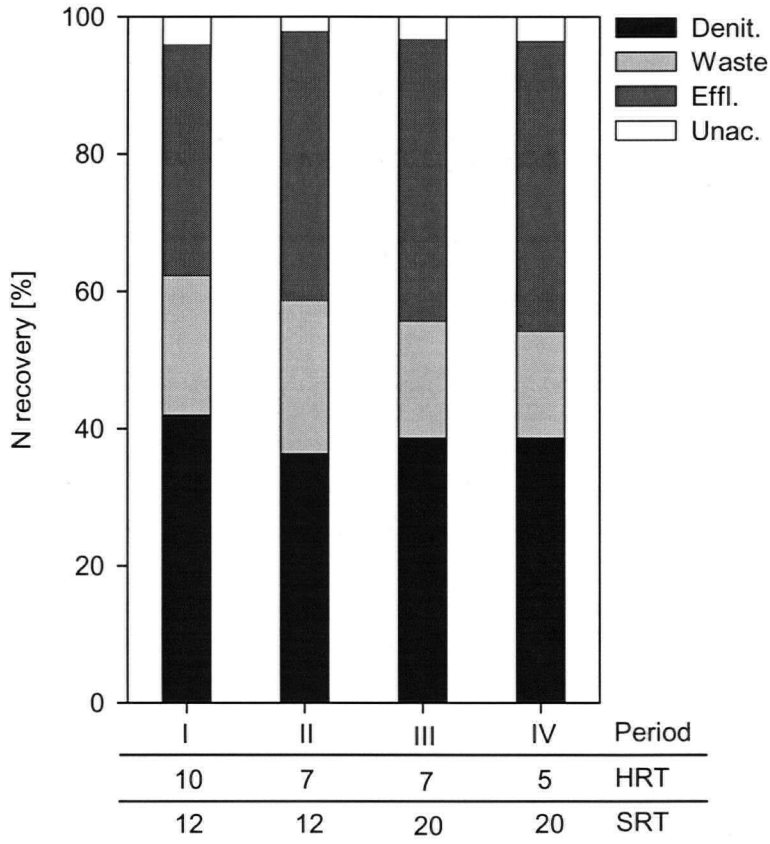

Figure 6.8 **N mass balances for the MEBPR systems over the four periods. Denit: denitrification; Waste: waste activated sludge; Effl: effluent flow; Unac: unaccounted for.**

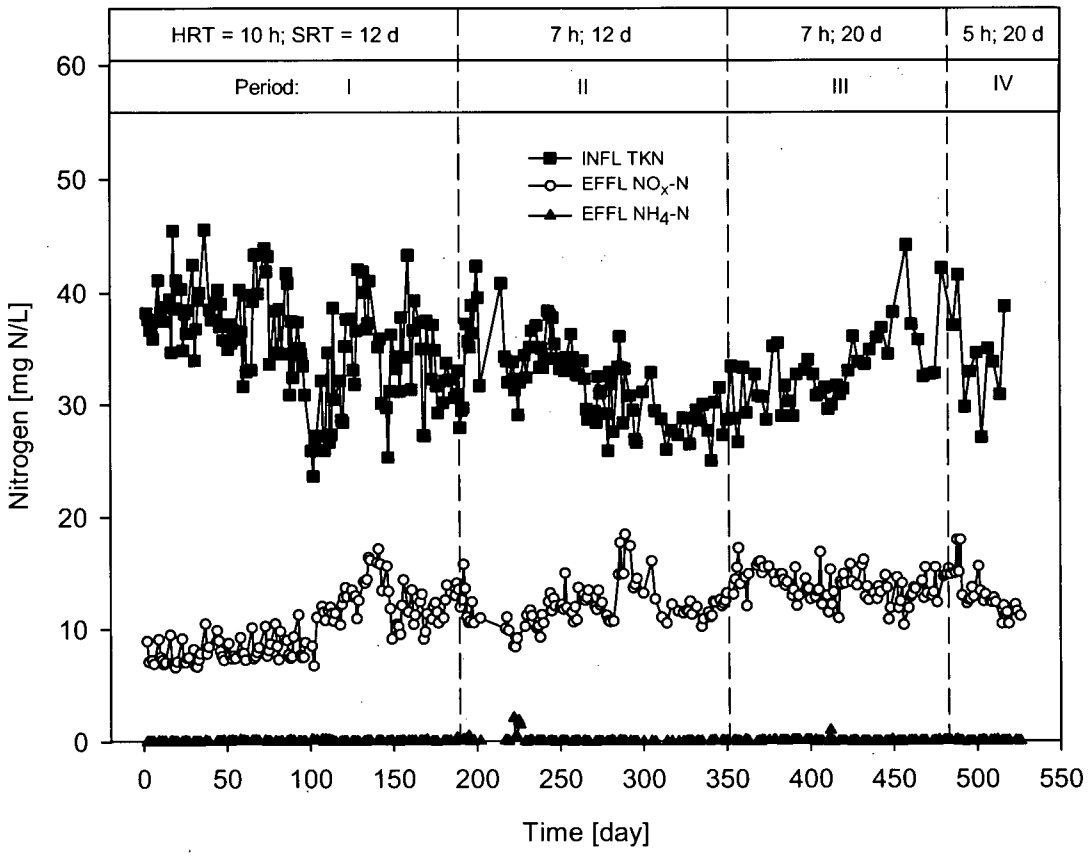

Figure 6.9 **Time series of influent TKN, effluent NH_4-N and TN over the entire duration of the study.**

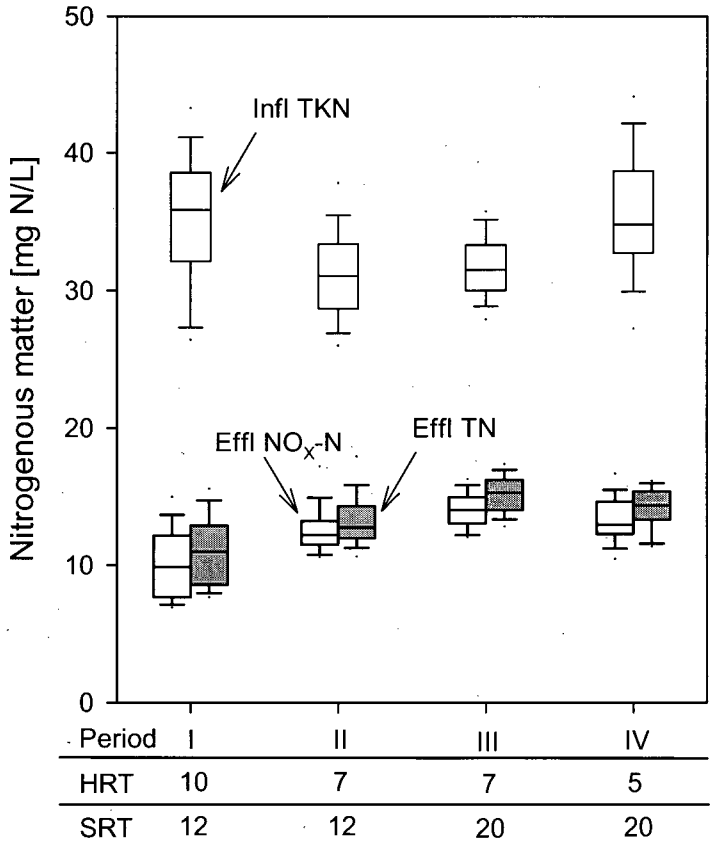

Figure 6.10 Distribution of influent TKN data and effluent NO_X-N and TN data during each experimental period.

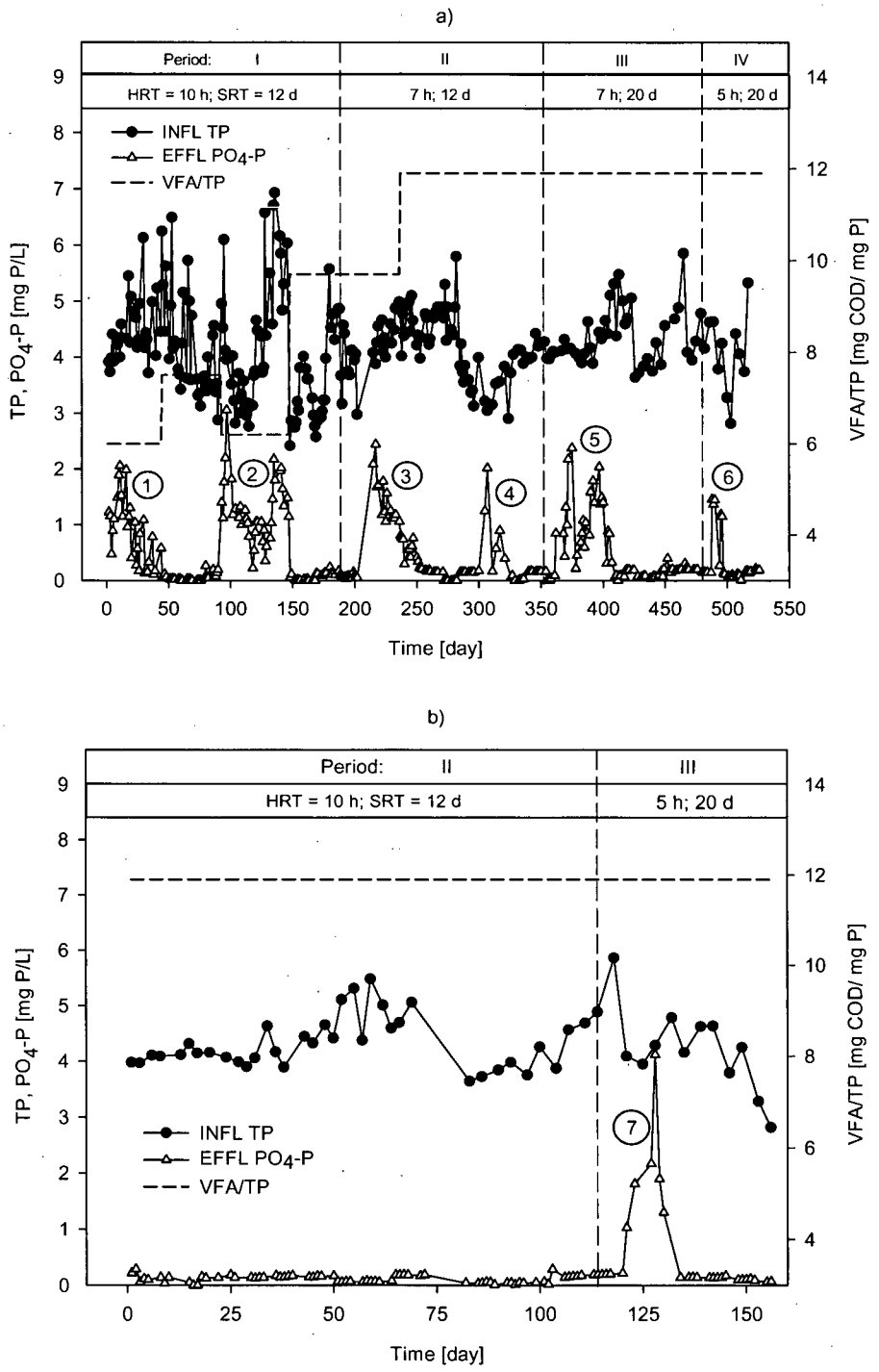

Figure 6.11 **(a) Total phosphorus (TP) in the influent and orthophosphate phosphorus (PO_4-P) in the MEBPR effluent Side A, and (b) Side B. The dash line represents the VFA to TP ratio prevailing in the incoming wastewater.**

