Role of External Carbon and Metal Salt Dosing in Membrane Bioreactor System to Achieve Limits of Technology Nutrient Removal from Municipal Wastewater

by

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Abstract

Membrane bioreactor (MBR) technology in conjunction with conventional biological nutrient removal has been demonstrated to be successful in recent years. However, the limits of technology (LoT) effluent goal, ≤ 3 mg TN/L (total nitrogen) and ≤ 0.1 mg TP/L (total phosphorus), could potentially push a system to the limits of its capability. The broad objective of the long-term PhD study was to investigate role of external dosing of alum in a membrane biological nutrient removal (MBNR) system targeting LoT effluent nutrient levels. Two parallel MBNR systems, modified Bardenpho configuration, were operated under similar process conditions with metal salt addition being the only difference.

The continuous flow MBNR system performance data signified the importance of external methanol and alum dosing in accomplishing the LoT nutrient removal goal. The stoichiometric methanol ratio, i.e. mg methanol required / mg NO_3-N removed, was calculated to be 6.1 in reducing average permeate NO_3-N concentration to 1.4 mg/L. Similarly, an average molar Al/TP ratio of 1.9 was required to reduce PO_4-P concentration to 0.07 mg/L in the permeate.

Chemical phosphorus removal did not have any influence on COD removal, nitrification (except for a brief period) and denitrification. The relationship between chemical P removal and enhanced biological phosphorus removal (EBPR) was dynamic and was dependent on alum dosage concentration. At high dosage levels (i.e. 80 mg/L), alum supplementation competed with and finally, inhibited EBPR until the MBNR system was converted to a chemical P removal system.

Activated sludge modeling was undertaken to analyze its suitability in predicting the performance of an MBNR system targeting LoT goals. The model was successful in predicting nitrogen removal, while parameter calibration was required for fitting of the

measured suspended solids and EBPR data. Moreover, the model could not predict the relationship between the simultaneous biological and chemical P removal accurately.

A direct batch DON measurement method, batch anion exchange resin adsorption followed by persulfate digestion, was developed and validated successfully. Using the method, the DON contribution to permeate total nitrogen was observed to vary from 7 percent to 96 percent in the parallel MBNR systems, when permeate TN concentrations were less than 3 mg/L.

Preface

This statement confirms that the author of this thesis is the primary person responsible for the research contained. All experimental designs and procedures were conceived by the author with input from the supervisory committee, namely Dr. Eric Hall, Dr. Donald Mavinic, Dr. Barry Rabinowitz, and Dr. Victor Lo. The specific names of those who assisted in conducting several of the experiments have been gratefully recognized in the Acknowledgments section of this thesis.

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List of Abbreviations

ADP	Adenosine Diphosphate
AOB	Ammonia-Oxidizing Bacteria
ASDM	Activated Sludge/Anaerobic Digestion Model
ASM	Activated Sludge Model
ATP	Adenosine Triphosphate
BNR	Biological Nutrient Removal
BOD	Biochemical Oxygen Demand
bsCOD	Biodegradable Soluble Chemical Oxygen Demand
CAS	Conventional Activated Sludge
CEBPR	Conventional Enhanced Biological Phosphorus Removal
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
DGGE	Denaturing Gradient Gel Electrophoresis
DO	Dissolved Oxygen
DIN	Dissolved Inorganic Nitrogen
DON	Dissolved Organic Nitrogen
EBPR	Enhanced Biological Phosphorus Removal
EDTA	Ethylenediaminetetraacetic Acid
EMP	Embden-Meyerhoff-Parnas
EPS	Extracellular Polymeric Substances
FISH	Fluorescence *In Situ* Hybridization
GAO	Glycogen-Accumulating Organism
HAO	Hydrous Aluminum Oxide
HAP	Hydroxy-Apatite
HDP	Hydroxy-Dicalcium-Phosphate
HFO	Hydrous Ferric Oxide
HRT	Hydraulic Retention Time
ICP	Inductively Coupled Plasma

ISS	Inorganic Suspended Solids
LoT	Limits of Technology
MEBPR	Membrane Enhanced Biological Phosphorus Removal
MBRs	Membrane Bioreactors
MBNR	Membrane Bioreactor Nutrient Removal
MLE	Modified Luzdack-Ettinger
MLSS	Mixed Liquor Suspended Solids
MW	Molecular Weight
NMP	Nuclear Magnetic Resonance
NOB	Nitrite-Oxidizing Bacteria
OLR	Organic Loading Rate
PAC	Polyaluminum Chloride
PAO	Phosphate-Accumulating Organism
PHA	Polyhydroxyalkanoate
PHB	Poly-β-Hydroxybutyrate
PHV	Polyhydroxyvalarate
QA	Quality Assurance
QC	Quality Control
RAS	Return Activated Sludge
rDON	Recalcitrant Dissolved Organic Nitrogen
SEM	Scanning Electron Microscopy
SERC	Staging Environmental Research Centre
SMP	Soluble Microbial Product
SPE	Solid-Phase Extraction
SRT	Solids Retention Time
TDN	Total Dissolved Nitrogen
TEM	Scanning Electron Microscopy
TKN	Total Kjedhal Nitrogen
TN	Total Nitrogen
TP	Total Phosphorus
TSS	Total Suspended Solids

UCT	University of Cape Town
VIP	Virginia Initiative Plant
VFA	Volatile Fatty Acid
VSS	Volatile Suspended Solids
WAS	Waste Activated Sludge
WRF	Water Reclamation Facility
WRP	Water Reclamation Plant
WWTP	Wastewater Treatment Plant

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I express my special gratitude to Fred Koch for his suggestions on biological processes. One wise man once told me that if you want to learn everything about phosphorus removal, take Fred for a drink. I definitely agree. Moreover, he made me realize that as researchers we have the big responsibility of creating sustainable technologies. I am also thankful to Paula Parkinson and Timothy Ma for their help. I cannot emphasize enough about how much they helped me in Environmental Engineering Laboratory.

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Dedication

Baba & Maa,

For their unconditional support, motivation & belief in me.

1 Introduction

1.1 Engineering Problem-Nutrient Challenge For Water Systems

Nitrogen and phosphorus are essential nutrients for any biological growth, including algae and aquatic plants in rivers, lakes, and shallow embayed areas of the marine environment. When discharged to surface water, these nutrients may promote eutrophication which (1) adversely affects fish growth, (2) causes undesirable tastes and odours and (3) reduces the value of water for domestic, industrial, agricultural and recreational use (Oldham and Rabinowitz, 2001). Recently, a study concluded that freshwater eutrophication-related economic loss was approximately $2.2 billion per year in the United States (Dodds *et al.*, 2009). Therefore, eutrophication control by limiting discharges of nitrogen and phosphorus to surface water is an important and challenging task for environmental engineers and scientists. The focus of this PhD research work was on wastewater treatment technology designed to remove nitrogen and phosphorus and the use of membranes for separation (filtration) of microbial solids from treated water.

1.2 Nutrient Removal in Wastewater Treatment Systems

1.2.1 Nitrogen

Municipal and industrial wastewaters contain significant amounts of nitrogen and phosphorus, and removal of these nutrients has become one of the major goals for wastewater treatment processes. Biological, chemical and physical treatment methods can be used to accomplish nitrogen removal from wastewater. Nitrogen removal can be achieved by processes such as ammonia stripping, ion exchange and membrane separation. Nonetheless, nitrogen removal by the activated sludge process is the most common method employed by environmental engineers and scientists due to the suitability of the process to most wastewaters and the relatively low cost of the application. In the activated sludge process, nitrogen removal is achieved by two

sequential biochemical reactions - nitrification and denitrification. During nitrification, ammonia is oxidized to nitrite and then to nitrate by two groups of autotrophic bacteria (ammonia-oxidizing bacteria – AOB and nitrite-oxidizing bacteria - NOB) under aerobic conditions. Denitrification, facilitated principally by heterotrophic microorganisms, occurs by the reduction of nitrate to nitrogen gas under anoxic conditions.

1.2.2 Phosphorus

Phosphorus removal by the activated sludge process was first reported in the 1950s. Greenburg *et al*. (1955) reported that the activated sludge process biomass could take up phosphorus in excess of its normal microbial growth requirements. Later, Srinath *et al.* (1959) observed that soluble phosphorus concentration could be reduced to less than 1 mg/L in batch experiments. Levin and Shapiro (1965) were the first researchers to report enhanced biological phosphorus removal (EBPR) during their work at the District of Columbia activated sludge plant. EBPR is defined as the ability of activated sludge microorganisms to remove a greater mass of phosphorus from the wastewater than that required for their basic metabolic purposes (Oldham and Rabinowitz, 2001). In the last two decades, different configurations of suspended growth biological processes have been used to remove phosphorus from wastewater. All of these biological processes include the basic steps of an anaerobic zone followed by an aerobic zone in a single sludge process. This practice is based on the original breakthrough in enhanced biological P removal technology reported by Barnard (1974). Barnard (1974) suggested that anaerobic contact between activated sludge and influent wastewater was required before aerobic degradation in order to accomplish enhanced biological phosphorus removal. The work by Barnard (1974) laid the foundation for the development of the Phoredox and Bardenpho process configurations, which form the basis of most of the EBPR process configurations in use today (Oldham and Rabinowitz, 2001).

Although enhanced biological phosphorus removal has been proven to be highly efficient, it can be sensitive to influent wastewater characteristics (especially the concentration of volatile fatty acids (VFA) in the influent and organic material that can be

fermented to VFA within the anaerobic zone), and subject to fluctuations in performance due to changes in environmental conditions and operation of the system. Therefore, in many cases, physiochemical treatment has been used with biological treatment to meet effluent phosphorus limits. Chemical methods like ion exchange, crystallization and metal salt addition have been used for removal of phosphorus from water and wastewater (Clark and Stephenson, 1998). In fact, the addition of metal salt has been adopted as a common practice in full-scale wastewater treatment plants due to the efficiency of phosphorus removal and the low cost of operation. The main metals used for chemical phosphorus removal are calcium hydroxide, iron (II), iron (III) and aluminum sulphates and chlorides (Clark and Stephenson, 1998). Chemical addition of precipitants can take place at one of three locations in a wastewater treatment process namely: pre-precipitation (before secondary treatment), co-precipitation (during secondary treatment) or post-precipitation (after secondary treatment) (de Haas *et al.*, 2000). Pre-precipitation and post-precipitation methods have very little direct impact on the biological process. However, co-precipitation can have multiple direct effects on the biological process in an activated sludge system. Some of the advantages of co-precipitation, compared to the other two precipitation methods, include: improved sludge settleability, reduced chemical consumption, relatively reduced sludge production and efficient removals of phosphorus (Clark and Stephenson, 1998). In some instances, a combination of the above approaches is employed with multiple points for metal injection for better control and operation of the process.

The effects of chemical addition on activated sludge process performance in terms of carbon oxidation, nitrification and denitrification are dependent on the chemical added, the amount of chemical used and the biological process configuration (Clark and Stephenson, 1998). Co-precipitation might have an adverse impact on the enhanced biological phosphorous removal process. The benefits of an EBPR system may be lost if simultaneous addition of chemical precipitant to achieve low effluent phosphorus concentration significantly inhibits the enhanced biological P removal mechanism (Clark and Stephenson, 1998; de Haas *et al.*, 2000).

1.3 Nutrient Removal with Membrane Bioreactor Technology

In recent years, membrane filtration technology has been successfully implemented in biological wastewater treatment processes. Increasingly stringent effluent discharge standards, scarcity of land in urban areas, and the need to reclaim and reuse water are promoting the use of membrane bioreactors (MBR) as alternatives to conventional wastewater treatment processes (Ahn *et al.*, 1999; Judd, 2006). MBRs offer some key advantages over conventional treatment processes that utilize secondary clarification – a gravity separation process. The complete retention of biomass in an MBR system decouples the solids retention time (SRT) from the hydraulic retention time (HRT), allowing biomass concentrations to increase in the reactor, resulting in a smaller reactor and a higher organic loading rate (OLR) (Adham *et al.*, 2001; Liao *et al.*, 2006). Also, the MBR process not only retains the solids but also some of the macromolecules in the mixed liquor (Monti *et al.*, 2006). The retention of macromolecules may impact the biological process as well as the membrane fouling characteristics of the mixed liquor. This may lead to degradation of slowly biodegradable retained organics, thus, leading to higher quality of effluents. However, it is also possible that the retained organics could influence the microbial population or the kinetics of the process. For example, it has been reported that nitrification kinetics are reduced in MBRs, potentially due to the inhibitory effect of retained organics and soluble microbial products (SMP) present in the MBRs (Ekama, 2009).

Many full scale MBR facilities have been designed and built for nitrification/denitrification and chemical phosphorus removal (Phagoo *et al.*, 2005). More recently, there has been significant interest in coupling the enhanced biological phosphorus removal process to MBR technology to capitalize on the benefits of the two advanced technologies (Barnard, 2006; Fleischer *et al.*, 2005; Monti *et al.*, 2006; Patel *et al.*, 2005; Peeters *et al.*, 2010; Sibag and Kim, 2012; Smith *et al.*, 2013). This combination is unique, because the EBPR process provides excess phosphorus accumulation in the biomass and the MBR process provides excellent solids-liquid separation which ensures that virtually no solids are present in the treated effluent

(Phagoo *et al.*, 2005). Nonetheless, many membrane biological nutrient removal systems (MBNR) employ supplemental metal salt and methanol addition to enhance phosphorus (P) and nitrogen (N) removal respectively (Crawford *et al.*, 2006; Fleischer *et al.*, 2005; Judd, 2006). The application of MBNR is a new development and many aspects of this technology are not very well understood. In addition, many questions with regards to the biological and chemical P removal processes, and the interactions between these two phosphorus removal mechanisms in a single process still remain to be answered, especially in the context of MBNR processes.

2 Background and Literature Review

2.1 Introduction

Membrane filtration technology along with the suspended growth activated sludge process can provide excellent nitrogen and phosphorus removal from wastewaters. However, the selection of an activated sludge process is dependent on influent quality and the effluent nutrient limits placed on a specific treatment plant. Nutrient limits can apply to either effluent nitrogen, or effluent phosphorus, or both, depending on the location and the limiting nutrient associated with the receiving environment. There is increasing pressure to achieve very low levels of nitrogen and phosphorus in effluents (Barnard *et al.*, 2008; Fleischer *et al.*, 2005; Scherrenberg *et al.*, 2008). This objective has been referred to as "limits of technology (LoT)" and is presently defined as effluent concentrations of $\leq$ 3 mg TN/L (total nitrogen) and 0.1 mg TP/L (total phosphorus) (Barnard *et al.*, 2008). The selection of an MBR process configuration is, therefore, crucial for achieving combined low effluent N and P concentrations. Research work has been focusing on extremely high nutrient removal in MBR systems treating municipal wastewater (Barnard *et al.*, 2008; Crawford *et al.*, 2006; Fleischer *et al.*, 2005; Lesjean *et al.*, 2005; Meng *et al.*, 2012; Patel *et al.*, 2005; Phagoo *et al.*, 2005; Sun *et al.*, 2013).

Another very important research area is the application of activated sludge modeling (ASM) to MBNR systems targeting LoT nutrient removal. ASM models have been successfully applied in design and operation of conventional activated sludge systems (CAS) for the last two decades. The general argument is that the basic process models are similar for both systems and the conventional activated sludge process model can be converted to that of an MBR by replacing the secondary clarifier with membrane filtration. Nevertheless, MBR models can have different kinetic and stoichiometric values due to the elevated sludge retention times and high mixed liquor concentrations applied, the accumulation of soluble microbial products (SMP) rejected by the membrane

filtration step, and the high aeration rates used for membrane fouling control (Fenu *et al.*, 2010).

The application of MBR technology for LoT nutrient removal is still an active area of research with many fundamental questions yet to be answered. The objective of this literature review is to summarize and critically evaluate the current state of MBR technology for membrane enhanced biological nutrient removal in wastewater treatment systems.

2.2 Membrane Biological Nutrient Removal Process Configurations

Initial MBR process designs often incorporated the Modified Ludzack-Ettinger (MLE) process for biological nitrogen removal in wastewater treatment plants (Barnard, 2006; Crawford *et al.*, 2006). Depending on the influent chemical oxygen demand (COD) and total nitrogen concentrations, the MLE process has the ability to achieve effluent total nitrogen (TN) concentrations between 5 and 10 mg/L (Tchobanoglous *et al.*, 2003). However, the major limitation of earlier MLE process designs was that the oxygen-rich recycle stream from the membrane tank consumed much of the readily available COD in the influent. As a result, the denitrification potential of the process was significantly reduced (Barnard, 2006; Crawford *et al.*, 2006). To overcome this limitation, in the current membrane-coupled MLE process, the RAS (return activated sludge) is sent to the aerobic zone, where the high dissolved oxygen (DO) is utilized. However, this change in the MBR process configuration results in the addition of an internal biomass recirculation loop, which increases the capital and operating costs of the system. Figure 2.1 shows a schematic diagram of the membrane-coupled Modified Ludzack-Ettinger (MLE) processes.

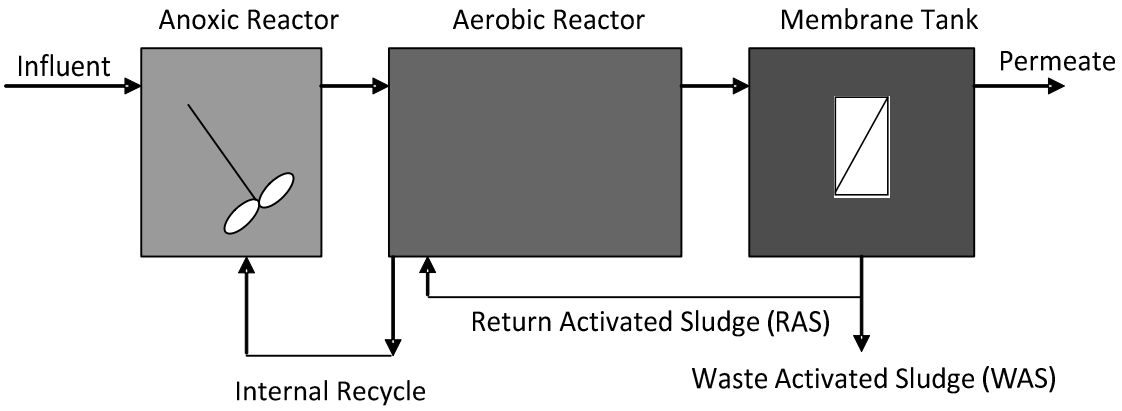


Figure 2.1 Modified Luzdack-Ettinger (MLE) MBNR system

The inclusion of biological phosphorus removal along with nitrogen removal added further complexity to the design of the MBR processes. Basic BNR configurations such as the A^2O, Johannesburg process, five-stage Bardenpho, University of Cape Town (UCT), modified UCT (MUCT) and Virginia Initiative Plant (VIP) processes can be coupled with membranes to achieve low nutrient concentrations in the effluent. The necessity of meeting increasingly stringent N and P effluent discharge permit requirements at wastewater treatment facilities has led to improvements of MBNR process configurations.

The Cauley Creek Water Reclamation Facility (WRF) in Georgia, USA, upgraded its MLE MBR process with chemical phosphorus removal to become an enhanced MBNR facility by employing the modified Johannesburg process (Phagoo *et al.*, 2005). In the conventional Johannesburg configuration, the return activated sludge (RAS) flows to an initial pre-anoxic zone. As a result, nitrate is minimized in the anaerobic reactor and biological phosphorus removal potential is optimized in the following zones. In the modified Johannesburg configuration, the process has been improved by introducing an additional recycle stream between the pre-anoxic zone and the anaerobic zone as shown in Figure 2.2 (Dr. James L. Barnard, pers. comm.). The additional biomass recycle improves denitrification potential by providing the denitrifying bacteria with residual biodegradable organic compounds not consumed by the phosphorus-accumulating

organisms (PAOs) in the anaerobic zone. Figure 2.2 provides a schematic of a modified Johannesburg MBR configuration.

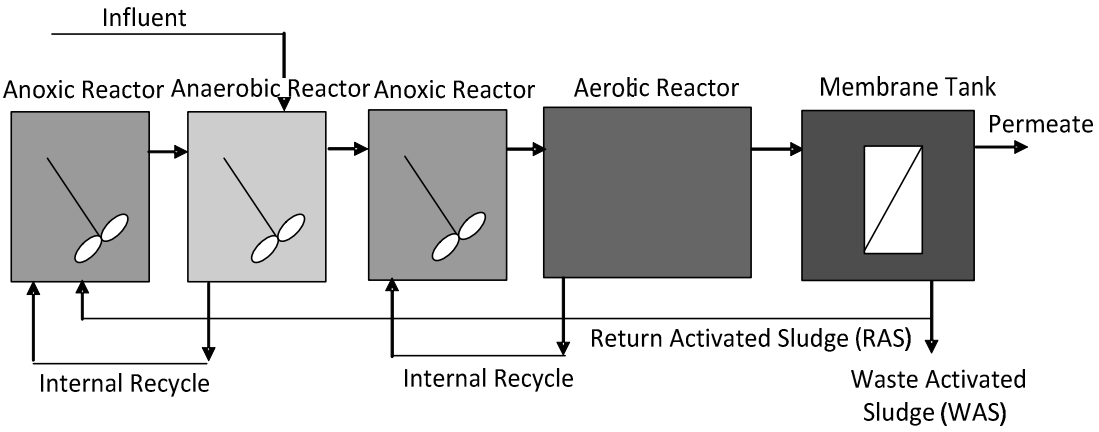


Figure 2.2 Modified Johannesburg MBNR System

Lesjean *et al.* (2005) studied two parallel MBNR configurations (pre-denitrification and post-denitrification without external carbon addition) in pilot scale facilities. The rationale behind using a post-denitrification configuration (Figure 2.3) offered by these authors included (1) higher and more stable nitrogen removal expected due to independence from the actual influent N/COD ratio and (2) lower mixed liquor suspended solids concentration at the membranes. The outcome from their study showed that very high P removal (0.05 mg/L TP in effluent) was feasible using both configurations. However, the wastewater used in the study had high concentrations of calcium and ferric ions (approximately 130 mg Ca/L and 10 mg Fe/L), which might have contributed significantly to the natural precipitation of phosphorus in the biological reactor. The same study also reported improved nitrogen removal (down to 5 mg/L TN in effluent) at high solids retention times (SRTs) in the post-denitrification MBNR configuration. Lesjean *et al.* (2005) postulated that the utilization of internally stored glycogen by some denitrifying bacteria in the anoxic zone resulted in improvement of denitrification rates. The next goal of their research was to achieve less than 0.05 mg TP/L and 5 mg TN/L in the effluent by using a post-denitrification MBNR configuration (Figure 2.3).

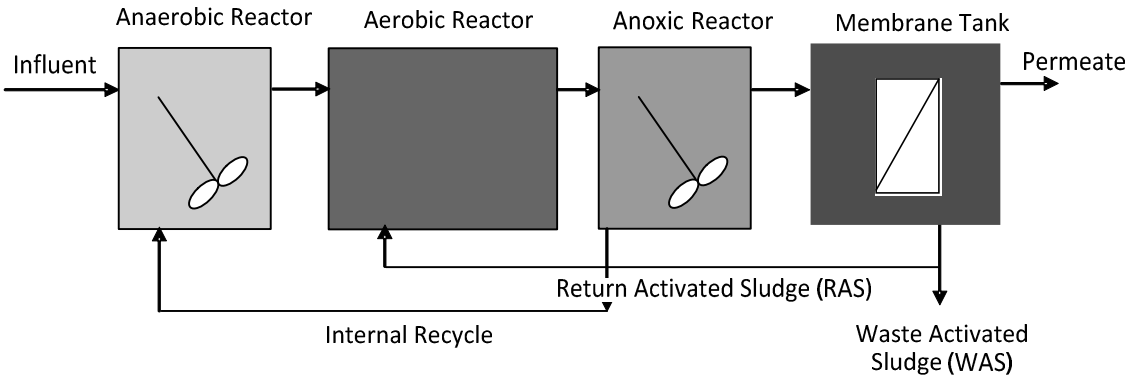


Figure 2.3 Post-denitrification MBNR System (Adapted from Lesjean *et al.*, 2005)

Fleischer *et al.* (2005) investigated the feasibility of a multistage MBNR system targeting extremely low effluent TN and TP concentrations. As shown in Figure 2.4, the six stage MBNR system had two pre-anoxic reactors and one post-anoxic reactor for optimization of nitrogen removal. Furthermore, methanol was added to provide an additional carbon source for denitrifying bacteria in the post-anoxic reactor. Phosphorus removal was achieved by employing both biological and chemical (alum) phosphorus removal processes. The study demonstrated the capability of the MBNR system to reliably produce effluent with TN less than 3 mg/L and TP less than 0.1 mg/L.

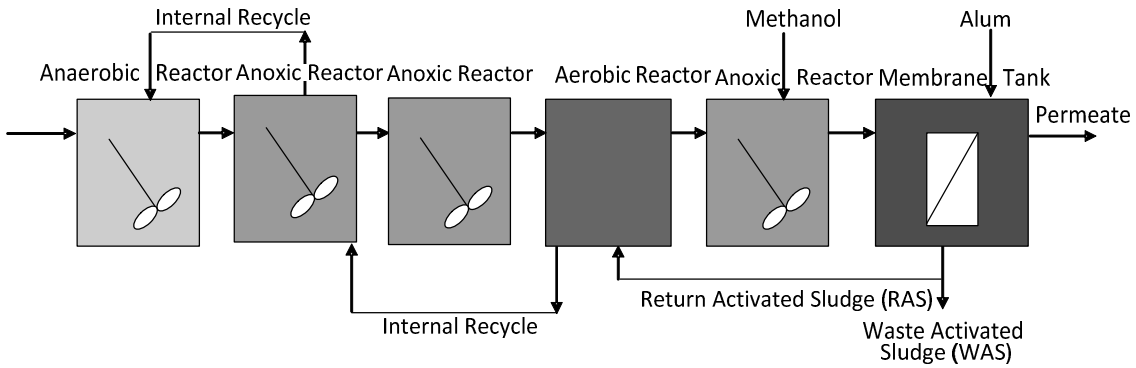


Figure 2.4 MBNR System (Adapted from Fleischer *et al.*, 2005)

A review of the recently designed multi-stage MBNR systems demonstrates that membrane bioreactor technology has the capability to meet stringent nutrient discharge limits. However, most full scale MBNR plants still make provision for phosphorus

removal by supplemental metal addition, even though they may be designed to remove phosphorus via the EBPR process (Crawford *et al.*, 2006; Phagoo *et al.*, 2005). Studies have indicated that over-dosing of metal salt can precipitate too much of the available soluble phosphorus, and as a result, reduce the competitive advantage of the EBPR mechanism (Crawford *et al.*, 2006; de Haas *et al.*, 2000; Röske and Schönborn 1994a; Röske and Schönborn 1994b). Therefore, controlling metal salt addition to the process is a key operational issue for MBNR systems. Similarly, for improving nitrogen removal potential, external carbon (e.g. methanol, acetate) is used in most MBNR plants to enhance denitrification. The use of chemicals in wastewater treatment plants designed for membrane biological nutrient removal adds complexity to the fundamental mechanisms occurring within the processes. Therefore, a comprehensive review of the current nitrogen and phosphorus removal technologies will help in understanding and optimizing treatment performance in MBR plants and identifying the gaps in fundamental understanding of the processes involved in N and P removal by membrane-coupled processes, especially, the dynamics between the chemical and biological processes.

2.3 Nitrogen Removal in BNR Plants

2.3.1 General introduction

Nitrogen in influent wastewater consists of ammonium (~60%) and organic nitrogen (~40%) (Tchobanoglous *et al.*, 2003). Typically, less than 1 percent is present as nitrate or nitrite unless the plant receives influent from industrial sources. Ammonium-nitrogen is removed by nitrification and the products of nitrification, nitrite and nitrate, are removed by denitrification in biological nutrient removal plants. On the other hand, removal of organic nitrogen is dependent on the biodegradability of the particular nitrogenous compound. Generally, the biodegradable organic nitrogen (both particulate and dissolved) fraction is removed when it is converted to ammonium-nitrogen by hydrolysis and mineralization in wastewater treatment plants (WWTPs) (Paredes *et al.*, 2007). Additionally, the non-biodegradable particulate organic nitrogen fraction is removed by efficient solids-liquid separation processes such as clarifiers, filters and

membrane systems. However, non-biodegradable dissolved nitrogen or recalcitrant dissolved organic nitrogen (rDON) remains in the effluent of BNR processes.