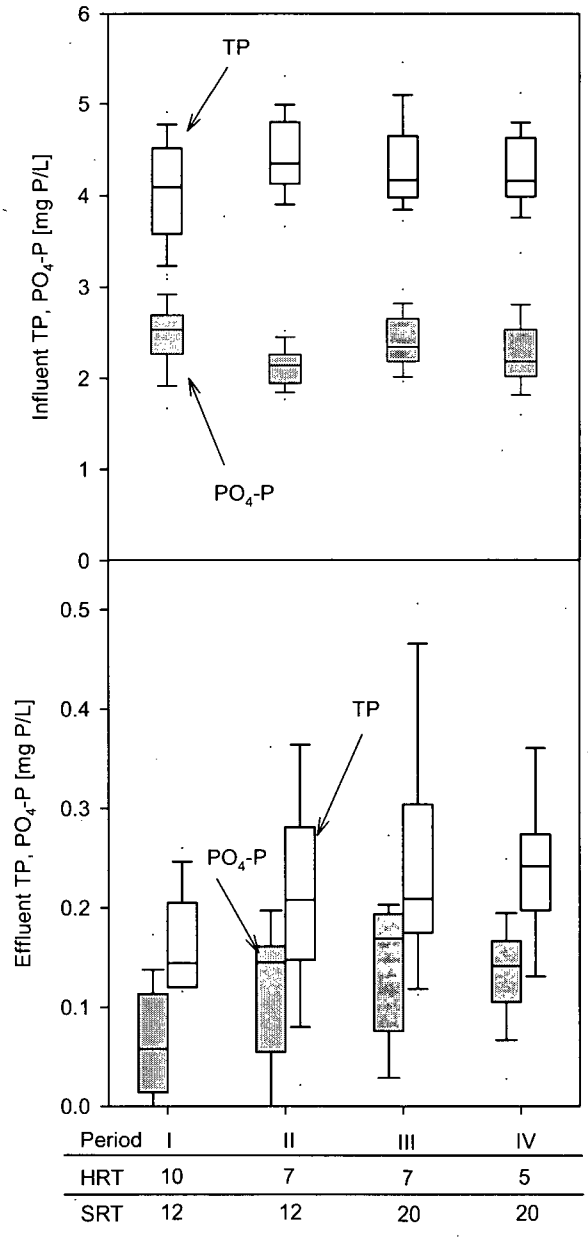

Figure 6.12 Distribution of TP and PO_4-P concentration data in the influent (above) and MEBPR effluent (bottom) during satisfactory performance in the four experimental periods.

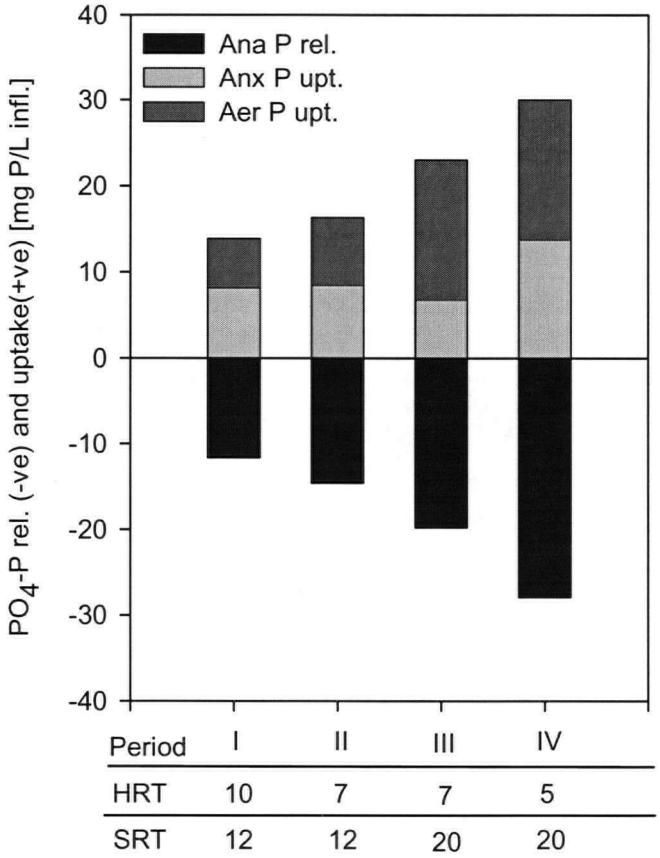

Figure 6.13 **Anaerobic PO_4-P release (ana), anoxic PO_4-P uptake (anx), and aerobic PO_4-P uptake (aer), during optimal P removal periods in each of the four experimental runs.**

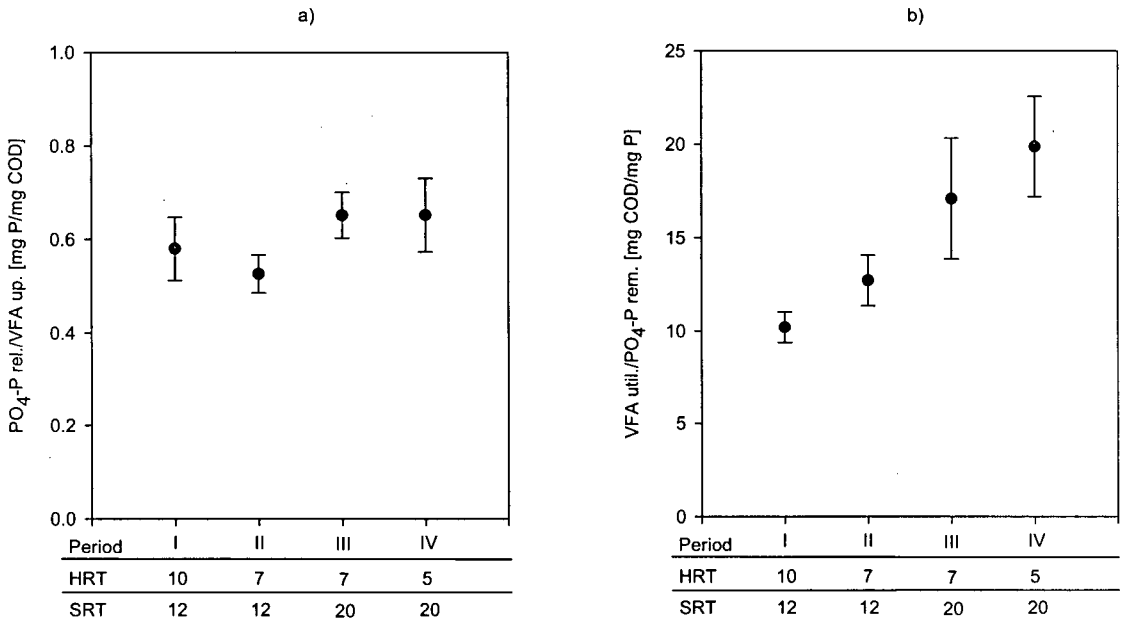

Figure 6.14 (a) Mass of PO_4-P released to VFA consumed in the anaerobic zone, and (b) mass of VFA utilized in the influent per unit of PO_4-P removed.

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Chapter 7 Characterization and production of anoxic foam in a membrane enhanced biological phosphorus removal process*

7.1 Introduction

Although conventional activated sludge processes (ASP) have been widely applied as a cost-effective wastewater treatment solution for many decades, they still suffer from solid-liquid separation shortcomings, typically caused by sludge bulking and foaming (Martins *et al.*, 2004; Lemmer, 2005). A large body of literature is available that documents bulking and foaming events in wastewater treatment plants all around the world (Blackbeard *et al.*, 1986; Seviour *et al.*, 1990; Eikelboom *et al.*, 1998; Madoni *et al.*, 2000; de los Reyes *et al.*, 2002; Mori *et al.*, 1992). In this regard, the advent of membrane filtration technology in the wastewater industry has marked a significant contribution for its ability to effect solids-liquid separation independently of the sludge settleability property (Krauth and Staab, 1993). Yet, if the settling problems associated with excessive growth of filamentous bacteria (i.e. sludge bulking) have been addressed in membrane bioreactors (MBRs), the formation of foam still poses a threat to the operational integrity of the process.

Stable and brown colour foam accumulating on the surface of the bioreactor is a three-phase material containing gas bubbles (air or nitrogenous gas), liquid (water), and solid particles (microorganisms) (Davenport and Curtis, 2002). With the use of molecular techniques, it has been shown that the formation of foam in conventional ASP systems may be accompanied by an increase in the level of mycolic acid-containing actinomycetes (mycolata), particularly *Gordonia* spp. (Davenport *et al.*, 2000; de los Reyes and Raskin, 2002). In addition, the filamentous

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organism *Candidatus Microthrix parvicella* (hereinafter referred to as *M. parvicella*) has also been widely implicated in foaming events (de los Reyes *et al.*, 2002; Rosetti *et al.*, 2005).