2.3.2 Fundamentals of biological nitrification

2.3.2.1 Stoichiometry

Nitrification can be defined as a two step biological process in which ammonium (NH_4-N) is oxidized to nitrite (NO_2-N) by autotrophic ammonia-oxidizing bacteria (AOB) and nitrite is oxidized to nitrate (NO_3-N) by nitrite-oxidizing bacteria (NOB) (Tchobanoglous *et al.*, 2003). The reactions for ammonium oxidation to nitrate are as follows (Henze *et al.*, 1996):

Ammonia oxidation:

$$NH_4^+ + 3/2O_2 \longrightarrow 2NO_2^- + 4H^+ + 2H_2O \quad (1)$$

$\Delta G^o(W) = -270$ kJ/mol NH_4^+-N

Nitrite oxidation:

$$NO_2^- + 1/2O_2 \longrightarrow NO_3^- \quad (2)$$

$\Delta G^o(W) = -80$ kJ/mol NO_2^--N

Total ammonia oxidation reaction:

$$NH_4^+ + 2O_2 \longrightarrow NO_3^- + 2H^+ + H_2O \quad (3)$$

Equation (3) illustrates a simplified picture of nitrification in wastewater treatment systems. However, nitrification is a biological process that requires external sources of carbonate and nitrogen compounds for the growth of autotrophic bacteria (Marquot, 2006). Bicarbonate (HCO_3^-) and ammonium act as carbonate and nitrogen sources respectively during nitrification. Therefore, the nitrification reaction with respect to bacterial ($C_5H_7NO_2$) growth is as follows (Marquot, 2006):

$$NH_4^+ + 1.86O_2 + 1.98\ HCO_3^- \longrightarrow 0.02\ C_5H_7NO_2 + 0.98\ NO_3^- + 1.88\ H_2CO_3 + H_2O \qquad (4)$$

2.3.2.2 Microbiology

In theory, nitrification can be carried out by both autotrophic and heterotrophic microorganisms, although under normal wastewater treatment conditions, nitrification is achieved mainly by the autotrophic microorganisms (Joo *et al.*, 2005; Joo *et al.*, 2007; Lin *et al.*, 2006; Su *et al.*, 2006). *Nitrosomonas europaea* is the most common species of autotrophic ammonia-oxidizing bacteria (AOB) in wastewater treatment plants (Henze *et al.*, 1996). Other autotrophic AOB genera include: *Nitrosococus, Nitrosospira, Nitrosolobus,* and *Nitrosorobrio* (Painter, 1970). Manser (2005) reported that *nitrosomonads* (including *Nitrosococcus mobilis*), not *nitrosospiras* (encompassing the genera *Nitrosospira, Nitrosolobus and Nitrosovibrio*) are in fact the predominant AOBs in wastewater treatment plants. Nonetheless, the advances in microbiological techniques and gene analysis in recent years has revealed that various other autotrophic bacteria have the ability to act as AOB in the activated sludge environment of WWTPs (Marquot, 2006; Tchobanoglous *et al.*, 2003).

Nitrobacter, belonging to the *Alphaproteobacteria*, was typically considered to be the most common species of autotrophic nitrite-oxidizing bacteria (NOB) in wastewater treatment plants (Henze *et al.*, 2002). Wagner *et al.* (1996) however, contradicted this notion when they reported that *Nitrobacter* could not be detected in samples from nine different WWTPs. In fact, Wagner *et al.* (2002) postulated that *Nitrospira* bacteria are dominant in most WWTPs due to their higher affinities towards nitrite and oxygen. Over the years, other researchers have also found NOB species like *Nitrospira* and *Nitrosococcus* to be prevalent in activated sludge treatment processes (Daims *et al.*, 2001; Daims *et al.*, 2000; Teske *et al.*, 1994).

2.3.2.3 Parameters influencing nitrification

Since autotrophic nitrifiers have a lower specific growth rate than heterotrophic denitrifying microorganisms, nitrification typically limits the overall biological nitrogen removal capacity of a system. In addition, the sensitivity of nitrifying microorganisms to factors like temperature, pH, alkalinity, ammonium or nitrite and heavy metals contributes to the variability of the nitrification mechanism (Carrera *et al.*, 2004; Hu *et al.*, 2004; Hu *et al.*, 2004; Tchobanoglous *et al.*, 2003). During nitrification, ammonium oxidation (first step) has been found to be the rate-limiting step, as AOB have lower specific growth rates than NOB (Henze *et al.*, 1996). For that reason, nitrification was considered to be a single step process (NH_4 to NO_3) in early process models. However, research has indicated that both reactions can be limiting at different stages of the process. Therefore it is necessary to consider individual oxidation reactions for the modeling of nitrification kinetics (Chandran and Smets, 2000a; Chandran and Smets, 2000b; Chandran and Smets, 2005).

2.3.3 Fundamentals of biological denitrification

2.3.3.1 Stoichiometry

Denitrification is carried out by facultative heterotrophic microorganisms when they use oxidized nitrogen (NO_2^--N or NO_3^--N) as electron acceptors in respiration (Henze *et al.*, 1996). Many facultative denitrifying microorganisms have the ability to use both oxygen and oxidized nitrogen as electron acceptors due to similarities in metabolic pathways. Nevertheless, microorganisms prefer oxygen as the final electron acceptor if both oxygen and nitrate are present in the reactor. Therefore, in a single-sludge process, oxygen should be absent for part of the process if denitrification by nitrate reduction is the design objective.

Microorganism-induced nitrate reduction can be either *assimilatory* or *dissimilatory* as illustrated in Table 2.1 (Zumft, 1997). During *assimilatory* reduction, nitrate is reduced

to ammonium for synthesis of microorganisms. The *dissimilatory* transformation of nitrate or nitrite to a gaseous species concomitant with energy conservation is the phenomenological definition of the denitrification process (Zumft, 1997).

Table 2.1 Dissimilatory and assimilatory nitrate reduction (Adapted from Zumft, 1997)

Dissimilatory branch		Assimilatory branch
Denitrification (energy conservation, electron sink)	Ammonification (electron sink, detoxification, energy conservation)	Assimilation (biosynthesis of nitrogen-containing compounds)
Respiratory nitrate reduction $NO_3^- \rightarrow NO_2^-$ (nitrite excreted or reduced further)		Assimilatory nitrate reduction $NO_3^- \rightarrow NO_2^-$ (nitrite reduced further)
↓	↓	↓
Denitrification sensu stricto, nitrate and nitric oxide respiration $NO_3^- \rightarrow NO_2^- \rightarrow N_2O$ (gases may be set free)	Ammonifying nitrite reduction $NO_2^- \rightarrow NH_4^+$ (ammonia excreted)	Assimilatory nitrite reduction $NO_3^- \rightarrow NO_2^-$ (nitrite reduced further) ↓ Assimilatory nitrite reduction $NO_2^- \rightarrow NH_4^+$ (ammonia incorporated)
↓	↑	↑
Nitrous oxide respiration $N_2O \rightarrow N_2$	Denitrification associated with both branches $NO_3^-/NO_2^- \rightarrow N_2O$	

The stoichiometric equation for denitrification in which organic matter in wastewater is used as the energy and carbon source for microorganisms is shown below (Henze *et al.*, 1996).

$$1/70\ C_{18}H_{19}O_9N + 1/5\ NO_3^- + 1/5\ H^+ \longrightarrow 1/10\ N_2 + 17/20\ CO_2 + 1/70\ HCO_3^- + 1/70\ NH_4^+ + 1/5\ H_2O \quad (5)$$

2.3.3.2 Microbiology

Denitrification can be achieved by both heterotrophic and autotrophic microorganisms (Zumft, 1997). However, the majority of the denitrification in wastewater treatment processes is achieved by heterotrophic organisms. The heterotrophic denitrifying bacteria genera include: *Achromobacter*, *Acinetobacter*, *Agrobacterium*, *Alcaligenes*, *Arhtrobacter*, *Bacillus*, *Chromobacterium*,

Corynebacterium, Flavobacterium, Hypomicrobium, Halobacterium, Methanomonas Moraxella, Neisseria, Paracoccus, Propionibacterium, Pseudomonas, Rhizobium, Rhodopseudomonas Spirillum, and *Vibrio* (Gayle, 1989; Payne, 1981). It is generally thought that *Pseudomonas* species is the most common microorganism for denitrification in WWTPs (Janda *et al.*, 1988; Gray, 1990; Lazarova *et al.*, 1992; Payne, 1981).

Recent research indicates the presence of two distinct types of denitrifiers (true denitrifiers and incomplete denitrifiers) in activated sludge treatment processes (Glass *et al.*, 1997; Drysdale *et al.*, 1999). Drysdale *et al.* (1999) found that even though many different heterotrophic bacteria contributed to denitrification in the Darvill BNR process, the majority of them were incomplete denitrifiers. The incomplete denitrifiers can only reduce nitrates to nitrites with no further reduction of the nitrites. According to the authors, denitrification was achieved via interactive associations between the complete and incomplete denitrifying heterotrophic bacteria. Their study also concluded that *Pseudomonas* was the most prevalent denitrifying species in the Darvill BNR process.

2.3.3.3 Parameters influencing denitrification

Reactor configuration and the nature and concentration of carbon substrates are two important parameters that typically influence denitrification rates (Henze *et al.*, 1996; Tchobanoglous *et al.*, 2003). Both pre- and post-anoxic reactor configurations have been used in wastewater treatment plants (Barnard *et al.*, 2008; Crawford *et al.*, 2006; Lesjean *et al.*, 2005). Denitrification by pre-anoxic design is achieved by creating an anoxic zone at the head end of the process and recycling the nitrified mixed liquor from the aerobic zone of the process. On the other hand, post-anoxic denitrification is achieved by either endogenous respiration or the addition of an external carbon source, by locating the reactor downstream of the aerobic zone and such an arrangement eliminates the need for recycling the nitrified biomass.

Denitrification by endogenous respiration becomes inadequate when the total nitrogen (TN) concentration is high in the influent wastewater (Barnard *et al.*, 2008). For

that reason, an external carbon source is essential for treatment plants with strict nitrogen discharge limits. Chemicals that have been used as external carbon substrates include methanol, acetate, ethanol, sucrose solution, glycerol, high-fructose corn syrup and commercially available organic carbon (Barnard *et al.*, 2008). Methanol is the most common external carbon substrate used in the denitrification process. Methanol is also preferred in wastewater treatment plants because it is widely available, cost-effective and the PAOs cannot use it for the EBPR mechanism (Baytshtok *et al.*, 2008; Dold *et al.*, 2008; Louzeiro *et al.*, 2003). On the other hand, methanol-utilizing microorganisms are slow growing, particularly at low temperatures and can be washed out at short anoxic hydraulic retention times (HRTs) and solids retention times (SRTs) (Dold *et al.*, 2008).

2.3.4 Fundamentals of dissolved organic nitrogen

Dissolved organic nitrogen (DON) is typically the main form of nitrogen in the effluent of an enhanced nitrification-denitrification process (Pehlivanoglu-Mantas and Sedlak, 2008). Pagilla *et al.* (2008) studied nitrogen speciation in primary and secondary effluents of three nitrifying plants in the United States and four BNR plants in Poland. The key objective of the study was to quantify the dissolved fraction of organic nitrogen in both influent and effluent. The authors observed DON fractions ranging from 9 to 50 percent of the TN in the treated effluent. Bratby *et al.* (2008) reported DON concentrations varying from 0.4 to 2.2 mg/L by reviewing data from their work as well other studies. The highly variable effluent DON concentration poses a challenge to future nutrient removal initiatives, as it is very difficult to remove DON in conventional BNR plants. Hence, characterization of DON along with its fate in wastewater treatment plants should be given more attention.

In the late 1970's and early 1980's, Stanford University researchers focused on factors affecting DON production and removal during activated sludge treatment (Parkin and McCarty, 1981a; Parkin and McCarty, 1981b; Randtke, 1977). The key contribution of their work was the development of a model which conceptualized the distribution of

DON constituents. The model is based on experimental work done in batch activated sludge systems and is illustrated in Figure 2.5.

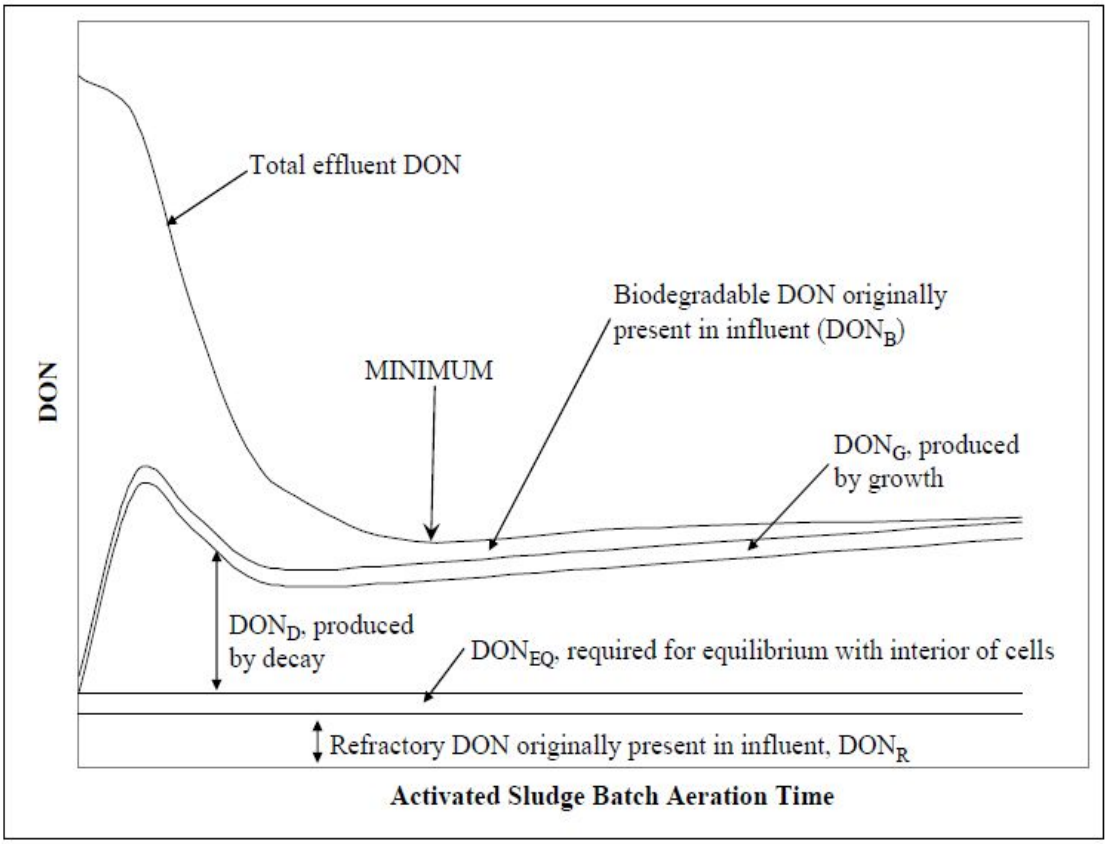


Figure 2.5 DON model (conceptualized by Parkin and McCarty, 1981a; Parkin and McCarty, 1981b) (Figure adapted from Bratby *et al.*, 2008)

The various individual DON constituents shown in the Figure 2.5 are the following.

- Influent wastewater DON that can be categorized as biodegradable DON (DON_B) and refractory DON (DON_R). DON_B is utilized rapidly in the activated sludge system while DON_R passes through the system unchanged.
- DON_{EQ} representing the DON excreted by microorganisms to maintain concentration equilibrium across the cellular membrane.
- DON_G representing the DON produced during the growth of microorganisms.
- DON_D represents production of DON related to microorganism decay. The model postulates that DON_D is refractory.