Physical barriers in the bioreactor, resulting in the accumulation of floating materials, have been reported to enhanced the growth of foam-forming bacteria, since they prevent the natural wash-out of foam from the system (Blackall *et al.*, 1991). Unlike conventional ASPs, with an overflow channel between the aeration basin and the secondary clarifier, membrane bioreactors (MBRs) are configured in such a way that microorganisms leave the system only through the sludge wasting line. Additionally, if the intake of the wasting line is located under the reactor surface, chances are that the microorganisms entrapped in the foam will have a retention time that is higher than the nominal sludge age and so they will be selectively enriched. Therefore, it is reasonable to expect that foam formation in MBRs is more favoured than in conventional ASP, when the potential for the proliferation of foam-formers exists. In a comparative study of conventional and membrane enhanced biological phosphorus removal (EBPR) processes, it was observed that the membrane system exhibited a significantly greater propensity to accumulate foam on the surface of the anoxic compartment than the conventional system (Monti *et al.*, 2005a, Chapter 2). After completely re-suspending the thick layer of foam into the liquid phase, the foam reappeared on the anoxic zone surface in similar amounts within hours. Recently, a Japanese study also reported foaming in the aeration tank of an MBR process, with extracellular polymeric substances as a putative causative agent (Nakajima and Mishima, 2005).

Given the stability of the anoxic foam and the relatively long sludge retention time (SRT) that is feasible in MBR processes, the regular mechanical removal of this material represents an interesting potential SRT control strategy and, at the same time, a method to minimize the retention of foam in the system. In addition, the concentrated floating material formed in

biological nutrient removal (BNR) plants may be viewed as a rich source of phosphorus, that can be recovered through downstream processes.

The present study aimed to explore the long-term feasibility of foam harvesting as a strategy to naturally generate thickened sludge for SRT system control. In addition, to assess the potential for downstream recovery, the foam was characterized and compared with the mixed liquor in terms of nitrogen and phosphorus content, specific nitrification and P-release rates, and microbial community composition. Even though significant contributions have been recently made in the understanding of the formation and stability of foam, there is limited knowledge of how the properties of this floating material compare with the bulk liquid activated sludge, particularly in MBR processes.

7.2 Materials and methods

7.2.1 Description of the pilot plant

The present study was conducted at The University of British Columbia (UBC) pilot plant, that was used as the experimental ground for the evaluation of a membrane enhanced biological phosphorus removal process. The UBC pilot plan is a dual train facility that initially was operated for a comparative study of a conventional and membrane process operated in parallel under identical operating conditions (Monti *et al.*, 2005a, Chapter 2). Subsequently, the conventional train was retrofitted to a second membrane process, so that different operating conditions could be tested simultaneously in the two trains, named Side-A and Side-B (Monti *et al.*, 2005b, Chapter 6).

A University of Cape Town (UCT) type bioreactor (Tchobanoglous *et al.*, 2003) with a total volume of 2,500 liters was divided into an anaerobic (11% v/v), anoxic (28% v/v), and aerobic zone (61% v/v). One ZeeWeed®-140 membrane module (Zenon Environmental Inc., Oakville, Ontario, Canada) was installed in the existing aerobic tank to perform the solids-liquid

separation step. The membrane fiber had a nominal pore size of 0.04 μm and the module featured 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 the permeate stored in the permeate tank. Intermittent coarse-bubble aeration (10 sec ON and 10 sec OFF) was provided to the module, at an air flow of 0.34 m^3/min (12 scfm), to reduce the cake of solids on the membrane surfaces and to prevent the packing of solids between the membrane fibers. For a detailed description of the pilot plant configuration and operating mode, refer to Chapter 6 (Monti *et al.*, 2005b).

7.2.2 Foam characterization

During the long-term study on EBPR in a membrane system (Monti *et al.*, 2005ab, Chapter 2 and 6), the stable layer of foam on the anoxic zone surface was characterized and compared with the mixed liquor by analyzing the following parameters: ratio of volatile suspended solids (VSS) to total suspended solids (TSS), specific nitrogen and phosphorus content, maximum specific nitrification and P-release rates, and microbial community composition. In addition, the mass of foam accumulating on the anoxic surface was quantified twice and expressed as a percentage of the total biomass residing in the bioreactor. The complete overview of the foam characterization study and the corresponding pilot plant operating conditions is presented in Table 7.1.

7.2.3 Batch test procedures

Off-line nitrification and P-release batch experiments with aerobic mixed liquor and foam were performed in a 5-liter reactor stirred with a magnetic bar, and a mechanical mixer turning at 60 rpm. The reactor was equipped with a portable VWR probe for temperature and pH measurement and with a YSI probe for monitoring the dissolved oxygen (DO) concentration. Mixed liquor samples from the aerobic zone and from the foam compartment were manually

transferred to two parallel batch reactors located in the immediate vicinity. The concentrated foam material was diluted 20 times with permeate effluent to achieve a final concentration between 2 and 4 g TSS/L. The experiments were conducted at the constant pH of 7, by dosing 0.1N HCl and 0.1N NaOH, and at ambient temperature (not controlled). Mixed liquor samples required for sampling were centrifuged for 3 minutes at 550 rcf and the supernatant was then filtered through a 0.45 μm filter to obtain 10 mL of soluble sample for subsequent measurements. Triplicate samples for TSS and VSS were collected from each batch reactor at the beginning and at the end of the experiment, and the 6 final values were averaged to estimate the suspended solids concentration of the batch test. All analyses were performed according to Standard Methods (APHA *et al.*, 1998).

P-release batch test. At time zero, 696 mg of sodium acetate were added to each reactor to establish an initial acetate concentration of roughly 60 mg COD/L. After 1-2 minutes from substrate addition, a sample was collected from each reactor to measure the initial concentration of acetate (HAc), and orthophosphate-P (PO_4-P). Anaerobic conditions were maintained for 120 minutes and samples were collected every 15 minutes until the completion of this phase. The maximum specific P release rate and the HAc uptake rate were calculated during the first 45 minutes of the experiment. The maximum reaction rates were determined as initial rates during the first 40-45 minutes of experiments, using linear regression methods.

Nitrification batch tests. At time zero, 380 mg of ammonium chloride were added to each reactor to establish an initial ammonium-N concentration of roughly 20 mg N/L. After 1-2 minutes from substrate addition, a sample was collected from each reactor to measure the initial concentration of ammonium-N (NH_4-N) and nitrite+nitrate-N (NO_X-N). Samples were then taken every 15-20 minutes until the completion of the reaction to measure the maximum nitrification rate of the investigated sludge.

7.2.4 Ribosomal intergenic spacer analysis (RISA)

A molecular fingerprinting method was used to characterize the bacterial community composition of the foam and the underlying activated sludge. Details on the principles and methodology of the RISA technique are provided in Chapter 5 (Monti *et al.*, 2005c). Samples from the two compartments were collected at the same time from the pilot plant and kept on ice until further analysis in the laboratory. As a part of the sample preparation before applying the RISA technique, foam samples were diluted with deionized water to a final concentration between 2 and 4 g TSS/L.

RISA fingerprint elaboration. The band patterns generated by RISA were analyzed for the richness, evenness and diversity indexes, as described in Chapter 5 (Monti *et al.*, 2005c). In addition, the Pearson's correlation coefficient was used to express the similarity between the fingerprints of bacterial communities contained in foam and mixed liquor samples.

7.2.5 Foam harvesting experiments

During the characterization study, the stable layer of foam on the anoxic surface was controlled by completely re-suspending it daily in the bulk liquid by manual mechanical mixing. For the foam harvesting experiment, the floating material was removed every day and transferred to a bucket until the anoxic surface was completely clean. The bucket contents were first weighted on a digital scale and then completely mixed to collect representative samples (triplicates) for percentage solids determination. The total mass of dry solids of the foam harvested was then included in the calculation of the sludge wasting volume required for system SRT control. Four distinct foam harvesting experimental runs were conducted (Table 7.2), which explored a combination of SRTs and HRTs typically applied to BNR systems for municipal wastewater treatment.

7.3 Results and discussion

As a part of a one-year comparative study between a membrane and conventional BNR process, a qualitative assessment was conducted on the bulking and foaming propensity of the two systems. This analysis is briefly presented, before focusing the discussion on the membrane foam characteristics and production.

7.3.1 Foam formation propensity

When a conventional EBPR (CEBPR) process was run in parallel with a membrane EBPR (MEBPR) system under identical operating conditions (Monti *et al.*, 2005a, Chapter 2), a clear difference was noticed in the propensity to form a layer of foam in the anoxic compartment. Although the surface of the CEBPR anoxic zone was basically free from any floating material at all times, the MEBPR counterpart was consistently covered by a thick layer of foam that accumulated after re-suspending it daily by manual mechanical mixing. Figure 7.1 shows how the surface of the anoxic zone appeared in the two treatment trains. As can be seen, the foam in the MEBPR system covered the entire surface and was approximately 10 cm thick, before daily mechanical resuspension. It appeared as a stable brown-colored layer, likely forming from the attachment of small gas bubbles generated during denitrification (Eikelboom, 2000). On the other hand, the anoxic zone surface of the CEBPR process featured only a few isolated chunks of foam floating or depositing along the border. It is worth mentioning that only a small portion of the aerobic zone surface was covered with foam in the two systems. Contrary to other cases reported in the technical literature, the foaming in the present MEBPR study did not follow any seasonal variation. It was speculated that, unlike in the CEBPR system, the absence of natural wasting of the floating material in the MEBPR process could have contributed to the accumulation of a significant amount of foam in the bioreactor. In addition, the use of vigorous coarse-bubble aeration in the membrane system resulted in a biomass with a smaller floc size distribution (data not shown) that might be more easily subjected to gas flotation in the anoxic

zone. It remains also unclear how the intermittent aeration contributed to backflows from the aerobic to the anoxic zone, potentially lead to foam formation.

A routine measurement of the sludge volume index (SVI) indicated that sludge bulking persisted in the two systems for almost the entire duration of the comparative study. Both processes exhibited an average SVI of more than 250 mL/g (± 9.5, 95 % confidence interval), with no noticeable seasonable variation. Through occasional microscopic examination of the sludge, it was noticed that both systems harbored an abundance of filamentous organisms, with an index between 4 and 5 (Eikelboom, 2000). Filamentous bulking has been reported as one of the main difficulties in the implementation of BNR technology (Ekama and Wentzel, 1999). Interestingly, the membrane and conventional EBPR processes in this study featured different dominant filamentous organisms: a *Gordonia* (formerly *Nocardia*) *amarae*-like organism (GALO) and *M. parvicella*, respectively. The dominance of GALO in a membrane EBPR sludge and foam was also reported by previous investigators at the UBC pilot plant (Po, unpublished data), with *M. parvicella* being the second most common filament in foam. The solid-liquid separation technologies and the aerator devices used, were the only two differences between the conventional and membrane systems. It is likely that these two factors played some role in favoring the growth of an activated sludge with distinct dominant filamentous organisms.

7.3.2 Foam characterization

The membrane anoxic foam was investigated in several aspects, including the solids inventory and composition, nitrification and P-release specific rates, and microbial community composition. The results are presented and discussed in the following sections in relation to the same property of the mixed liquor.

7.3.2.1 Foam inventory and content

In order to quantify the contribution of foam to the total MEBPR solids inventory, the weight of the stable floating material was estimated two times by measuring the TSS concentration in each bioreactor zone before and after completely re-suspending the foam in the anoxic zone. Figure 7.2 illustrates the results of both experiments and the percentage distribution of suspended solids in the bioreactor. As can be seen from the top graph, the mixing of the foam layer resulted in a overall increase in the TSS concentration of the three compartments, with the largest increase occurring in the anoxic zone. The total biomass inventory calculated after the mixing was compared to the inventory before the mixing to estimate the portion associated with the anoxic foam. From the bottom graph of Figure 7.2, it can be observed that the floating material accounted for approximately 8% of the total solids inventory of the membrane system, representing a significant portion. The suspended solids content of the foam was also estimated to be between 4 and 6%, which is an impressively thickened sludge, given the completely natural process leading to it. Similar solids concentrations have been reported by Eikelboom (2000) for extreme cases of scum formation in conventional activated sludge plants. The results of this analysis suggest that, if consistently formed over time, the anoxic foam represents a concentrated source for phosphorus (P) recovery, particularly for P-enriched sludge typical of EBPR processes. For this reason, further evaluation of its composition was conducted by measuring volatile solids fraction and the specific nitrogen (N) and phosphorus (P) contents.

Foam and anoxic mixed liquor samples were simultaneously collected from the plant at different times over the investigation period, and assessed for VSS/TSS ratio and the specific N and P content. As can be observed in Figure 7.3, the specific composition of the foam material was similar to that of the underlying activated sludge. According to the paired *t*-test at a 95% confidence level, the VSS/TSS ratio of the foam was the only parameter that was statistically higher in the foam than in the mixed liquor portion, indicating the propensity of the floating

material to accumulate more organic material. Based on these three parameters, it appears that, overall, the foam formation could be characterized by an homogenous transport of sludge particles from the liquid to the surface of the anoxic compartment. The only significant difference is the suspended solids concentration which can be more than 10-fold greater in the case of the foam fraction.

7.3.2.2 Foam activity

The biological activity of the foam was assessed by comparing the results from nitrification and P release batch tests with those relative to the aerobic mixed liquor. Nitrification batch tests were conducted in parallel with foam and mixed liquor taken from the aerobic zone every two weeks for a period of four months. The selection of the aerobic mixed liquor was justified by the fact that nitrifiers exhibit the highest activity under aerobic conditions. The comparison of the maximum specific nitrification rates of the two types of sludge is illustrated in Figure 7.4. As can be observed from the first column in the graph, the overall nitrification potential was comparable in the foam and the mixes liquor. Even though a few individual batch test results showed a clear difference between the two sludges, a *t*-test performed on eight measurements over the four month period indicated no significant difference at a 95 % confidence level. These observations suggest that the concentration of nitrifying organisms in the foam was comparable to that of the mixed liquor fraction.

To evaluate the P-release potential of the foam, four batch tests were performed in parallel with the aerobic mixed liquor over a period of four months. The aerobic mixed liquor carried the highest suspended solids concentration and specific P content compared to the other two bioreactor zones, therefore representing an alternative source for P-recovery in the system. The comparison of the maximum specific P release rate is presented in Figure 7.4. In contrast to the nitrification rates, the anoxic foam exhibited a consistently lower activity for P release, which

was found to be only 25 to 30 % of that of the aerobic mixed liquor. Further experimental work to explain this reduction was beyond the scope of this investigation. However, the following hypotheses are advanced to account for the reduced P release rate measured in the anoxic foam. The floating material originated from sludge previously exposed to anoxic conditions and, therefore, with the poly-P pool only partially replenished. In a UCT-type reactor, the orthophosphates released in the solution under anaerobic conditions are partially taken up in the anoxic compartment and then, further sequestered in the final aerobic zone (van Loosdrecht *et al.*, 1997). It follows that the poly-P pool of PAOs present in the anoxic sludge is a fraction of that in the aerobic sludge, and this value depends on the extent of anoxic P uptake prevailing in the process. The P release rate of an EBPR sludge is a function of the amount of poly-P accumulated in the cell, as widely demonstrated in the technical literature (Henze *et al.*, 2000). In addition, foam is likely to be characterized by an environment with more limited nutrient availability than in the activated sludge mixed liquor. With a combination of *in situ* techniques, Eales *et al.* (2005) observed that filamentous bacteria enriched in foam and capable of EBPR were metabolically inactive. Therefore, it is possible that some microorganisms residing in the foam may have been characterized by an overall reduced metabolic activity.

If the recovery of P from the foam is to be accomplished through P release methods followed by struvite formation (Liao *et al.* 2003), the lower specific release activity compared to the aerobic sludge is likely to be counterbalanced by the high suspended solids concentrations in foam, which were approximately 10-fold higher than in the underlying mixed liquor.

7.3.2.3 Foam bacterial composition

The third aspect of the comparison of membrane foam and the underlying activated sludge was the investigation of the bacterial composition based on RISA community fingerprinting. This molecular technique helped to address the question of whether or not the two bacterial

populations differed and, additionally, to identify unique bands which may be associated with foam and/or mixed liquor.

Eleven samples were collected from each of the foam and the aerobic compartment over a period of eight months. In this case, the selection of which mixed liquor zone to choose for the comparison was irrelevant since the composition of the microbial community in the three bioreactor zones was demonstrated to be nearly identical (Monti *et al.*, 2005c, Chapter 5). Figure 7.6 shows all the ribosomal intergenic spacer (RIS) fingerprints for mixed liquor and foam samples. The paired fingerprinting images presented in Figure 7.6 allow for a comparison between the two bacterial populations. It can be seen that band pattern in the foam and activated sludge fraction correspond fairly well at any given time, indicating that the bacterial composition of the two communities shared a high degree of similarity. This aspect was quantified with the similarity index based on the Pearson's correlation coefficient. In this study, an average similarity index value of 80.0% (95% confidence interval of 7.9%) was observed between the two populations residing in the foam and in the mixed liquor. According to the visual elaboration of Figure 7.6, two bands were observed to be significantly and consistently darker in the foam than in the corresponding activated sludge, indicating an enrichment of specific microorganisms. Similarly, one band that was noticed to be associated with the mixed liquor was absent or less concentrated in the foam sample, suggesting that some microorganisms were not transported from the liquid to the surface.

Based on these observations, it can be concluded that foam formation was characterized by an homogenous flotation of microorganisms from the bulk liquid, with only a few species being more abundant or uniquely present in either the foam or mixed liquor fraction. Previous studies reported in the technical literature have reached similar conclusions using other molecular techniques such as fluorescence in-situ hybridization (FISH). For example, in a survey of 40 different wastewater treatment plants in Germany experiencing intermittent or stable foaming,

Muller *et al.* (2005) reported that nocardioform actinomycetes and *Actinobacter* spp. accumulated in high numbers in the foam compared to the mixed liquor fraction. On the other hand, organisms like *M. parvicella* and type 0092, that were dominant in the activated sludge, were enriched in the foam layer in only 50% and 8% of the plants, respectively.

A more quantitative assessment of the fingerprints of the two bacterial communities is given by the richness, evenness, and Shannon diversity indices. As presented in Table 7.3, the RISA fingerprints from the anoxic foam exhibited a narrower diversity than those from the mixed liquor portion. RISA Shannon diversity indices were statistically different between fingerprints from the two sludge fractions at a 90% confidence level ($p = 0.058$). Again, this is in conformity with the expectation that the foam fraction enriches some particular populations, therefore reducing the overall microbial diversity compared to the underlying mixed liquor.

The change of the foam bacterial community over time was evaluated by comparing the similarity of a given foam sample with the first one collected at time zero. Figure 7.7 illustrates this analysis for the first eight samples, before the membrane process was re-seeded with new sludge. During the first 80 days, the foam fraction exhibited a significant variation in the microbial community composition. Interestingly, this was followed by similar dynamics in the corresponding population harbored in the mixed liquor fraction, suggesting that external environmental conditions caused the observed dynamics of the two communities. On the other hand, in the second half of the investigation, the microbial population forming the foam layer remained very consistent, whereas the corresponding community in the underlying mixed liquor experienced continuous fluctuation. As documented in Figure 7.6, the relatively stable community in the foam was explained by the presence of permanent abundant species that were described earlier as distinctive microorganisms of the anoxic floating material.

7.3.3 Foam production

Daily anoxic foam harvesting was explored as a strategy for process SRT control and, at the same time, for foam containment. Therefore, it was critical to investigate whether the production of floating material was persistent when it was continuously removed from the system and, if this was the case, to estimate the mass generated at steady state.

Before the first foam harvesting experiment was conducted, the MEBPR system was operated at an SRT of 20 days and an HRT of 7 hours, with the foam being re-suspended every day in the anoxic compartment. At the onset of the harvesting experiment, all the floating material was manually collected from the anoxic surface and permanently removed. The evolution of the dry mass of foam production from the first three-week-long harvesting experiment is presented in Figure 7.8. It can be noted that, after a slight initial increase, the daily foam production suddenly decreased and then stabilized at a new equilibrium value of about 0.4 kg/day. This type of response was also confirmed in the other three experiments performed under different operating conditions (data not shown). The initial phase of the response suggests that the potential of foam formation remained unchanged for a certain interval of time; this was likely due to the large quantity of foam-forming organisms that had accumulated in the bioreactor over time. Once the accumulated biomass responsible for foaming was severely depleted, foam production reached a new steady state level which represented the actual amount of foam formation from the process on a daily basis. The present experiment demonstrated that continuous foam removal from the system is successful in minimizing the foam production and its related nuisance. In a survey of bulking and foaming in full scale wastewater treatment plants, Madoni *et al.* (2000) reported that mechanical removal of foam was successful in 63% of the plants in reducing problems associated with foam production.