In recent years, researchers have been expending effort to understand the structure and behaviour of wastewater-derived DON, even though challenges remain due to the difficulties associated with the measurement of DON (Pehlivanoglu-Mantas and Sedlak, 2006; Pehlivanoglu-Mantas and Sedlak, 2008). Pehlivanoglu-Mantas and Sedlak (2008) reported that total amino acids and ethylenediaminetetraacetic acid **(**EDTA) accounted for less than 30 percent of the DON in effluent. Approximately, 70 percent of the DON could not be identified in their work. The authors put forward the idea that the unknown DON compounds consisted of a complex suite of partially metabolized compounds of biogenic origin. Using solid-phase extraction (SPE) and molecular weight (MW) fractionation techniques, Pehlivanoglu-Mantas and Sedlak (2008) concluded that most of the DON compounds were of low-molecular weight and hydrophilic in nature. Westerhoff *et al.* (2006) estimated that 78 percent of the DON was less than 1,000 Daltons in size and approximately 7.5 percent was larger than 10,000 Daltons in effluent from the Mesa WWTP in Arizona.

2.3.4.1 Parameters influencing DON removal

The fate of DON in the activated sludge process can be influenced by operating conditions (solids retention time (SRT), hydraulic retention time (HRT)), membrane vs conventional treatment and coagulant addition. According to Parkin and McCarty (1981a), SRT is a crucial operating parameter as it controls DON_B to DON_R ratio in the effluent. Longer SRTs will reduce DON_B in the effluent, whereas, decay may increase the DON_R fraction. Therefore, SRT optimization is imperative to minimize concentrations of DON in the effluent. The authors suggested an optimum range of 6 to 10 days for SRT in wastewater treatment systems. Sattayatewa *et al.* (2009) investigated the role of reactor HRT in DON transformation in a four stage Bardenpho-type WWTP. The researchers found that DON was produced in the pre-anoxic zone and to a lesser extent in the first aerobic zone.

Membrane bioreactors can influence DON removal because (1) complete retention of suspended solids improves degradation of slowly biodegradable retained organics which

may include DONs and (2) MBRs are operated with longer SRTs than conventional activated sludge systems. Kim and Nakhla (2010) observed lower effluent DON concentrations in MBR systems as compared to a parallel conventional system. The research work was conducted on a UCT-MBR system, their patented MBR system and a conventional A^2O system. The authors also concluded that there was no correlation between different MBR process configurations, system biomass concentration (ranged from 1.6 g/L to 2.7 g/L) and system HRT (6 to 8 hours) and effluent DON concentration.

Aluminum- or iron-based coagulants are used in wastewater treatment plants with the primary objective of removing phosphorus from the effluent. Depending on the structure of DON, these coagulants can also be helpful in reducing DON concentration in the effluent. Coagulants are typically more efficient in removing higher MW (molecular weight) compounds. The current knowledge indicates that most of the effluent DON consists of low molecular weight compounds, which may limit the effectiveness of coagulant addition. By reviewing the relationship between coagulant addition and DON removal, Bratby *et al.* (2008) observed a wide spread in effluent DON concentrations. They hypothesized that different wastewaters contain a wide variety of DON constituents and it is particularly evident at lower DON concentrations. Nonetheless, currently a lot of work is needed to fully understand the role of coagulants with lower effluent DON concentrations.

2.3.4.2 Measurement of DON

DON in WWTP effluent is typically estimated by subtracting ammonium-nitrogen from soluble total Kjeldhal nitrogen (soluble-TKN). Although this method gives satisfactory results for secondary treatment plant effluent, it has drawbacks when effluent is derived from a nitrifying treatment plant or effluent with very low total nitrogen concentrations. Effluent samples with NO_3-N concentrations higher than 6 mg/L have been reported to interfere with the TKN test (Bratby *et al.*, 2008). The interference occurs in the digestion step, in which a reaction between ammonium and nitrate causes reduction of ammonium. During the reduction reaction, ammonium is converted to nitrous oxide

(N_2O) and disappears with the digestion gas (Bratby *et al.*, 2008). As a result, estimated TKN concentrations can be smaller than ammonium concentrations determined separately. When effluent TN concentration is around, or less than, 3 mg/L, the detection limit is the major issue with the TKN method. DON can also be determined by subtracting dissolved inorganic nitrogen (DIN) from the total dissolved nitrogen (TDN) concentration. However, when effluent concentrations are close to limits of technology levels (≤ 3 mg TN), the individual constituents are present at very low concentrations. This will present significant analytical challenges in terms of detection limits, measurement precision and expensive instrumental methods (Sattayatewa and Pagilla, 2008).

The methodologies discussed above have been adopted by most of the studies related to DON determination in wastewater effluent. Nonetheless, the move towards very low TN concentration means that the indirect methods may not provide reliable DON data. For that reason, Sattayatewa and Pagilla (2008) modified the column anion exchange resin-persulfate digestion method proposed by Crumption *et al.* (1992) for wastewater effluent DON determination. The steps of the Crumption *et al.* (1992) method are the following.

- Acidification (reduce pH < 2) water samples with HCl. This pH is well below the pK_a of most of the organic constituents and as a result, they will not be adsorbed to basic anion resin.
- Passage of the acidified water samples through a very basic anion exchange resin column for NO_3-N removal.
- Digestion of the nitrate-free samples with persulfate reagents during which all forms of nitrogen are converted to nitrate.
- Measurement of nitrate concentration by one of several different methods such as diphenylamine spot plates, spectrophotometric, nitrate-selective electrode, high-performance liquid chromatography, and cadmium reduction.

The results from the Sattayatewa and Pagilla (2008) study showed excellent nitrate removal by ion exchange treatment. In addition, they reported organic nitrogen recoveries ranging between 96.9 to 105.8 percent, when anion exchange resin was applied to solutions containing urea, glycine, glutamic acid, sulphanilamide and methionine. Although the column method described above can be very useful in determining DONs in WWTP, it is very tedious and requires considerable time to analyze large numbers of samples.

2.4 Limits of Technology Nitrogen Removal

The likelihood of achieving the LoT total nitrogen concentration (TN $\leq$ 3 mg/L) in wastewater treatment plants is significantly limited by the recalcitrant dissolved organic nitrogen concentration. In fact, in order to achieve TN $\leq$ 3 mg/L, the wastewater effluent should not contain combined ammonium, nitrates, nitrites and residual degradable dissolved organic nitrogen concentrations of more than 1 to 1.5 mg/L (Barnard *et al.*, 2008). The key design parameters towards that objective are longer SRTs for ammonium removal and external carbon addition for nitrate and nitrite removal. Depending on the recalcitrant nature of the DON, more external carbon will be needed for nitrate removal at greater cost. Research in the area of bioavailability and biodegradability characteristics of DON will help in shaping the future realistic effluent TN regulations.

2.5 Phosphorus Removal in BNR Systems

2.5.1 General introduction

Municipal and industrial wastewater discharges are two of the major point-sources of phosphorus to surface water. In order to remove phosphorus effectively in wastewater treatment plants, it is necessary to understand the various forms of phosphorus in the influent wastewater, as summarized in Table 2.2.

Table 2.2 Phosphorus species in wastewater (Denham, 2007; Neethling *et al.*, 2007; Thistleton, 2000)

Phosphate Group	Species	Liquid/Solid	Remark
Orthophosphate	PO_4^{3-}, HPO_4^{2-}, $H_2PO_4^-$, H_3PO_4 (depending on pH)	Liquid	• Weak acid, most dominant form, reactive; • Final product of the phosphorus cycle; • Most readily available for biological utilization
Polyphosphate/ Condensed Phosphate	Pyrophosphate, tripolyphosphate, metaphosphate, intracellular polyphosphate granules	Liquid/solid	• Complex large molecule; • Precipitate in condensed form or hydrolysis to orthophosphate; • Found in agriculture, water treatment processes and cleaning compounds
Organic phosphate	Cell material and organic material	Solid/liquid	• Associated with biological growth; • Can exist in particulate or soluble form
Chemically bound phosphorus	Phosphorus precipitants, typically Al, Ca, Fe, struvite and others; adsorption to sorbent or to metal hydroxides, form complex	Solid	• Includes precipitated, co-precipitated and adsorbed forms

Table 2.2 indicates that there is no gaseous form of phosphorus through which it can be removed from wastewater treatment systems. Biological, physical and chemical reactions convert different forms of phosphorus to particulates that can be removed from wastewater in a solids-liquid separation step. In this review, biological and chemical phosphorus removal processes will be discussed comprehensively to identify potential knowledge gaps.

2.5.2 Enhanced biological phosphorus removal (EBPR) process

2.5.2.1 Biochemical pathways of EBPR

The concept of bacterial-induced enhanced biological phosphorus removal was first published by Fuhs and Chen (1975) during their study on the Phostrip process. The authors proposed that an excess biological phosphorus removal mechanism occurred through complex biochemical pathways, mediated by microorganisms that were later referred to as phosphate-accumulating organisms (PAOs). The key biochemical reactions in the EBPR process (with acetate as substrate) are presented below. Acetate has been identified as the model carbon substrate in the vast majority of EBPR biochemical pathways studies. This can be attributed to acetate being typically the most common VFA species present in influent wastewater (Ahn and Speece, 2006; Comeau *et al.*, 1987; Cuevas-Rodríguez and Tejero-Monzón, 2003; Thomas *et al.*, 2003; Zeng *et al.*, 2006).

Anaerobic Metabolism

- Under anaerobic conditions, PAOs assimilate acetate that has been produced by fermentation of biodegradable soluble chemical oxygen demand (bsCOD) (Tchobanoglous *et al.*, 2003).
- Activation of acetate to acetyl-CoA follows the assimilation phase. The activation step happens simultaneously with adenosine triphosphate (ATP) catabolism. In the anaerobic reactor, ATP generation takes place mostly due to the conversion (or hydrolysis) of energy-rich phosphoric group (i.e. polyphosphate) to adenosine diphosphate (ADP) (Oehmen *et al.*, 2007).
- K^{+}, Mg^{+2}, and $H_2PO_4^{-}$ are released to the external medium during the hydrolysis of polyphosphates (Oehmen *et al.*, 2007).
- Two molecules of acetyl-CoA condense to form acetoacetyl-CoA, and subsequently they are reduced by NADH to form 3-hydroxybutyryl-CoA. The final product of anaerobic metabolism is 3-hydroxybutyryl-CoA, also known as poly-β-hydroxybutyrate (PHB) (Oehmen *et al.*, 2007).

Aerobic Metabolism

- Cell growth of PAOs occurs by utilizing stored PHB as an energy and carbon source under aerobic conditions (Mino *et al.*, 1998; Tchobanoglous *et al.*, 2003).
- Energy released from PHB oxidation facilitates formation of intracellular polyphosphate bonds following the uptake of soluble orthophosphate from the solution (Tchobanoglous *et al.*, 2003).
- Additionally, some stored PHB and energy produce glycogen in EBPR process (Oehmen *et al.*, 2007; Tchobanoglous *et al.*, 2003).

During anaerobic metabolism, reduction power is required for conversion of acetate to polyhydroxyalkanoate (PHA) compounds (Mino *et al.*, 1998). To explain the production of reducing equivalents in EBPR processes, two different biochemical models have been proposed (Mino *et al.*, 1998; Oehmen *et al.*, 2007). According to the *Comeau/Wentzel model*, the TCA cycle functions to oxidize a part of the acetate to CO_2 and generates reducing power in the form of NADH under anaerobic conditions (Comeau *et al.*, 1986; Wentzel *et al.*, 1986). Alternatively, the *Mino model* proposed that the reducing equivalents in the form of NADH are produced when internal carbohydrate (i.e. glycogen) is converted to pyruvate via the Embden-Meyerhoff-Parnas (EMP) pathway. Also, pyruvate is converted to acetyl-CoA and CO_2 in the EBPR process (Mino *et al.*, 1987). Over the years, several experimental research studies have supported the *Mino model* by demonstrating the involvement of glycogen in the EBPR process (Maurer *et al.*, 1997; Satoh *et al.*, 1992; Satoh *et al.*, 1996; Smolders *et al.*, 1994b).

Using *in vivo* ^{13}C and ^{31}P nuclear magnetic resonance (NMR) techniques, Pereira *et al.* (1996) offered new insights on TCA and glycogen utilisation in EBPR processes. Based on redox balance reactions, the authors concluded that the reducing power generated by degradation of glycogen cannot solely account for the PHA production. Therefore, Pereira *et al.* (1996) suggested that both glycogen and the TCA cycle were used for the production of reducing equivalents, merging the two initial models. In recent years, researchers have studied other metabolic pathways involving utilization of glucose and propionate substrates (Lemos *et al.*, 2003; Wang *et al.*, 2002).

2.5.2.2 Microbiology

The early research identified microorganisms involved in EBPR based on culture-dependent techniques (Seviour *et al.*, 2003). Using these techniques, Fuhs and Chen (1975) identified *Acinetobacter* sp. as the primary organism responsible for the EBPR process. The observations by Fuhs and Chen (1975) were subsequently supported by other microbiologists who tried to identify EBPR microorganisms (Wenztel *et al.*, 1988). However, the use of more sophisticated techniques such as biomarkers, fluorescence *in situ* hybridization (FISH), 16s rRNA-based clone libraries or denaturing gradient gel electrophoresis (DGGE) has shown that *Acinetobacter* is not primarily responsible for EBPR (Mino *et al.*, 1998; Oehmen *et al.*, 2007; Seviour *et al.*, 2003). Although many studies reported isolation of pure PAO cultures, none of them exhibited all of the EBPR characteristics, which they should theoretically possess (Mino *et al.*, 1998). In most cases, anaerobic acetate metabolism was the key characteristic lacking in the isolated pure cultures (Jenkins and Tandoi, 1991). Initially, it was thought that only a single dominant group of microorganisms could exhibit enriched P removal in an EBPR process. However, subsequent evidence indicated that the microbial community of the EBPR process is phylogenetically very diverse (Loy *et al.*, 2002). Using a genus-specific FISH probe ACA23a, Wagner *et al.* (1994) found that the bacterial population was dominated by *Actinobacteria* (36%) and *Betaproteobacteria* (36%) groups. On the other hand, *Acinetobacter* accounted for only 3-6 percent of the total bacterial community.

In the last decade, a great deal of EBPR microbial population research has focussed on the *Rhodocyclus* group from subclass 2 of the *Betaproteobacteria*. Bond *et al.* (1995) were the first to highlight presence of the *Rhodocyclus* group in clone libraries from the EBPR biomass. The authors postulated that *Rhodocyclus* organisms played a role in EBPR, as it represented the major difference between EBPR and non-EBPR microbial communities. Using FISH, Bond *et al.* (1999) later showed that subclass 2 of the *Betaproteobacteria* comprised 55 percent of all bacteria in a laboratory scale EBPR reactor. This particular phosphate-removing community is known as *Candidatus Accumulibacter phosphatis* (Hesselmann *et al.*, 1999) or often abbreviated to

Accumulibacter in the literature (Oehmen *et al.*, 2007). *Accumulibacter* has been found to be an abundant PAO in both lab scale (Onuki *et al.*, 2002) and full scale studies (Lee *et al.*, 2002; Saunders *et al.*, 2003; Zilles *et al.*, 2002).