For the experiment reported in Figure 7.8, it was attempted to control the process SRT only through foam wasting to see what minimum SRT could be maintained without regular mixed

liquor withdrawal. The daily production of 0.4 kg of dry weight foam resulted in an SRT of 33 days, which can be considered to be within the range of values typically applied to membrane bioreactors.

To investigate the effect of suspended solids concentration in the bioreactor on the steady state foam production, four experiments were performed by varying the system SRT and HRT, while keeping all other conditions unchanged (Table 7.2). Figure 7.9a illustrates the equilibrium dry matter foam production as a function of the solids-concentration factor (i.e. SRT/HRT). As can be observed, the foam being generated at the anoxic zone surface greatly depended on the operational conditions of the process. Higher values of the SRT/HRT ratio, indicating higher TSS in the treatment system, resulted in more foam being produced, although following an asymptotical function. Interestingly, the suspended solids content of the foam did not vary with the SRT/HRT ratio. As shown in Figure 7.9b, the anoxic foam removed on a daily basis exhibited a consistent solids content of about 4%, with the exception of one experiment in which the solids content was measured at nearly 3%.

According to the present study, the practice of removing foam on a regular basis helped to reduce the propensity of foam formation significantly. At the same time, the harvesting of concentrated foam could be applied as a strategy for system SRT control, which contributed to a substantial reduction in the overall sludge wasting volume. However, the quantity of foam formation decreased at lower SRT/HRT ratios. In the longest foam harvesting experiment, conducted at an SRT of 20 days and an HRT of 10 hours, the foam wasting represented only 15% of the overall required sludge wasting mass. On the other hand, the potential for long SRT and short HRT operating conditions (i.e. high SRT/HRT ratio) feasible in membrane processes opens interesting possibilities for the implementation of foam wasting as the main strategy for SRT control.

7.4 Conclusions

In contrast to the conventional EBPR process, the parallel membrane system was found to consistently accumulate large quantities of foam on the surface of the anoxic zone, accounting for about 8% of the overall sludge inventory. Based on this observation, the foam fraction was characterized and compared with the underlying activated sludge, with the main findings reported in the following points.

- The flotation of particles from the bulk liquid to the anoxic surface appeared to be an homogenous phenomenon, resulting in a similar content of N, P, organic matter, and nitrifying organisms in the mixed liquor and the foam. However, the activity of the PAOs in the foam exhibited a significant reduction compared to the activated sludge, likely caused by the starving conditions prevailing in the floating material.
- The calculated similarity of bacterial community composition of the two fractions was about 80%, with two populations being highly enriched in the foam and one population more abundant in the underlying activated sludge.
- The production of foam was demonstrated to be a dependable phenomenon even if foam was continuously removed from the system. The quantity of foam generated at steady state was found to be a function of the suspended solids concentration in the bioreactor. The practice of foam harvesting could be an interesting option for sludge age control in MBR processes operated at long SRTs and short HRTs.

Table 7.1 Day and number of batch tests and RISA samples, with corresponding plant operating conditions.

Day	Batch test number		SRT[+] (day)	Day	RISA sample	SRT; HRT (day; hour)
	Nitrification[*]	P-release[*]				
139	1		12	1	1	
201	2		12	15	2	12; 10
277	3a			36	3	
	3b			49	4	
286		1a		80	5	
		1b		116	6	
298	4a		a = 20 b = 12	156	7	12; 7
	4b			170	8	
308	5a	2a		203	9	
	5b	2b		224	10	
				244	11	20; 7

[*]a = membrane process Side-A; b = membrane process Side-B

[+]The process HRT during the period of batch tests was set at 7 hours

Table 7.2 **Experimental runs of continuous anoxic foam harvesting with corresponding operating conditions.**

Run	Duration (day)	HRT (hour)	SRT (day)	SRT/HRT (day/day)
I	23	7	33	113
II	25	15	30	48
III	45	10	25	60
IV	90	10	20	48

Table 7.3 **Richness, evenness, Shannon diversity indices for the RISA fingerprinting data. The Pearson's correlation coefficient between sludge and foam indicates the similarity index.**

	Richness	Evenness	Shannon	Pearson correlation
Sludge	16	0.973	2.67	79.92
Foam	14	0.967	2.52	

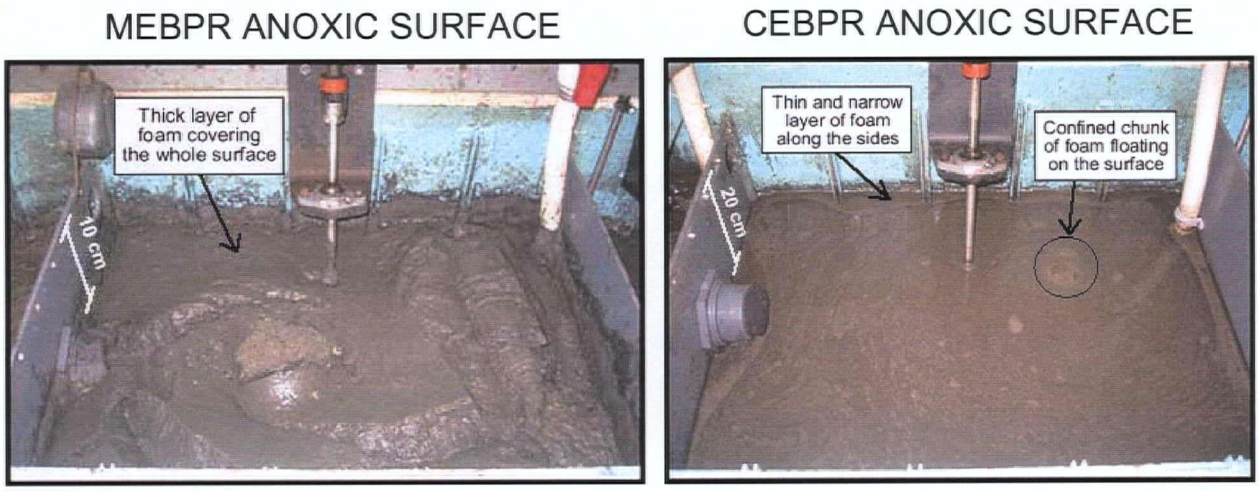

Figure 7.1 **Picture of the membrane (left) and conventional (right) anoxic surface representing a typical situation of foaming in the two systems operated in parallel under identical conditions.**

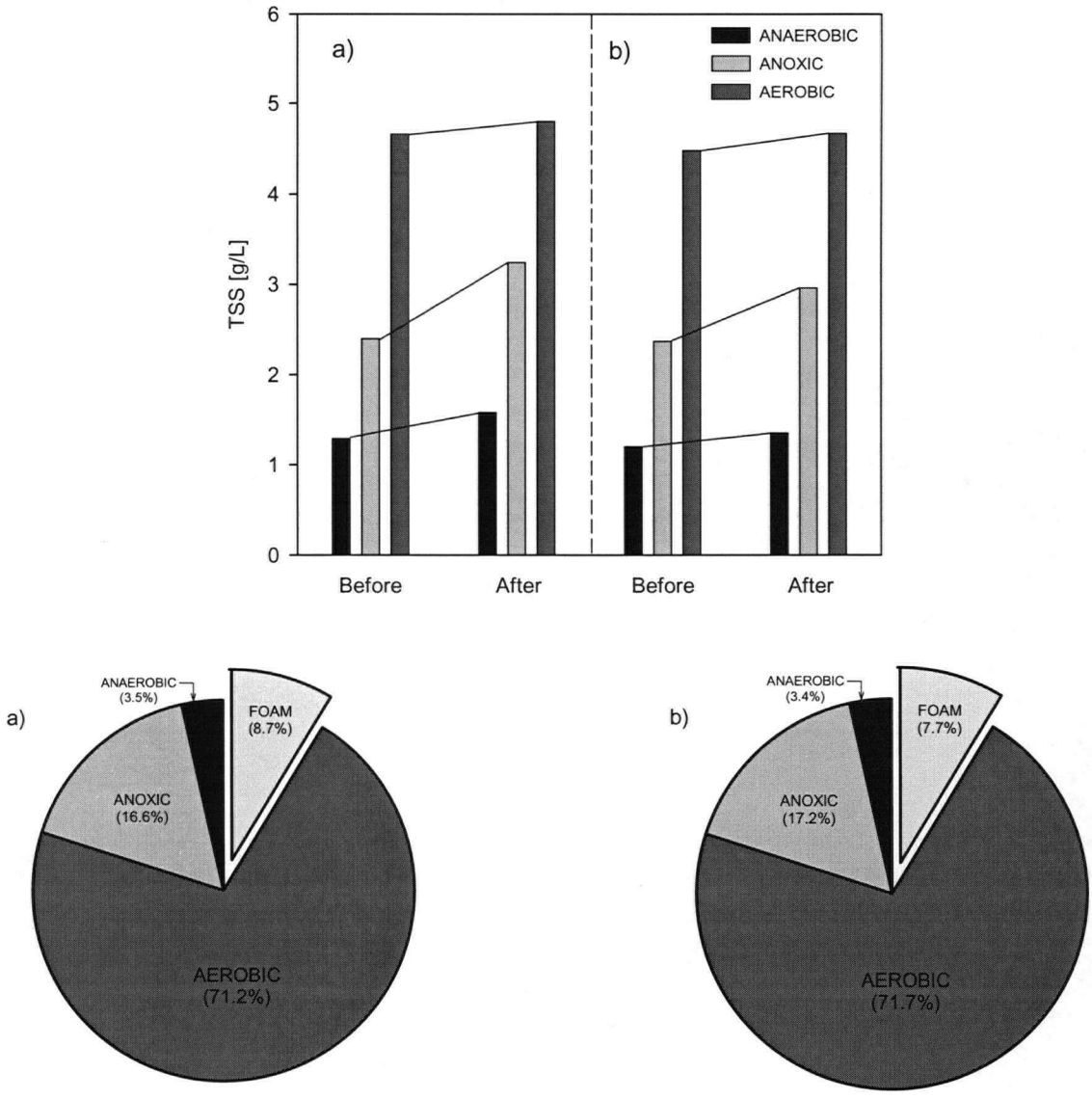

Figure 7.2 **Top: two measurements at different times of TSS in the three MEBPR reactor zone, before and after the mixing of the foam layer. Bottom: sludge inventory of the system, including the foam compartment. a) day 140 and b) day 150.**

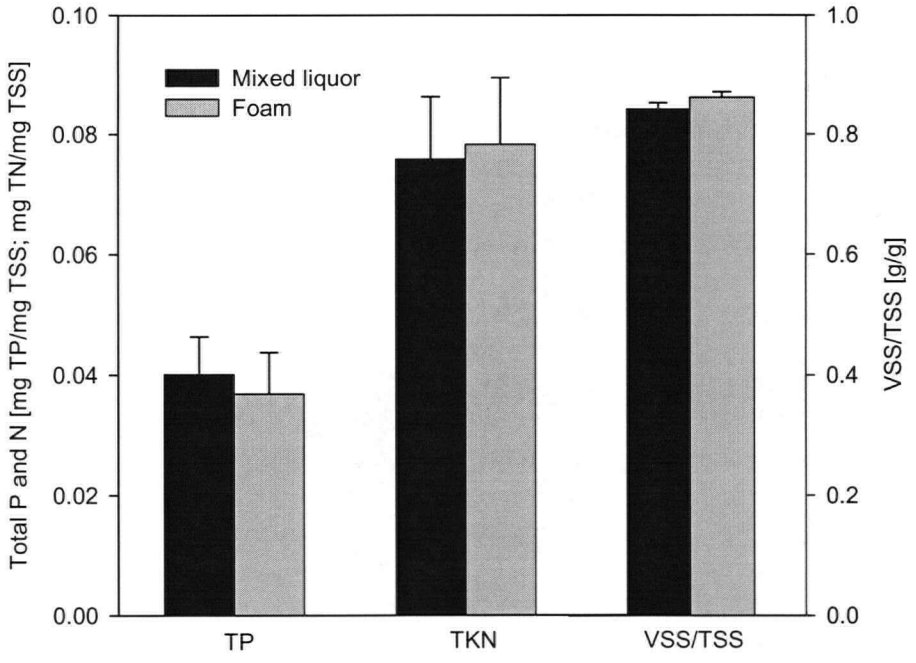

Figure 7.3 Comparison of specific nitrogen (TKN) and phosphorus (P) content, and VSS/TSS ratio of foam and mixed liquor.

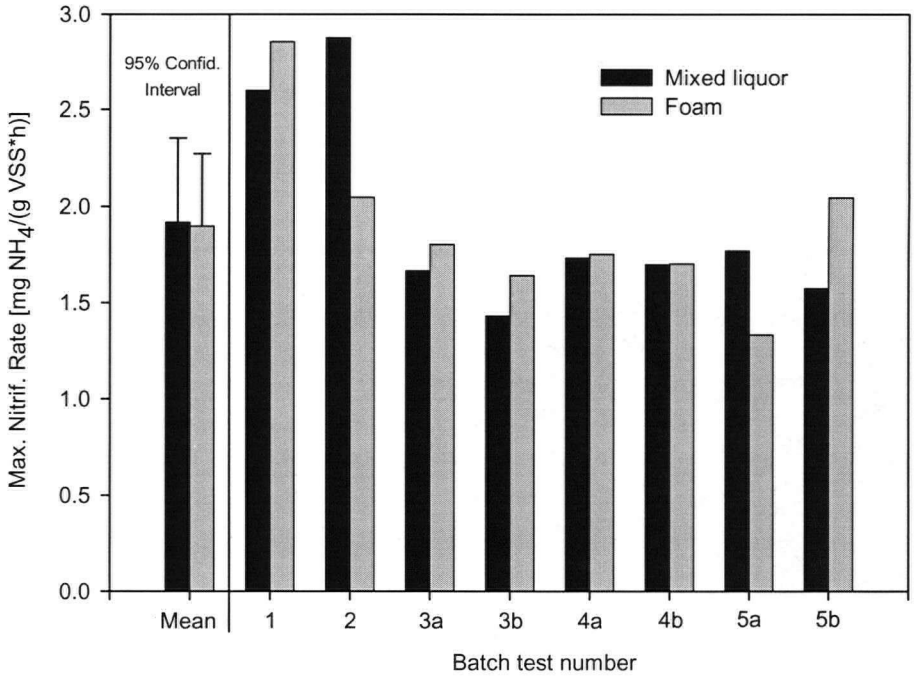

Figure 7.4 **Comparison of maximum specific nitrification rates between foam and aerobic mixed liquor in eight batch tests. Average value with 95% confidence interval is reported in the first column.**

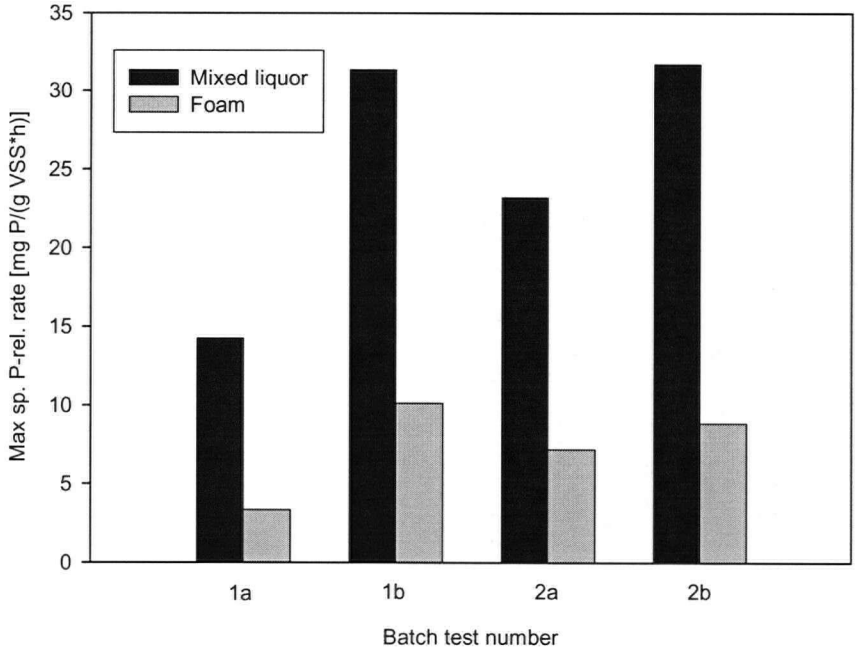

Figure 7.5 **Comparison of the maximum specific P release rate between the foam and the aerobic mixed liquor.**

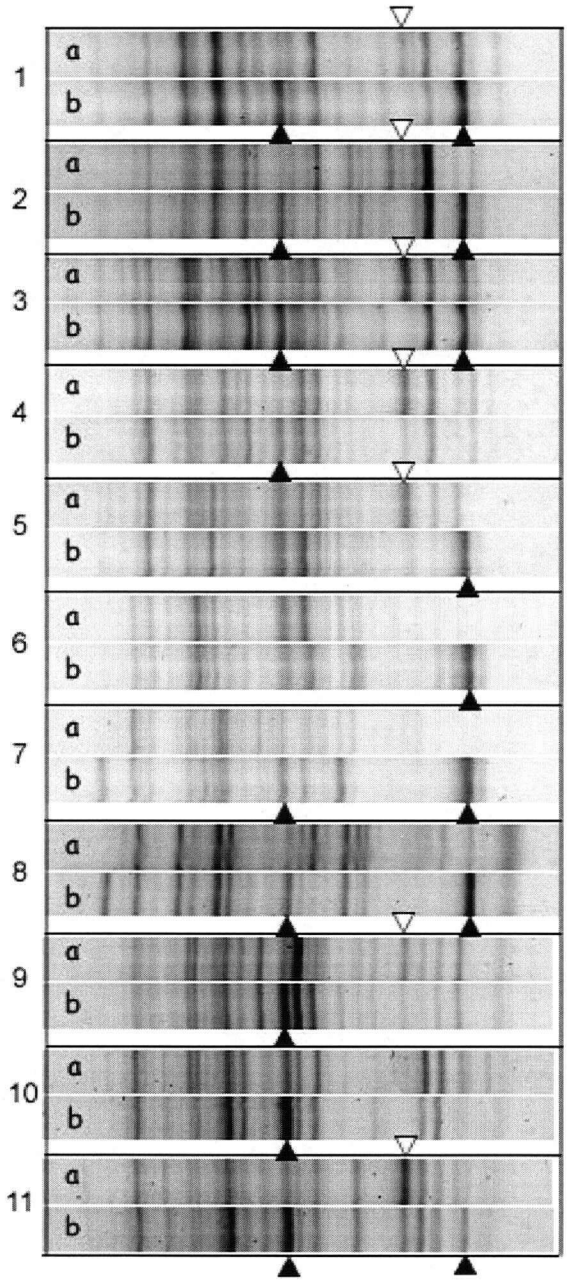

Figure 7.6 **Comparison of bacterial community composition in the mixed liquor (a) and foam (b) according to RISA technique. Two bands appeared consistently darker in the foam (▲) and one in the underlying mixed liquor (▽).**