Genomics has been proposed as a new tool for gathering information on the metabolism of an individual cell or a microbial population, along with an understanding of the associated phylogeny (Oehmen *et al.*, 2007). Martin *et al.* (2006) obtained almost the complete genome of *Accumulibacter* from two enriched EBPR mixed liquors. By using the metagenomic data, they were able to analyze the important metabolic processes such as: (1) the transport of polyphosphate through the cell membrane and its degradation/generation under anaerobic/aerobic conditions, (2) the metabolic pathways for glycogen degradation and for the generation of additional reducing power necessary for anaerobic PHA production, (3) extracellular polymeric substances (EPS) production and (4) denitrification and nitrogen fixation ability. The current knowledge regarding metagenomics of dominant populations of EBPR systems is still limited and development in this area of research will provide full scale plants with an important tool for evaluating the EBPR potential of a sludge population.

2.5.2.3 Parameters influencing EBPR removal

Successful operation of an EBPR process depends on numerous environmental and operational factors. Review of the literature indicates that parameters such as (1) microbial competition between glycogen-accumulating organisms (GAOs) and phosphate-accumulating organisms (PAOs), (2) cation concentration and (3) solids retention time (SRT) and hydraulic retention time (HRT) are crucial for EBPR processes, in addition to the need for VFAs in the anaerobic zone.

PAOs and GAOs competition

The link between EBPR failure and microbial competition was first reported by Cech and Hartmann (1993), who noticed large numbers of “G-Bacteria” in a glucose-fed

reactor. The "G-bacteria" term in the literature now commonly refers to glycogen-accumulating organisms (GAOs). Due to their influence on EBPR performance, the biochemical pathways of GAOs have been studied extensively over the years. Several of these studies concluded that PAOs and GAOs have about the same functional pathways (Mino *et al.*, 1998; Oehmen *et al.*, 2007; Seviour *et al.*, 2003). Like PAOs, GAOs can take up VFAs and store them as PHA under anaerobic conditions. However, the key difference between the two organisms is the source of energy; PAOs use polyphosphate to generate energy whereas GAOs ferment glycogen to PHA and CO_2 to generate energy. Recently, Erdal *et al.* (2004) noted that the two organisms store different forms of PHA. According to the authors, the main storage product of PAOs is poly-β-hydroxybutyrate (PHB) while that in GAOs is polyhydroxyvalerate (PHV). Lately, studies have focussed on the factors influencing the microbial competition between PAOs and GAOs (Filipe *et al.*, 2001c; Oehmen *et al.*, 2005; Lu *et al.*, 2006). Influent composition, pH and temperature have been reported in the literature as the major parameters that can influence microbial competition dynamics in EBPR processes (Oehmen *et al.*, 2007).

The presence of volatile fatty acids (VFAs) such as acetate, propionate and butyrate is a prerequisite for biological phosphorus removal (Henze *et al.*, 1996). Wastewaters with high concentrations of VFAs or a large fraction of readily fermentable substrate possess high phosphorus removal potential (Tanyi, 2006). This is the case because VFAs constitute the substrate for PAOs and subsequently provide the PAOs a competitive advantage over the GAOs. Therefore, VFA content of the influent is the most important design parameter in the sizing of the anaerobic zone in an EBPR process (Monti, 2006). VFAs are typically present in the feed or are produced through fermentation (hydrolysis and acidogenesis) of readily biodegradable COD (COD_{Rb}) in the anaerobic zone or in a side-stream sludge fermenter.

In EBPR systems, the pH value strongly affects the PAO-GAO competition. Typically, an increase in pH enhances PAOs selection over GAOs (Oehmen *et al.*, 2007). The selection of PAOs can be explained by the fact that a higher pH will cause higher energy demand for acetate and simultaneously, this will negatively affect the ability of

GAOs to take up acetate. Since polyphosphate is the only extra energy source for PAOs as compared to GAOs, the PAOs will expend polyphosphate to meet higher energy demand at increased pH values (Filipe *et al.*, 2001b). Additionally, Filipe *et al.* (2001c) proposed that a pH value of 7.25 is the critical point in an anaerobic zone of an EBPR process. Below the critical pH value, GAOs have the capability to take up VFAs faster than the PAOs. In the aerobic zone, a higher pH (7-7.5) is also beneficial for the PAOs, as low pH (6.5) will inhibit PHA utilization and biomass growth (Filipe *et al.*, 2001a). Similar results have been reported by other researchers, who observed shifting of microbial populations from GAOs to PAOs and subsequent higher P removal at increased pH values (from $\leq$ 7 to 7.5–8.5) (Bond *et al.*, 1999; Jeon *et al.*, 2001; Schuler and Jenkins, 2002; Serafim *et al.*, 2002).

The influence of temperature on microbial competition has been investigated in both low and high temperature operations. Erdal (2002) reported that low temperature (particularly less than 10 ^{0}C) improved biological phosphorus removal by providing the PAOs with an advantage over the GAOs. This can be attributed to the fact that the glycolysis reaction in the GAOs is more temperature sensitive than the energy reaction in the PAOs (Dr. James L. Barnard, pers. comm.). Consequently, PAOs outperformed the GAOs at 5 ^{0}C, even though metabolism of PAOs was slower at 5 ^{0}C than at 20 ^{0}C (Erdal, 2002). On the other hand, EBPR performance tends to slow down or diminish completely at warmer temperatures (Panswad *et al.*, 2003; Rabinowitz *et al.*, 2004). Predominance of GAOs at higher temperatures has been reported as the cause for the decline in phosphorus release and uptake in EBPR systems (Whang *et al.*, 2007).

Cation concentration

Cations such as magnesium (Mg^{2+}) and potassium (K^+) must be present above critical concentrations in influent wastewater for a successful EBPR process (Machnicka *et al.*, 2004). This is because Mg^{2+} and K^+ are essential counter-ions for polyphosphate in the cell, and play a major role in energy generation (Schönborn *et al.*, 2001; Van Groenestijn

et al., 1988). The role/fate of cations in biological phosphorus removal is explained as follows.

- Potassium defines cell membrane permeability and is critical for phosphate transport between the surrounding environment and the cell (Medveczky and Rosemberg, 1971).
- The enzyme polyphosphate kinase catalyzes polyphosphate biosynthesis in the presence of magnesium ions (Machnicka *et al.*, 2004).
- Magnesium is taken up and released simultaneously with phosphate in the EBPR process (Machnicka *et al.*, 2004).

Solids retention time (SRT)/ hydraulic retention time (HRT)

Barnard (1988) noted that EBPR systems can operate with an SRT in the range from 2 to 40 days. However, efficient phosphorus uptake typically requires a minimum SRT of 3 to 4 days and this value can vary with mixed liquor temperature in the EBPR process (Dr. James L. Barnard, pers. comm.). Wentzel *et al.* (1988) proposed that successful EBPR process operation at high SRT could be attributed to the low endogenous decay rates of PAOs as compared to those of aerobic heterotrophic bacteria. At high SRTs, a proportionally higher percentage of active biomass will consist of PAOs; as a result, the phosphorus content of the biomass will increase with an increase in SRT. On the other hand, if SRT is increased to a level at which the endogenous biomass decay rate is significant, secondary phosphorus release may lead to decreased effluent quality in EBPR systems (Dr. James L. Barnard, pers. comm.).

Hydraulic retention time (HRT) selection can influence both the formation of PHAs in the anaerobic zone and phosphorus uptake rate in the aerobic zone of an EBPR process. If sufficient HRT is not allowed in the anaerobic zone, formation of PHA will not be adequate to support the desired phosphorus uptake in the aerobic zone. Similarly, if aerobic HRT is too small, phosphorus uptake could be limited (Dr. James L. Barnard, pers. comm.). However, Erdal (2002) noted that a long aerobic HRT actually reduced

EBPR efficiency. The author indicated that long HRT in the aerobic phase causes depletion of glycogen reserves, which can limit PHA storage in the anaerobic zone.

2.5.3 Chemical phosphorus removal

2.5.3.1 General introduction

Chemical phosphorus removal relies on the transformation of soluble, colloidal and quasi-colloidal forms of phosphorus to a particulate form and the subsequent removal of this form (along with any phosphorus already present in particulate form) by solids-liquid separation processes (Takács *et al.*, 2006). The advantage of chemical phosphorus removal is its simplicity of operation and ease of implementation in wastewater treatment systems. Depending on the physical configuration of the plant, chemical cost factors, and the effluent quality requirements, phosphorus removal can be accomplished by pre-precipitation, co-precipitation (simultaneous precipitation) or post-precipitation at wastewater treatment facilities (Nutt, 1991). Co-precipitation along with filtration is the most commonly used precipitation process that can meet effluent concentrations of 0.5 mg/L or lower on a consistent basis (Denham, 2007). For co-precipitation to occur, metal salts can be added to (1) the effluent from primary sedimentation facilities, (2) the biological reactor and (3) the effluent from a biological treatment process before secondary sedimentation (Tchobanoglous *et al.*, 2003). Aluminum (III) and ferric (III) salts are typically used for phosphorus precipitation in wastewater treatment facilities. Aluminum (III) compounds used for phosphorus removal include alum [$Al_2(SO_4)_3 \cdot 18\ H_20$], sodium aluminate ($NaAlO_2$), and polyaluminum chloride (PAC). Ferric (III) is used as ferric chloride ($FeCl_3$) or ferric sulphate [$Fe_2(SO_4)_3$] in wastewater treatment facilities (WEF and ASCE, 2005).

2.5.3.2 Mechanism

The classic model describing precipitation of phosphorus with aluminum (III) and ferric (III) is as follows (Tchobanoglous *et al.*, 2003; WEF and ASCE, 2005).

Precipitation with aluminum (III):

$$Al^{3+} + H_nPO_4^{3-n} \leftrightarrow AlPO_4 + nH^+ \quad (7)$$

Precipitation with ferric (III):

$$Fe^{3+} + H_nPO_4^{3-n} \leftrightarrow FePO_4 + nH^+ \quad (8)$$

From Equation (7) and (8), it can be observed that 1 mole aluminum (III) or ferric (III) will precipitate 1 mole of phosphate. However, Maurer and Boller (1999) and Smith *et al.* (2008) reported that the above precipitation reactions cannot explain phosphorus removal under the conditions in a wastewater treatment plant. The precipitation of phosphorus in a liquid environment is a complex phenomenon due to the formation of a range of metal phosphorus and hydroxyl complexes, as well as adsorption and co-precipitation of phosphorus onto the precipitates and complexes (Neethling *et al.,* 2007). The different simultaneous pathways that are most likely responsible for phosphorus removal by aluminum (III) and ferric (III) species include (Maurer and Boller, 1999; Smith *et al.*, 2008; Takács *et al.*, 2006):

- Adsorption (surface complexation) of phosphates and organic dissolved P onto hydrous aluminum oxide (HAO) or hydrous ferric oxide (HFO); HAO/HFO is formed by rapid precipitation of acidic aluminum/ferric solution in wastewater in the presence of sufficient alkalinity;
- Co-precipitation of phosphate into the HAO and HFO structure;
- Precipitation of ferric phosphate and mixed cation phosphates (i.e. calcium, magnesium, aluminum phosphate or hydroxyphpsphates);
- Coagulation/flocculation of primary precipitate colloidal particles and organic colloidal phosphorus.

2.5.3.3 Parameters influencing chemical phosphorus removal

In spite of its widespread application, chemical P removal is not well understood and is a complex process that is difficult to predict with respect to the net chemical reactions and their results. This can be attributed to the influence of variables like metal dose-to-phosphorus ratio, contact time, mixing intensity, pH, alkalinity and age of flocs on the precipitation process (Maurer and Boller, 1999; Smith *et al.*, 2008; Thomas *et al.*, 1996). Szabó *et al.* (2008) conducted batch and continuous tests to understand the influence of the different parameters on phosphorus precipitation. A brief review of their findings along with other related research work is presented in the following paragraphs.

Metal/phosphorus ratio

Generally, the dose of metal required for phosphorus removal is dependent on the effluent phosphorus discharge permit and the design specifications of the wastewater treatment plant. Other factors that can affect the stoichiometric ratio include (Bratby, 2006):

- Phosphorus speciation, i.e. influent wastewater dominated by orthophosphates is more readily removed by chemical precipitation than condensed and organic phosphorus;
- pH correction, i.e. acid/base should always be added before metal addition, as precipitation reactions are irreversible; and
- Efficiency of mixing at the point of metal addition.

During their investigation, Szabó *et al.* (2008) observed a linear relationship between dosage of coagulant and residual phosphorus at small metal dosages. However, the authors found that specific phosphorus removal decreased with an increase in concentration of metal salt. Maurer and Boller (1999) hypothesized that higher dosages typically result in an over-saturation of metals in water and therefore, will lead to an accelerated growth of precipitated flocs. Accelerated floc growth will facilitate fast conversion of micro flocs to macro flocs, including preferential binding of hydroxide

groups into the precipitates. Subsequently, specific phosphorus removal per dose decreases during high metal salt dosages (Maurer and Boller, 1999).

Contact time

Lijklema (1980) demonstrated that HFO flocs continued to adsorb orthophosphates after nearly 1,000 hours of contact time. Similar results were reported by Szabó *et al.* (2008), who observed the adsorption of orthophosphate onto HFO flocs even after over 100 hours of contact time. Furthermore, the authors observed two distinct phosphorus removal mechanisms during chemical precipitation. The initial mechanism was "instantaneous" whereby very fast P removal occurred under ideal mixing conditions. The second mechanism was a "slow removal or polishing step", where a further significant decrease in soluble phosphorus occurred via precipitation. The slow removal can take a few hours to days to achieve the target residual phosphorus concentrations. The slow removal step or precipitation contact time in wastewater treatment systems can be reduced by using higher metal/P ratios. However, using continuous flow reactors under similar mixing conditions, Szabó *et al.* (2008) demonstrated that a system with longer HRT and SRT can provide more efficient P removal than one with a shorter HRT and SRT. Therefore, it was recommended that additional removal of residual phosphorus should be reached by a slow removal step, rather than by adding massive dosages of excess metal salts.

Mixing energy

Complete mixing along with mixing intensity are key parameters for phosphorus precipitation reactions. Smith *et al.* (2008) suggested that mixing should be done at the site of addition of the acidic metal solution to attain the required complexation. Otherwise, incomplete mixing could result in low sorptive capacity and thus affect the efficiency of chemical precipitation. Similarly, rapid mixing is critical, especially for the initial "instantaneous" step, as it provides the metal and phosphate ions ample opportunity for complexation (Szabó *et al.*, 2008). In their experimental work, Gillberg *et*

al. (1996) demonstrated that rapid mixing significantly increased the percentage of orthophosphate removed, as compared to slow mixing. Moreover, rapid mixing was found to be more important to both aluminum and ferric ions at higher pH values.

pH

The effect of pH during chemical precipitation is very important in achieving extremely low soluble phosphorus residuals in wastewater effluent. Under favourable conditions, excellent phosphorus removal can be accomplished over a wide pH range for both water (phosphorus solution) and raw wastewater (Szabó *et al.*, 2008). Nonetheless, the prospect of complexes forming with phosphorus is highest for an optimum pH value (Bratby, 2006). Szabó *et al.* (2008) suggested that the most favourable orthophosphate removal can be accomplished with pH values between 5 and 7. On the other hand, for pH values in the acidic range, a soluble phosphate complex was the most predominant form along with a limited quantity of metal hydroxide complexes. In addition, higher metal salt addition (subsequent pH decrease) can dissolve already precipitated phosphate compounds (Bratby, 2006; Szabó *et al.*, 2008). For pH values between 7 and 10 (alkaline range), the formation of negatively charged soluble iron hydroxide [i.e. $Fe(OH)_4$] adversely impacts phosphorus removal in wastewater (Szabó *et al.*, 2008). Altundogan and Tümen (2001) reported a similar mechanism in their work whereby reverse desorption of orthophosphate occurred from bauxite under increased pH conditions. For pH values greater than 10, calcium and magnesium can form precipitates with orthophosphate (Fettig *et al.*, 1990) and consequently, phosphorus removal can be attained without iron or coagulant addition.