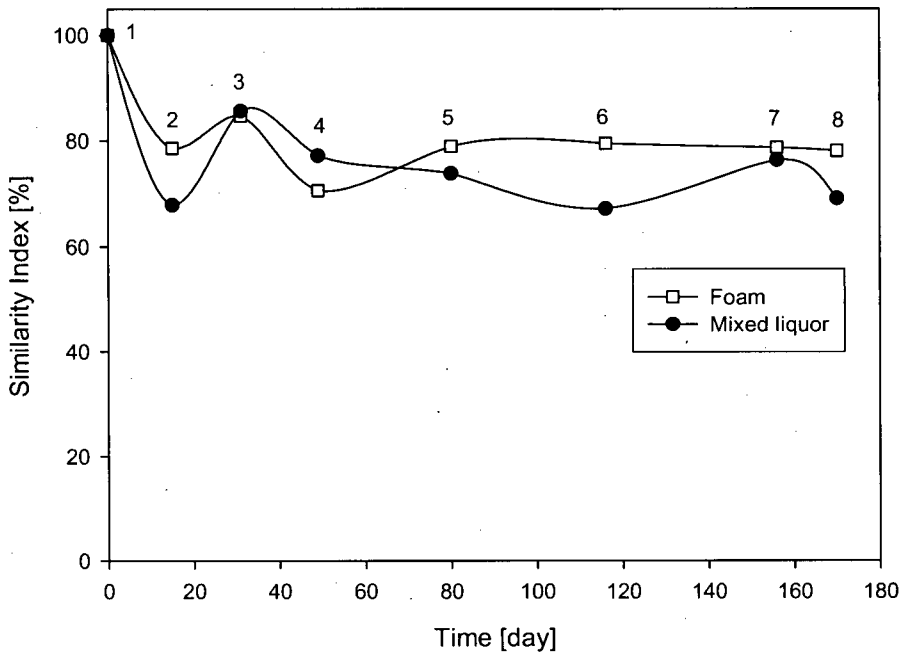

Figure 7.7 **Changing of the foam and mixed liquor bacterial community composition based on the Pearson's correlation coefficient before the re-seeding of the process.**

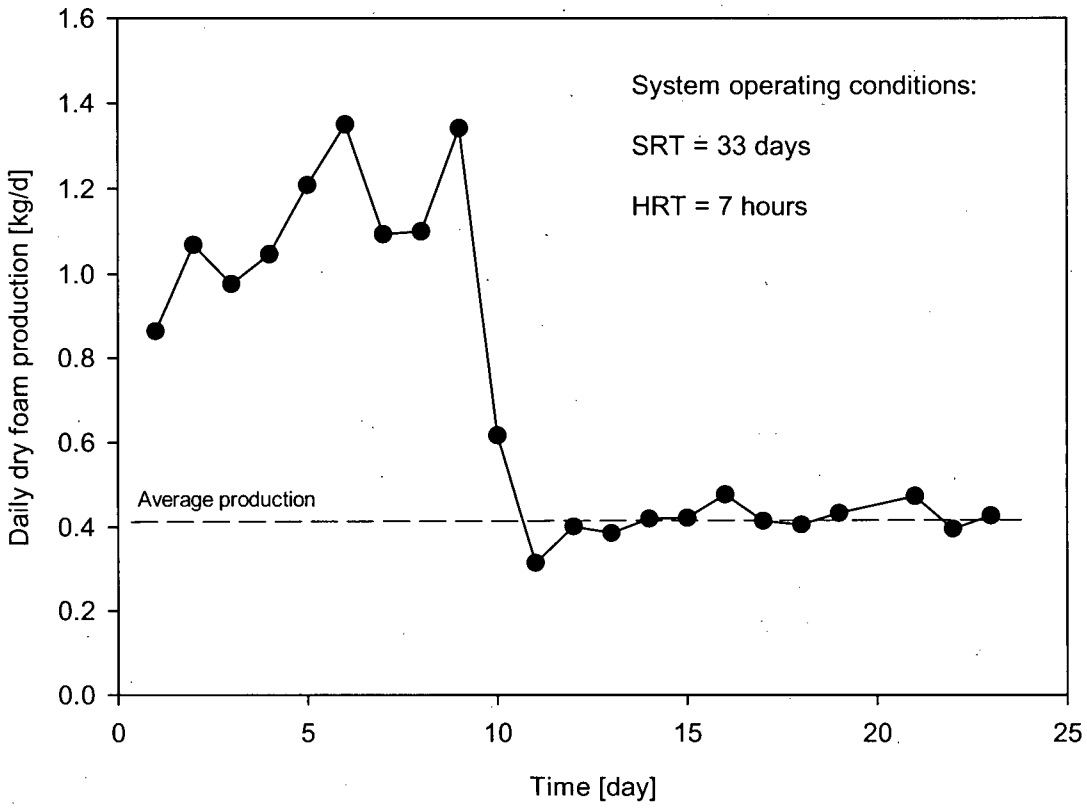

Figure 7.8 **Evolution of foam production as a result of daily removal of foam from the system.**

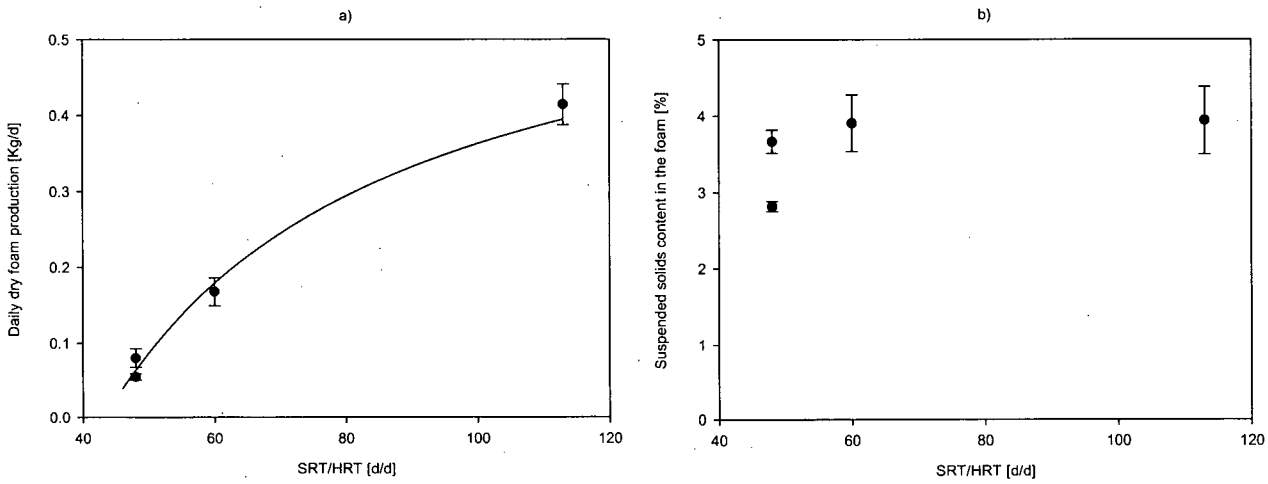

Figure 7.9 **(a) Steady-state production and (b) suspended solids percentage of foam as a function of the total solids-concentration factor (SRT/HRT).**

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Chapter 8 General conclusions and direction for future research

8.1 Introduction

In light of an increasing demand to expand the capacity of existing conventional biological nutrient removal (BNR) plants, a two-year-long study was conducted to explore the potential of membrane processes to perform BNR satisfactorily and in a cost-efficient manner.

When a wastewater treatment plant is designed as a membrane bioreactor, two main and radical differences occur: (1) the solids-liquid separation is performed through membrane filtration as opposed to gravity separation, and (2) the process is operated at relatively high rate conditions (i.e. short HRTs) and at a low food-to-microorganism ratio (i.e. long SRTs). The numerous studies reported in the technical literature on membrane bioreactors typically focused on point 2), that is the investigation of the bioreactor performance at operating conditions which are generally difficult to achieve in conventional systems. On the other hand, there is still a very limited understanding of the impact of membrane solids-liquid separation, per se, on critical aspects such as process performance, removal kinetics, and microbial community composition. This represents a clear knowledge gap in the field of wastewater engineering. In addition, the application of membranes in BNR plants is particularly of interest for effluent polishing, which results in a total phosphorus concentration similar to the orthophosphate concentration. Therefore, it is likely that membrane-based BNR processes are to be operated at conditions similar to those applied in conventional BNR plants, making the understanding of membrane impact even more critical.

The potential of membrane processes to function at high rate conditions with significantly reduced sludge production, opens new avenues for BNR facility designers to consider a set of previously unexplored operating conditions. According to the literature on conventional BNR

plants, it seems that long SRTs have a negative impact on the process performance, whereas the effect of short HRTs on process stability is basically unknown. Therefore, a thorough study on the impact of the HRT and SRT on the BNR process performance and stability at progressively challenging conditions was warranted.

To address the research question on the role of a different solids-liquid separator in biological nitrogen and phosphorus removal, a pilot-scale membrane process was run in parallel to a conventional process under comparable operating conditions and with the same primary effluent feed. Subsequently, the conventional train was retrofitted to be a second MEBPR process, such that different combinations of HRT and SRT could be simultaneously explored. This permitted the response of the BNR process to be determined when operated under previously unexplored high rate conditions. It was believed that such a sequential study could permit a thorough evaluation of the coupled membrane EBPR process, leading to a solid and sustainable design.

8.2 Overall conclusions

8.2.1 Impact of a membrane solids-liquid separator

From the comparative study of a membrane and conventional process, the following conclusions were reached on the impact of membrane filtration on the biological nitrogen and phosphorus removal.

- The utilization of membranes transformed the environmental conditions of the solids-liquid separator from anoxic to aerobic, and it eliminated the sludge blanket normally present in a secondary clarifier. This resulted in an overall reduced denitrification activity and a reduced observed sludge yield in the membrane process, due to the larger mass held of aerobic sludge in the system. As a consequence, the removal of phosphorus suffered

under critical conditions when the influent wastewater was limited in volatile fatty acids (VFAs) concentrations (Chapter 2 and 3).

- No intrinsic differences were found in the EBPR mechanism as documented by the comparable kinetic values for P release and uptake, and by the stoichiometric coefficients. It follows that the mechanistic EBPR theory developed for the conventional processes can be confidently applied to membrane processes (Chapter 3).
- Although the nitrification was stable and complete at the pilot plant under pseudo-steady state conditions, the maximum specific nitrification activity in the membrane sludge was significantly lower than that of the conventional sludge. It was hypothesized that lower activity might have been the result of reduced numbers of nitrifying organisms, due to more extensive decay originating from high shear conditions (Chapter 4).
- The use of membrane solids-liquid separation resulted in a significantly different and less diverse bacterial community composition. The latter may have contributed to the process instability during periods of carbon-limited conditions (Chapter 5).
- The separation of biomass from the treated water through membrane filtration triggered the formation of a significant amount of foam on the surface of the anoxic zone (Chapter 2). The composition of the foam layer was found to be similar to that of the underlying mixed liquor, suggesting that foam formation was governed by a homogenous transport of particles to the liquid surface (Chapter 7).