Alkalinity

Szabó *et al.* (2008) investigated the role of alkalinity by conducting a series of jar tests on model wastewater (phosphorus solution). The authors reported that for a specific value of pH, higher alkalinity resulted in higher residual soluble phosphorus concentrations. Szabó *et al.* (2008) suggested that in higher alkalinity waters, the

probability of metal hydroxide precipitation is greater than that of co-precipitation of phosphate and metal hydroxides. Nonetheless, the impact of alkalinity in a chemical phosphorus removal process has not been researched extensively and it requires further evaluation and analysis.

Age of flocs

According to various researchers, the aging of flocs adversely affects the long term slow phosphorus removal mechanism (Berkowitz, 2006; Lijklema, 1980; Szabó *et al.*, 2008; Smith *et al.*, 2008). Lijklema (1980) demonstrated that one-day old floc has half the sorption capacity of fresh HFO flocs. Similarly, Szabó *et al.* (2008) reported that the sorption capacity of HFO flocs decreased by 25 percent after 30 minutes of aging. The decrease in sorption capacity of old flocs might be due to the following factors:

- The HFO molecules become denser with age (Dzombak and Morel, 1990); and
- Higher density limits the ability of orthophosphate diffusion within the molecular structure (Makris *et al.*, 2004).

To understand the above mechanisms better, Smith *et al.* (2008) employed scanning electron microscopy (SEM) and transmission electron microscopy (TEM) techniques to examine HFO particles of different ages. The image analysis results indicated that fresh HFO flocs were indeed much less dense than older flocs, confirming the hypothesis.

2.5.4 Interaction between EBPR and chemical phosphorus removal

Since the early days of EBPR operation, it has been discussed and debated whether, and to what extent, chemical precipitation contributes to the phosphorus removal that is seen in the process (Arvin, 1983; Marais *et al.*, 1983; Rabinowitz and Marais, 1980). Although the occurrence of simultaneous P removal mechanisms may be beneficial for many wastewater treatment facilities, a knowledge gap still exists concerning the mechanisms involved in achieving target effluent phosphorus concentrations.

Rabinowitz and Marais (1980) were probably one of the first groups to study the influence of simultaneous metal salt dosing on EBPR systems. The key findings from their work included: (1) chemical phosphorus removal and biological phosphorus removal mechanisms were independent of each other and (2) chemical phosphorus removal was observed (persistence effect) even after cessation of metal dosing in the reactors. However, Lötter (1991) observed that sustained iron dosing reduced biological phosphorus removal capability in activated sludge treatment processes. The author did not provide any explanation regarding the exact mechanism for the decline in phosphorus removal performance. Boyd and Lötter (1993) later hypothesized that inhibition of biological phosphorus removal by ferric salt in EBPR systems was caused by the formation of ferric hydroxide precipitates. The ferric hydroxide precipitates take up hydroxyl ions which are necessary for the hydroxyl-mediated transport process of phosphate across bacterial cell membranes (Boyd and Lötter, 1993). During their research on interactions between the chemical and enhanced biological phosphorus elimination processes, Röske and Schönborn (1994a) concluded that biological phosphorus removal was not affected by low Fe and Al concentrations (up to 3 mg/L) but was out-competed at Al concentrations of more than 6 mg/L. Their conclusion was based on the development of an analytical P-fractionation technique, which could distinguish between biologically and chemically bound phosphate in activated sludge systems. In their second paper of the series, Röske and Schönborn (1994b) reported that the extent of phosphorus release (to the supernatant) under anaerobic conditions was lower in systems operating with simultaneous Fe addition. However, the authors were not able to determine whether this was due to a purely biological effect or to a chemical effect whereby biologically-released P formed a complex with Fe salt in the sludge mass. de Haas *et al.* (2000) have also studied the impact of Al and Fe salts on the EBPR mechanism in activated sludge systems. The authors found that chemical precipitation improved the net P removal of the EBPR processes. However, similarly to the previous research findings, de Haas *et al.* (2000) observed a negative influence of metal salts on the biological phosphorus removal mechanism. Additionally, de Haas *et al.* (2000) noted that inhibition of biological P removal was greater during periods of phosphate limitation (i.e. low effluent P concentration) conditions.

The review of the literature indicates that the research work on interactions between EBPR and chemical phosphorus removal has focused on conventional BNR systems. However, very little is known regarding the influence of metal salt addition on biological phosphorus removal in MBNR systems, where the membrane forms a physical barrier to passage of particulates and the systems are operated at higher MLSS concentrations. Also, a much higher concentration of metal salt will be needed than reported in the studies for LoT effluent TP concentrations. Therefore, research into simultaneous phosphorus removal mechanisms in MBNR systems will provide useful insight for understanding and optimizing phosphorus removal in wastewater treatment facilities.

2.6 Limit of Technology Phosphorus Removal

A total phosphorus (TP) concentration of 0.01 to 0.02 mg/L in effluent is being proposed as a future nutrient removal goal in wastewater treatment facilities (Barnard, 2006; Neethling *et al.*, 2007). However, current biological systems can only achieve effluent TP less than 1.0 mg/L reliably in full scale systems (Neethling *et al.*, 2007). In fact, the lowest effluent TP observed in biological wastewater treatment plants is 0.1 to 0.3 mg/L (Neethling *et al.*, 2007). For that reason, researchers are currently focusing on the coupling of the biological P removal process with other advanced processes to achieve extremely low residual P concentrations. A summary of various process combinations reported in the literature along with the effluent phosphorus limits achievable are shown in Table 2.3.

Table 2.3 Possible limits for phosphorus removal technologies (Adapted from Barnard, 2006)

Process Configurations	**Phosphorus Limits (mg/L)**
Biological treatment with chemical addition + filters	0.09-0.1
Biological or chemical treatment with post chemical + filters	0.05
Membrane reactors with biological and/or chemical treatment	0.04-0.05
Biological treatment plus iron oxide-coated sand filters	0.01-0.02
Reverse osmosis	<0.01

Based on the information in Table 2.3, it can be concluded that apart from the application of reverse osmosis, technological challenges persist in achieving LoT TP limits. Alternatively, Neethling *et al.* (2007) proposed that fundamental understanding of different phosphorus species could help in interpreting and optimizing phosphorus removal technologies. Using standard filtration and chemical analysis, Neethling *et al.* (2007) assessed the different phosphorus species in water and wastewater and compared phosphorus speciation values from different treatment processes. The key conclusions from their work were: (1) advanced tertiary treatment processes achieved approximately 0.02 mg/L effluent TP and (2) refractory dissolved organic phosphorus (rDOP) compounds were the most dominant in the effluent. However, currently, very little is known about the characterization and treatability of rDOP compounds. Therefore, Neethling *et al.* (2007) proposed that rDOP should be given more attention in future phosphorus removal research initiatives.

2.7 MBR Process Modeling

2.7.1 Introduction

The development of the activated sludge process has expanded from carbon oxidation alone, to nitrification, denitrification and enhanced biological phosphorus removal (EBPR). These mechanisms added further complexity via involvement of three different

groups of microorganisms (PAOs, non-PAOs and nitrifying autotrophs) and three distinct environmental regimes (anaerobic, anoxic and aerobic) (WEF MOP 31). Modeling has therefore, become an inherent part of the design of a wastewater treatment plant. The advantages of modeling are the provision of insight into plant performance, process design, trouble shooting and operator training. Nonetheless, successful implementation of models is dependent on the information flow between the models and real world systems as demonstrated in Figure 2.6. Influent data, physical sizes of facilities, operating data, and effluent data are the information engineers/designers can obtain from real world systems. These data can be used in a model (through influent fractioning and plant configuration interfaces) to achieve specific objectives. Subsequently, model information can be used to compare and improve real world system performance. Models can be classified as *mechanistic,* when based on physical description of the process, or *empirical,* when based on quantitative description of the process. Mechanistic models are generally used in activated sludge system modeling.

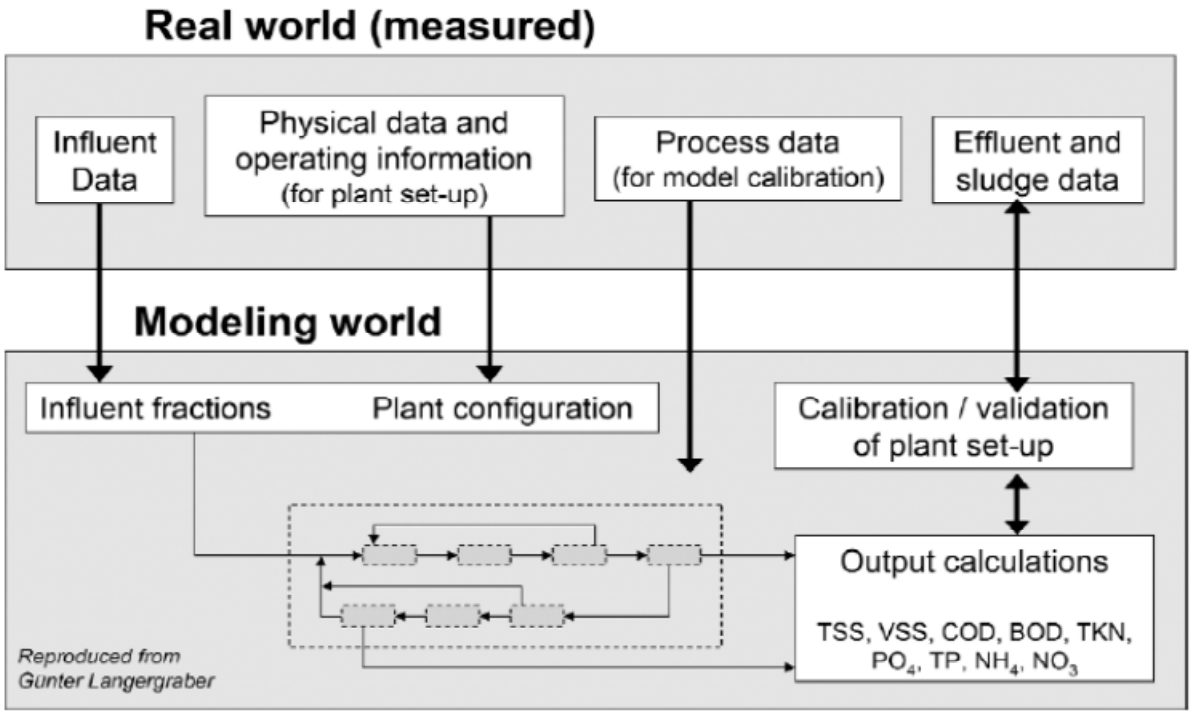


Figure 2.6 Information flow between real world and modeling (Adapted from WEF MOP 31)

The early efforts in developing models for wastewater systems utilized only two state variables where degradation of substrate and formation of biomass was considered with first order kinetics (McKinney, 1962). Research progress in the area of activated sludge enabled modelers to incorporate additional state variables and process descriptions. The model structure was based on Monod-type kinetics. The model was developed for both steady state (Marais and Ekama, 1976) and dynamic simulation environments (Dold *et al.*, 1980). The above work had set the platform for development of the ASM series of models.

The Activated Sludge Model No. 1 (ASM1) is typically considered as the reference model for wastewater treatment systems. ASM1 was developed to describe carbon oxidation, nitrification and denitrification in activated sludge wastewater treatment systems. Influent carbon and nitrogenous compounds were subdivided into different fractions based on biodegradability and solubility. Chemical oxygen demand (COD) was selected to represent the concentration of organic matter in the model. ASM2 was developed to explain biological phosphorus removal in activated sludge systems. The major principles of the bio-P mechanism according to ASM2 are (Figure 2.7):

(1) Growth of PAOs (X_{PAO}) can only occur with cell internal organic matter (X_{PHA}).

(2) The storage of cell internal matter is possible when fermentation products like acetate (S_A) are present in the system. This implies that X_{PHA} storage will only take place in the anaerobic zone of real world activated sludge systems.

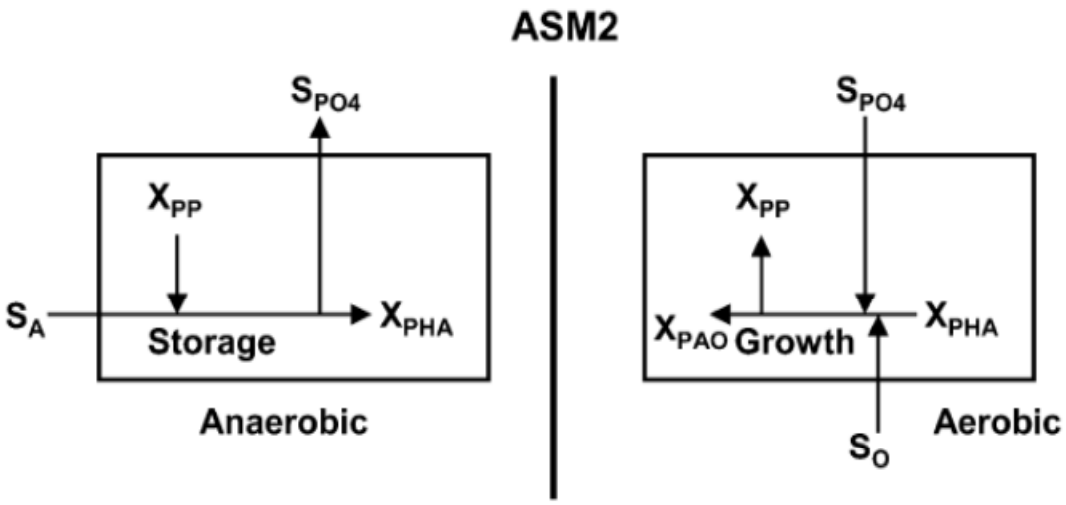


Figure 2.7 Bio-P mechanism described in ASM2 (X_{pp}: Polyphosphate; S_{PO4}: Orthophosphorus; S_O: Oxygen) (Adapted from Henze *et al.*, 1995)

ASM2d was later developed to include the denitrifying abilities of PAOs. Additionally, chemical phosphorus removal via precipitation was introduced in the ASM2 series models. The purpose of ASM3 development was to address three major defects in the ASM1 model (Gujer *et al.*, 1999). First, a single decay process (lysis) had been used in the ASM1 model to explain the decay process in both aerobic and anoxic conditions, while endogenous respiration was the selected mechanism in ASM3. Second, ASM3 recognizes the importance of storage, i.e. all readily biodegradable substrate (S_S) is taken up and stored as X_{STO} in the activated sludge process. Third, the circular growth-decay-growth model (also known as death regeneration model) in the case of ASM1 was replaced with an easy-to-calibrate growth-endogenous respiration model in ASM3 (Gernaey *et al.*, 2004). According to Koch *et al.* (2000), ASM3 performs better when the storage of readily biodegradable substrate is significant (industrial wastewater) or in the case of wastewater treatment plants with substantial non-aerated zones.