The above results clearly demonstrated that the presence of membrane filtration is more that a mere replacement of the secondary clarifier. It can be concluded that its impact on the biological nutrient removal process is significant and should be considered for a sound and reliable design.

8.2.2 Impact of HRT and SRT on the MEBPR process

From the long-term development study of the MEBPR process at different HRT and SRT set-points, the following conclusions were drawn.

- With a favorable ratio of VFA to P in the influent, the MEBPR process could maintain satisfactory nitrification and phosphorus removal performance under high rate conditions, with the lowest HRT tested being at five hours (Chapter 6). Operating at these high rate conditions is considered to be a significant technological advance beyond conventional BNR technology.
- When the SRT was extended from 12 to 20 days, the MEBPR process could continue to generate an effluent quality with low P concentrations. However, this resulted in increased VFA utilization per unit mass of P removed, with the SRT having a significantly larger impact than the HRT. The reduced observed sludge yield, the large mass of P released in the anaerobic zone, and the significant contribution of PAO maintenance were thought to be critical factors in the variation of the VFA/P ratio (Chapter 6).
- Foam production was consistent under all the operating conditions tested, with an average concentration of 4% (w/v) suspended solids in the foam. The mechanical removal of foam from the anoxic zone was implemented as part of the SRT system control, and this minimized the foam formation and the volume of mixed liquor wasted (Chapter 7).

8.3 Engineering significance

The engineering problem leading to the present research study revolved around the question of whether existing conventional BNR plants could be upgraded with membrane technology to accommodate more flow capacity. The results obtained from the comparative and

assessment study suggested that these plants can be retrofitted with membrane solids-liquid separation, while maintaining satisfactory effluent quality at high influent flow rates. It should be noted that the utilization of membranes also eliminates the need for tertiary treatment normally included in BNR plants to polish the final effluent. However, the resulting partial loss of denitrification and the reduced sludge yield may affect the biological P removal in those treatment plants treating carbon-limited wastewater.

The application of long SRTs in the operation of a bioreactor has the peculiar advantage of reducing the observed sludge yield and, therefore, the cost associated with sludge handling facilities. Unfortunately, it was demonstrated that increasing the SRT in an EBPR process results in a significant consumption of carbon per unit mass of P removed from the influent wastewater. Typically, municipal wastewaters do not contain sufficient carbon and, for a successful implementation of the EBPR mechanism, the addition of extra VFAs through a pre-fermented sludge stream is often necessary. Even for those isolated cases of carbon-rich wastewaters, the operation of a EBPR process at long SRTs, entails the accumulation of a large mass of P in the bioreactor; this may result in high and prolonged peaks effluent in P concentration in the event of process failure. The information generated from the present study suggests that the operation of an MEBPR process at such at long SRTs is not recommended. So, does it mean that we will never see a low sludge yield MEBPR processes coming into practice? The recent development of sustainable P mouse traps has the potential to overcome some of these technical hurdles.

Phosphorus, a conservative element, is typically removed from the wastewater with the wasted sludge, which is then often disposed of in lands used for agricultural purposes. The development of fluidized bed reactors for the formation of struvite (i.e. magnesium ammonium phosphate) has been shown to be a cost-effective method to recover P from the anaerobic supernatant of municipal wastewater treatment plants (Britton *et al.*, 2005). The implementation of such technology in the MEBPR bioreactor has the potential to change substantially the layout

and the operation of existing BNR plants. It is here proposed an innovative treatment scheme, whereby P-recovery merges with the MEBPR process to exploit the advantages of both technologies. As illustrated in Figure 8.1, the new combined process features a main membrane bioreactor, operated in the EBPR mode, and a side-stream P-recovery unit for the formation and extraction of struvite. A stream of mixed liquor is continuously pumped from the bioreactor to an anaerobic unit where VFAs are added (either from the influent or a pre-fermented stream) and P is released. The mixture is then separated in a clarifier and the settled biomass is returned to the main process or wasted as a part of the system SRT control. The P-rich supernatant is fed to a P-recovery column where struvite is formed and harvested. The final effluent is recycled back to the main process for further treatment. The following is the list of significant breakthroughs brought about by this innovative coupling.

- *High rate process*. The presence of the membrane solids-liquid separator guarantees the operation of the EBPR process at low HRTs typical of high rate treatment systems.
- *Long SRT process*. Contrary to the present study, the presence of a P-recovery unit allows for satisfactory P removal from the wastewater at long SRTs, while keeping the P content of the sludge low. This should bring the ratio of P removed per unit mass of VFAs utilized, back to those values typically observed for conventional BNR plants
- *Minimal P wasted*. The significant reduction of sludge yield at long SRTs permits the minimal uptake of P for biomass growth, therefore making it available for poly-P storage and subsequent recovery. In addition, the practice of wasting the mixed liquor from the underflow of the side clarifier (poly-P pool emptied) for the SRT control contributes further to minimize the waste of P from the system.
- *Low P effluent concentrations*. Assuming a well functioning EBPR sludge, the membrane process generates a final effluent of superior quality, without the need for costly tertiary treatments.

- *Reduced foam production.* The implementation of regular removal of foam from the anoxic surface has the potential to minimize the overall foam production, therefore reducing the nuisance associated with it. In addition, when the MEBPR process functions at long SRTs and short HRTs (i.e. high suspended solids in the bioreactor), the mass of solids-rich foam generated on a daily basis can account for the total required sludge wasting.

8.4 Resaerch needs

As is the nature of research, the present study generated new exciting research questions that could not be addressed within the allocated time or were beyond the scope of this research work. Below is a list of working hypotheses and research needs that are likely to improve our knowledge of membrane processes coupled to biological nitrogen and phosphorus removal.

- *Nitrification process.* Based on the reduced observed maximum specific nitrification activity, it was hypothesized that the high shear conditions created by vigorous coarse bubble aeration increased the nitrifiers decay rate. It is therefore essential to measure the decay rate associated with nitrifying organisms and compared with that of the conventional sludge (Dold *et al.*, 2004). In addition, the characterization and quantification of the nitrifying community composition with molecular techniques is likely to improve our understanding on the observed differences (Boon *et al.*, 2002; Rowan *et al.*, 2003; Schramm *et al.*, 1998).
- *Bands sequencing.* During the comparative study of the bacterial communities in the two systems, several bands were recognized as distinct in either the membrane or the conventional process. The identification of the microorganisms associated with those bands would be useful to better understand the impact of membrane filtration on the process. This new knowledge can be gained through band excision, coupled with direct

sequencing or cloning of the genetic material associated with these bands (Feris *et al.*, 2004).

- *Dynamic loading pattern.* The present research study was conducted at pseudo-steady state conditions dictated by constant influent flow rates with variable wastewater characteristics. For a complete evaluation of the MEBPR process, the response of the system under dynamic conditions typical of a full-scale WWTPs is of essence. It is expected that the reduced maximum specific nitrification activity could result in an ammonium breakthrough, when a step increase in the ammonium influent load is experienced.
- *Optimization of the process configuration.* In the present research work, the MEBPR process was studied in a simple UCT type configuration in order to achieve biological nutrient removal in the simplest possible manner. However, the consequence of such a design was that the biomass distribution was skewed toward aerobic conditions. The process changes that come with the utilization of a membrane solids-liquid separation require a more appropriate configuration, aiming toward a more even distribution of the biomass and to an improved denitrification activity (Oldham and Rabinowitz, 2001)
- *P removal and recovery.* The coupling of the MEBPR process with the P-recovery unit (Figure 1) needs to be investigated to determine the feasibility of this innovative treatment scheme and its impact on the process stability. Important questions at the design stage include: (1) the best zone in the bioreactor from which to draw the mixed liquor toward the P-release reactor and the subsequent P-recovery column, (2) percentage of flow, relative to the influent, to send to the P-recovery unit, and (3) sizing and operating conditions of the P-release unit.

It is expected that the introduction of membrane technology and P-recovery in the BNR processes will change dramatically by optimizing the way in which these systems are designed and operated.

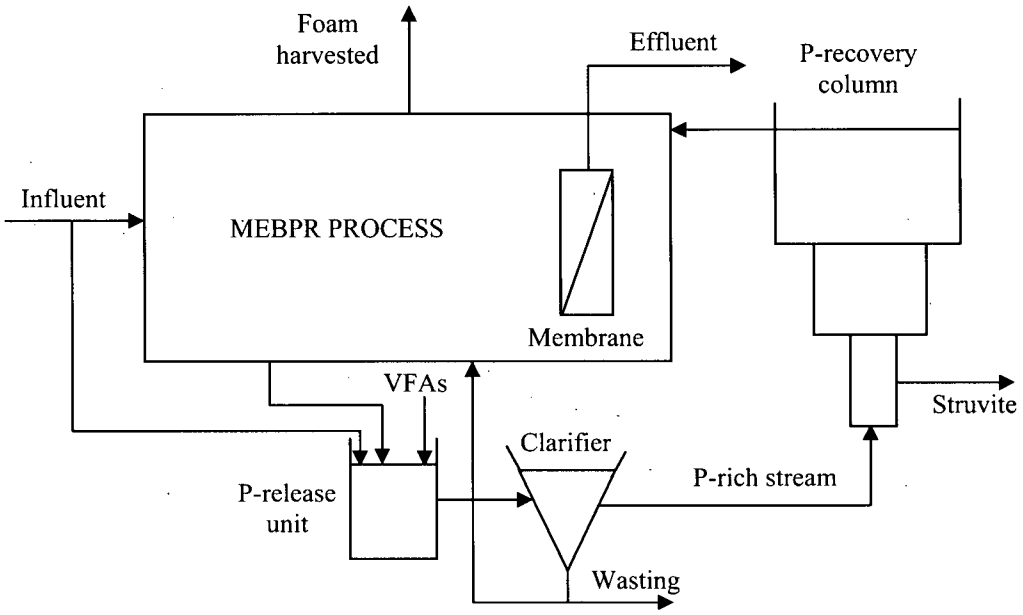

Figure 8.1 The coupled MEBPR process with the on-line P-recovery unit.

8.5 References

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