Note: The above discussion only addresses the high level concepts of the ASM series models. Complete information regarding model development, structure and influent COD, TN and TP fractionation can be found in Henze *et al.* (2000). Furthermore, a recent review of the models can be found in Hauduc *et al.* (2013).

2.7.2 Application of ASM modeling in MBR Systems

ASM models have been developed and implemented successfully in conventional activated sludge systems (CAS) for the last two decades. In recent years, ASMs have also been used to model membrane bioreactors (Jiang *et al.*, 2009; Lobos *et al.*, 2009; Ng and Kim, 2007; Nopens *et al.*, 2007; Spérandio and Espinosa, 2008; Wintgens *et al.*, 2003). It is important to note that ASMs have been developed for CAS systems operating with SRTs in the range of 3-15 d, an HRT range of 3-5 h and an MLSS range of 1.5-4 g/L (Tchobanoglous *et al.*, 2003). On the other hand, Itokawa *et al.* (2008) reported that MBR plants are operating with HRT between 4 and 6 h (13 plants), MLSS in the range of 7 to 13.5 g/L (11 plants), and an SRT between 15 and 40 days (7 plants) in European municipal plants. Fenu *et al.* (2010) subsequently suggested that MBRs can have different kinetic and stoichiometric values due to high sludge retention times, high mixed liquor concentration, accumulation of soluble microbial products (SMP) rejected by the membrane filtration step, and high aeration rates for scouring purposes. It has also been hypothesized that complete sludge retention can affect biomass population selection, settling characteristics and growth kinetics (Parco *et al.*, 2006) and biomass with a higher substrate affinity and lower growth rate may have a competitive advantage over those with a lower substrate affinity and higher growth rate in MBR systems (Jiang *et al.*, 2009). Due to the fundamental difference in operating conditions, various research groups have evaluated and estimated (both experimentally and with a trial-error approach) the suitability of ASM in MBR solids production, nitrification, denitrification and phosphorus removal.

Net sludge production typically depends on non-biodegradable particulate COD (X_I), heterotrophic yield (Y_H) and heterotrophic decay rate (b_H). As sludge production estimation is an important goal of modeling, the determination of correct values for the above parameters is crucial for MBR systems. According to Fenu *et al.* (2010), the high SRTs in MBRs can cause hydrolysis of the particulate COD component that is generally considered to be inert in CAS and as a result, can influence the suspended solids concentration. However, Witzig *et al.* (2002) did not observe hydrolysis in their work and

postulated that bacteria went into maintenance mode at high sludge ages. From a modeling perspective, the maintenance mode impacts the sludge yield in the same manner as hydrolysis. Nonetheless, accurate determination of X_I can be done effectively by comparing measured and predicted sludge production values in wastewater treatment system (Henze *et al.*, 2000).

Jiang *et al.* (2005) reported the Y_H value to be 0.72 g COD/g COD (at 23 ^{0}C) by conducting respirometric measurements with acetate. On the contrary, Zhang and Hall (2006) experimentally determined (with influent municipal wastewater) a Y_H value of 0.5 g COD/g COD in their membrane enhanced biological phosphorus removal (MEBPR) system. They reported a higher heterotrophic yield value (=0.59 g COD/g COD) for a parallel conventional enhanced biological phosphorus removal (CEBPR) system. Since the nature of the carbon source present in influent wastewater can impact the Y_H value, Fenu *et al.* (2010) suggested that a range of 0.63-0.67 g COD/g COD can be used for MBR systems. Zhang and Hall (2006) also determined heterotrophic decay rate (b_H) with the batch respirometric method of Ekama *et al.* (1986), and reported 0.24 d^{-1} and 0.31 d^{-1} for MEBPR and CEBPR respectively. These values are lower than the default for b_H in ASM2 (=0.4 d^{-1}). Jiang *et al.* (2005) observed a similarly low b_H (= 0.25 d^{-1}) value in their MBR system. A summary of studies on MBR system non-biodegradable particulate COD (X_I), heterotrophic yield (Y_H) and decay rate (b_H) is presented in Table 2.4.

Due to the very sensitive nature of autotrophs to environmental conditions, their performance in nitrogen removal has been widely studied in MBR systems. While Monti and Hall (2008) observed that nitrification rate was 15 to 75 percent greater in CAS as compared to a parallel MBR system, other researchers have reported that nitrifier growth was significantly higher in a submerged MBR and nitrification was more effective and stable than in a CAS (Gao *et al.*, 2004; Munz *et al.*, 2008; Parco *et al.*, 2006). Though Manser *et al.* (2005) did not find any difference in MBR and CAS system maximum specific ammonium uptake rates, the MBR system performed better during transient shock loads, especially at low temperature and relatively low dissolved oxygen (DO).

The discrepancy in nitrifier activity in MBR vs CAS can be explained by hypotheses such as difference in microbial population selection, bioavailability of substrates due to the smaller size of flocs in MBRs (Manser *et al.*, 2005) and their tendency to grow in clusters in different areas of the floc (Fenu *et al.,* 2010). Along with the experimental work, researchers have attempted to model MBR nitrification with default ASM parameters. Jiang *et al.* (2005) observed autotrophic yield value (Y_A) of 0.25 g N/g COD, which is closer to the ASM default value of 0.24 g N/g COD. Manser *et al.* (2005) reported similar ammonium-oxidizing bacteria (AOB) decay values (b_A) (0.13 d^{-1}) for both CAS and MBR systems. The authors however found nitrate-oxidizing bacteria (NOB) decay rate to be slightly different with 0.28 d^{-1} for CAS and 0.17 d^{-1} for MBR systems. The influence of autotrophic growth rate (μ_A) and decay rate (b_A) was studied by Sperandio and Espinosa (2008), whereby they calibrated these parameters for a wide range of SRTs. They concluded that ASM1 default values (0.8 d^{-1}; 0.04 d^{-1}) overestimated ammonium removal for all the SRTs studied, whereas ASM3 (1 d^{-1}, 0.15 d^{-1}) gave better results but minimized the SRT influence. The authors proposed new values for nitrifier growth rate (μ_A = 0.45 d^{-1}) and decay rates (b_A = 0.04 d^{-1}) for MBRs. Another approach to nitrification modeling has been to calibrate half saturation constants K_{NH} and K_{OA}, which directly influence the model effluent ammonium concentration. The calibration is primarily based on the principle that lower transfer resistance is observed in MBRs due to smaller floc size. Nonetheless, Fenu *et al.* (2010) suggested that selection of K_{NH} and K_{OA} values should depend on careful examination of operational parameters such as SRT, MLSS concentration, viscosity, oxygen concentration and floc size distribution. Table 2.4 summarizes different studies focusing on MBR nitrification modeling along with their calibration data.

Modeling of denitrification in MBR systems with default ASM parameters has received limited attention from researchers. The general conclusion has been that denitrification is not affected by membrane configuration and hence, default values for reduction factor for anoxic growth, and the anoxic heterotrophic yield can be used for modeling (Fenu *et al.*, 2010; Parco *et al.*, 2007). Parco *et al.* (2007) demonstrated that the denitrification rate in an MBR system was similar to that of CAS process (= 0.25 mg

NO_3/mg SS·d) while operating with an SRT of 20 days and mass load of 0.14 g COD/g MLSS·d. However, it is important to note that process configurations with sludge recirculation directed from the DO-saturated membrane tank to the anoxic tank can negatively impact denitrification potential (Sarioglu *et al.*, 2008). This mechanism can be addressed by calibrating the parameter K_{OH} (Table 2.4).

Table 2.4 Model parameters from literature on MBR in municipal wastewater treatment (Modified from Fenu *et al.*, 2010)

	Model Parameter	**Unit**	**Default ASM1**	**Jiang *et al.*, 2005**	**Zhang and Hall, 2006**	**Spe´randio et al., 2005**	**Manser *et al.*, 2005**	**Jiang 2007**	**Sarioglu *et al.*, 2008**	**Delrue, 2008**	**Jimenez *et al.*, 2008**	**Erftverband 2001, 2004**	**RWTH 2008**	**Range of values**
				ASM1	ASM2	ASM1; ASM3	ASM1	ASM2d	ASM1 endogenous decay model	ASM1	ASM1 modified	ASM1	ASM1	
	SRT	D		20	17-25	10-110	20		38	30-60	15			
Nitrification	μ_{maxA}	d^{-1}	0.8			0.45			1		0.8			0.45-1.00
	b_a	d^{-1}	0.05-0.15	0.08		0.04		0.055	0.06		0.15			0.04-0.15
	K_{NH}	mg N-NH_4/l	1			0.25-0.6		0.2	2	1		0.1		0.10-2.00
	K_{OA}	Mg O_2/l	0.4				0.18	0.2	1.25	1				0.18-1.25
	Y_A	g COD/ g N	0.24	0.25										0.24
Denitrification, COD oxidation, Sludge production	% X_I	% COD	15								17.5			
	Y_H	g COD/ g COD	0.67	0.72	0.50				0.66			0.67	0.52-0.9	0.63-0.67
	b_H	d^{-1}	0.62	0.25	0.24			0.4	0.24				0.03-0.47	0.24-0.4
	$K_{O,NOB}$	mg N/l	0.5				0.13		2		1			0.13-2
	K_{OH}	mg O_2/l	0.2				0.05		1		0.22			0.05-1

Research efforts in the area of MBR EBPR modeling with default ASM parameters have provided mixed results (Cosenza *et al.*, 2013; Zuthi *et al.*, 2013). Parco *et al.* (2007) found that MBR system anaerobic P-release rate, acetate consumption rate, anoxic P-uptake rate and aerobic P-uptake rate were very close to CAS EBPR rates with mixed cultures. In addition, they reported that different volatile suspended solids concentrations have no impact on the above mentioned rates. The authors subsequently concluded that EBPR kinetic parameters are comparable in both MBR and CAS systems. On the other hand, modeling work by Jiang *et al.* (2008) with default ASM2d parameters resulted in overestimation of nitrate concentration and underestimation of phosphorus concentration. Using a trial and error calibration approach, they simultaneously reduced anaerobic acetate production and the aerobic/anoxic phosphorus uptake rate (q_{fe} = 1 d^{-1}, q_{pp} =1.1 d^{-1} and $\eta_{NO3,PAO}$ = 0.4) for data fitting.

Both EBPR and chemical phosphorus removal is being used increasingly in WWTPs and studies related to their interplay have been summarized in **Section 2.5.4**. Modeling work in this area can provide further insight into the process phosphorus removal performance, optimization of metal salt dosage and the viability of EBPR when higher concentrations of metal salt are added for achieving the effluent objectives (in the context of LoT phosphorus removal). Liu *et al.* (2011) used an activated sludge model combined with a chemical precipitation model to study the impact of alum on biological phosphorus removal, nitrification and denitrification in an MBR system targeting 0.025 mg/L TP in the effluent. The authors conducted their experimental work in a pilot scale UCT MBR system (SRT of 51 days and alum dosage of 17.5 mg/L) and modeling work was completed in BioWin^{TM}. The two major conclusions from their work were (1) sludge production, COD, nitrification and denitrification performance were predicted reasonably by calibrating only AOB growth rate and (2) alum dosing, as predicted, inhibited EBPR while the measured data did not provide such evidence. However, the authors did not provide detailed information on EBPR activity such as anaerobic release and aerobic uptake potential of PAOs before and after alum addition.

This literature review shows that current MBR process modeling is predominantly based on experience with conventional activated sludge models and researchers have successfully implemented these with calibration of stoichiometric and kinetic parameters. However, LoT nutrient removal will require modeling of nitrification, denitrification by both influent and external carbon addition, EBPR and chemical phosphorus removal. This very complex task will require detailed understanding of the predicted vs measured data, careful calibration of the parameters based on current literature and process knowledge and conclusions regarding the process mechanisms and performance.

2.8 Conclusions

Based on the literature review presented above, following conclusions can be drawn.

Currently known

- The future BNR technology will move in the direction of achieving very low effluent nitrogen (i.e. $\leq$ 3 mg TN/L) and phosphorus (i.e. $\leq$ 0.1 mg TP/L) concentrations. Membrane biological nutrient removal (MBNR) is a novel technology that can contribute to the achievement of these goals. Nevertheless, external carbon and metal salt supplementation has been proposed as necessary to meet stringent nitrogen and phosphorus discharge limits respectively, in MBNR systems.
- Metal salt addition is imperative when an EBPR system is targeting very low effluent TP concentrations. The relationship between the two simultaneous phosphorus removal mechanisms has been investigated in conventional BNR systems and some results indicate inhibitory effects on the bio-P mechanism. It is however important to mention that the impact is dependent on the added metal salt concentration.
- ASM models have been developed and implemented successfully in conventional activated sludge systems (CAS). MBRs, on the other hand, may require different kinetic and stoichiometric values due to the long sludge retention times, high mixed liquor suspended solids concentrations, the accumulation of soluble microbial products (SMP) rejected by the membrane filtration step, and the high aeration rates

used for membrane scouring purposes. Researchers have used both experimental and trial-error approach to estimate parameters for sludge production, nitrification, denitrification, EBPR and chemical phosphorus removal in MBR systems.

- Effluent dissolved organic nitrogen (DON) concentration becomes the dominant nitrogen fraction (varying from 0.4 to 2.2 mg/L) for wastewater treatment systems targeting LoT nitrogen removal. That means effluent should not contain combined ammonium, nitrates or nitrites of more than 1 to 1.5 mg/L. For design considerations, complete nitrification and denitrification, aided by supplementation with an external carbon source, are necessary.
- DON in WWTP effluent can either be calculated by subtracting ammonium from soluble Kjeldahl nitrogen (sol-TKN) or by subtracting dissolved inorganic nitrogen (DIN) from the total dissolved nitrogen (TDN) concentration. However, when effluent concentrations are close to the limits of technology level (≤ 3 mg TN), this will represent significant analytical challenges in terms of detection limits, measurement precision and expensive instrumental methods. Recently, a combined column anion exchange resin (for residual nitrate removal) and persulfate digestion (for conversion of DON to nitrate) method has been proposed for reliable measurement of effluent DON concentrations.

Knowledge gaps

- MBRs in conjunction with conventional nitrification/denitrification and EBPR have been demonstrated to be successful in recent years. However, the LoT effluent goal could potentially push a system to limits of its capability. Currently, little information exists regarding performance of an MBR system in such a scenario. Moreover, the significance of external carbon and metal salt dosing for enhanced denitrification and phosphorus removal respectively, has not been explored in the context of the LoT objective.
- The interactions between simultaneous biological and chemical phosphorus removal are often very complex and poorly understood. Due to the poor reliability of EBPR in meeting ≤ 0.1 mg/L TP goal consistently, metal salt might be added at greater than

stoichiometric requirements. In addition, the longer SRTs maintained in MBNR systems will enable build up of metal salt inventory. The impact of such levels of metal salt dosing on EBPR and nitrogen removal is currently unknown.

- ASM modeling can be very useful in predicting nitrogen and phosphorus removal capabilities of a selected MBNR configuration. In particular, the requirement for and the efficiency of external carbon and metal salt dosing can be assessed. Nevertheless, there is very little in the current literature describing efforts to model an MBNR system targeting LoT effluent concentrations.
- The importance of the DON fraction in LoT level TN effluent has been discussed extensively. However, a knowledge gap exists regarding how it is produced or utilized in reactors of an MBNR system. Moreover, high alum dosing, originally meant for LoT phosphorus removal, can potentially reduce DON concentrations in permeate (via a coagulation effect). This theory has not been investigated yet. Another key issue regarding DON is the development of a direct and reliable measurement method, which can be used to process bulk samples from reactors and permeates. The direct measurement method that has been proposed recently is ill equipped for the processing of large numbers of samples.

3 Research Objectives

Four fundamental research questions were developed following the literature review.

1. **Is external carbon and metal salt dosing a significant requirement in an MBNR system targeting LoT TN and TP removal?**
   i. Could LoT level nitrogen and phosphorus removal be achieved in an MBNR system without supplemental additions of carbon and/or metal salt?
   ii. If external carbon dosing is needed, is the stoichiometric requirement known for achieving extremely low effluent nitrate levels?
   iii. If external metal salt dosing is needed,
      a. Is the stoichiometric requirement known for achieving extremely low effluent phosphorus levels?
      b. Is the relationship with EBPR defined by dosing concentrations?
      c. Does it influence COD removal, nitrification and denitrification?

2. **Do EBPR kinetics become progressively inhibited when alum addition to the mixed liquor of an MBNR system is increased from small dose to a high dose in a stepwise manner?**

3. **Does ASM-based modelling successfully predict the influence of external carbon and metal salt dosing on COD, nitrogen and biological phosphorus removal performance of an MBNR system targeting LoT effluent goals?**

4. **Is there a direct method that might be applied to measure DON concentrations reliably in an MBNR system targeting LoT nitrogen goals?**

4 Materials and Methods

4.1 Introduction

The methodology for the project is presented in four parts: (1) design, construction and operation of two parallel laboratory scale MBNR systems (2) off-line sequential anaerobic/aerobic batch tests, (3) modeling of the MBNR system and (4) development of a reliable and direct technique for DON measurement. The following sections provide detailed descriptions of the methodologies.

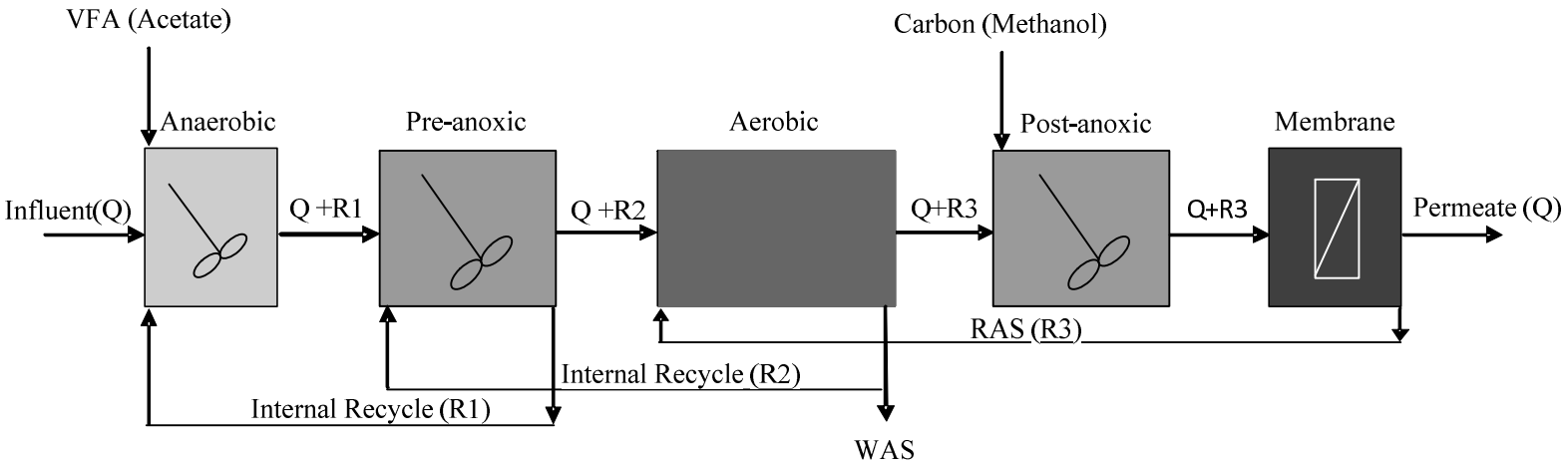
4.2 MBNR System

4.2.1 Design and operation

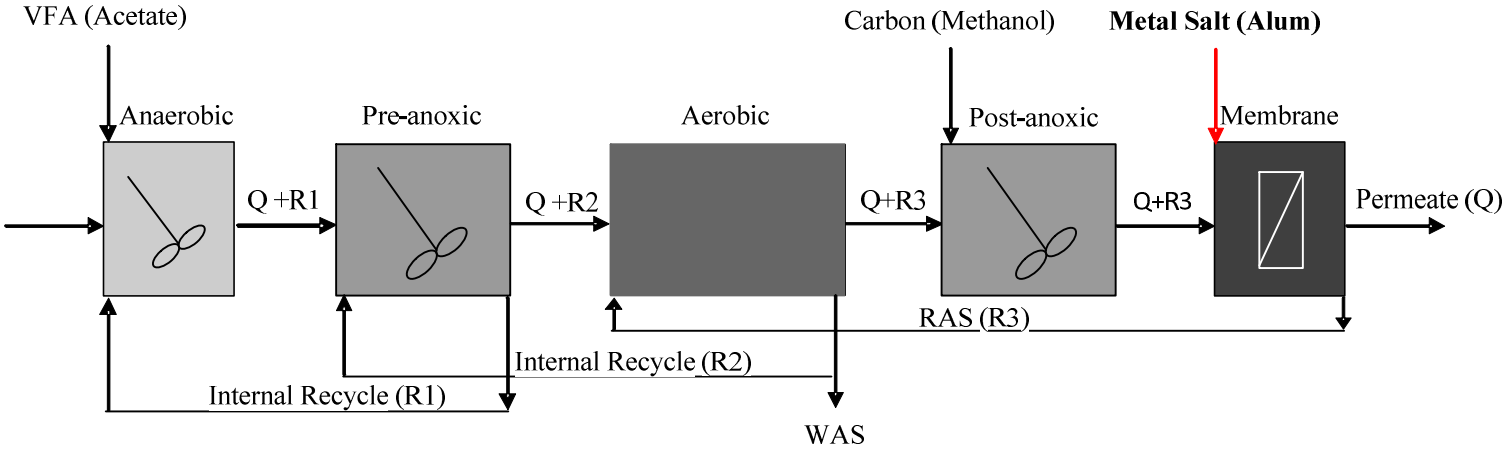
There are a number of process configurations that can be used for enhanced BNR along with membranes for solids-liquid separation. For the present study, the chosen process configuration was a modified Bardenpho-type reactor with five different zones: (1) anaerobic, (2) pre-anoxic, (3) aerobic, (4) post-anoxic and (5) membrane tank. The following modifications were made to the conventional configuration:

- The 2nd aerobic reactor was replaced by a membrane tank which also functioned as solids-liquid separator;
- The return activated sludge (RAS) flow from the membrane tank was directed to the aerobic zone, rather than to the upstream anaerobic zone;
- A second recirculation flow was added from the pre-anoxic zone to the anaerobic zone.

The two parallel MBNR systems were operated under similar process conditions, with metal salt addition being the only difference as shown in Figure 4.1.



a. MBNR (Biological) system



b. MBNR (Chemical) system

Figure 4.1 Schematic of parallel MBNR systems

The MBNR systems were studied at lab-scale with municipal wastewater as the influent to each treatment line. The influent was municipal wastewater collected from the Staging Environmental Research Centre (SERC), South Campus, University of British Columbia. Initially, the wastewater was collected two times per week and stored in the refrigerator at 4 ^{0}C. However, unsteady influent VFA concentrations (which impact EBPR performance) and the need for data for steady state modeling necessitated the shifting of influent collection to once per week on operation day 193. The influent supply to the MBNR systems was renewed every day. The common influent tank had a mixer operating continuously that provided gentle mixing to keep particles in suspension. Also, the influent line was provided with a fine screen (pore size 1 mm) to hold back material that might clog the membranes. The whole set-up was operated in a temperature controlled-room at a constant temperature of 20 ^{0}C that was maintained during the whole study period. All the reactors were cylindrical in shape and built from plexiglass. The forward flow of the wastewater was achieved by gravity and peristaltic pumps were installed for influent flow, recycle flow, permeation and nutrient addition. The flow rate of the influent was controlled by level sensors employed in the membrane tank. The sensors facilitated on/off control of both the influent and permeate pumps depending on the mixed liquor level in the membrane tank. The rationale was to stop overflow of mixed liquor or emptying of the membrane tank during off-hours and weekends. Mixers were placed in all the reactors to achieve ideal continuous stirred tank reactor (CSTR) conditions, with the exception of the membrane tanks. Air supply to the aerobic reactor was intermittent in the MBNR systems. Using an on-line dissolved oxygen (DO) probe (calibrated), solenoid valve and a controller, the set point was fixed at 2 mg/L. The on-line DO probe reading was compared with an external DO probe value on a daily basis and it was cleaned periodically to avoid potential oxygen depletion in the aerobic reactor. The membrane tank received a constant air supply of 8 L/minute which resulted in oxygen-saturated mixed liquor in the membrane tank.

The design operating conditions of the bench-scale MBNR systems are summarized in Table 4.1. The reactor volumes were calculated based on the design net flow and the desired HRT. The MBNR systems were operated with constant SRT and variable MLSS

concentration during the whole study period. **APPENDIX A** provides detailed information on the sizing of the reactors based on net flow and HRT and mixer dimensions. Furthermore, photographs are included to illustrate different component of bench scale parallel MBNR systems.

Table 4.1 Design operating parameters of the bench-scale MBNR system

Parameter	Value
Net flow (Q)	33.45 L/day
Anaerobic reactor volume	2.5 L
Pre-anoxic reactor volume	5.6 L
Aerobic reactor volume	14.65 L
Post-anoxic reactor volume	5.6 L
Membrane tank volume	1.5 L
Total hydraulic retention time (HRT)	13.83 h
Anaerobic HRT	1.5 h
Pre-anoxic HRT	3 h
Aerobic HRT	6 h
Post-anoxic HRT	3 h
Membrane HRT	1/3 h
Solids retention time (SRT)	25 days
Temperature	20 ^{0}C
Membrane module	ZW-1, submersible
Membrane pore size (nominal)	0.04 µm
Membrane flux	15 L/m$^{2.}$hr
Membrane Operation	Relaxation Mode (5 min ON/1 min OFF)

During the experimental work, MBNR operating data were collected manually each day and recorded in log sheets. The log sheets were also used to record major operations and maintenance events for reference. The parameters monitored were as follows:

- Date
- pH (influent, reactors and permeate) (by using portable pH Testr BNC (r))
- DO (aerobic reactor) (by using portable Hach HQ30d DO probe)
- Permeate Flux
- Transmembrane pressure (TMP) before/during/after relaxation

- Sludge wasting
- Recycle rates (once/week)

The sampling schedule for the MBNR systems is shown in Table 4.2 and Table 4.3. The schedule was developed to obtain a consistent and comprehensive evaluation of the performance of the MBNR systems. Influent and effluent grab samples were collected on Mondays, Wednesdays and Fridays for COD, ammonium, nitrate/nitrite and orthophosphate analysis. The same schedule was followed for VFA in the influent and the anaerobic zone of both MBNR systems. On the other hand, samples for TKN, TP and reactor scan were collected twice i.e. on Mondays and Fridays. Measurement of total suspended solids (TSS)/ volatile suspended solids (VSS) was undertaken only on Fridays.

Table 4.2 Influent/Effluent monitoring program

Parameter	Number of sampling events/week	Influent	Permeate
Total suspended solids (TSS)/ Volatile suspended solids (VSS)	1	1	-
Volatile fatty acid (VFA)	3	1	-
Total COD (COD_{Tot})/ Filtered Flocculated COD (COD_{FF})/ Soluble COD (COD_{Sol})	3	1	1
Total Kjeldahl nitrogen (TKN)/ Dissolved TKN	2	1	1
NH_4-N	3	1	1
NO_3-N	3	1	1
Total phosphorus (TP)/ Dissolved TP	2	1	1
PO_4-P	3	1	1

Table 4.3 Reactor scan schedule

Parameter	Number of sampling events/week	Anaerobic Zone	Pre-anoxic Zone	Aerobic Zone	Post-anoxic Zone	Membrane Tank
VFA	3	1	-	-	-	-
NH_4-N	2	1	1	1	1	1
NO_3-N	2	1	1	1	1	1
PO_4-P	2	1	1	1	1	1

4.2.2 Sample analysis

Table 4.4 shows detailed information on final sample volume, preservation and chemical analysis procedures

Table 4.4 Detail of sample analysis procedure

Parameter	Sample Volume	Filtration (0.45 µm)	Preservation	Chemical Analysis (APHA *et al.*, 2005)	Equipment Information
COD_{Tot}/ COD_{Sol}/ COD_{FF}*	2 mL	No for COD_{Tot}/; Yes for COD_{Sol}/COD_{FF}		5220 D. Closed Reflux, Colorimetric Method	Hach DR/2000 Direct Reading Spectrophotometer
VFA	1-2 mL	Yes	2% Phosphoric acid solution (H_3PO_4)	5560 D. Gas Chromatography Method	HP 5890 Series II Gas Chromatograph FID (Flame Ionization Detector)
NH_4-N	5-8 mL	Yes	5 % Sulfuric acid solution (H_2SO_4)	4500-NH_3 G. Automated Phenate Method	Lachat QuikChem 8000 Flow Injection Analyzer
NO_3-N	5-8 mL	Yes	0.1 g mercuric acetate in 20 mL acetone and 80mL water solution	4500-NO_3^-F. Automated Cadmium Reduction Method	Lachat QuikChem 8000 Flow Injection Analyzer
PO_4-P	5-8 mL	Yes	0.1 g mercuric acetate in 20 mL acetone and 80mL water solution	4500-P F. Automated Ascorbic Acid Method	Lachat QuikChem 8000 Flow Injection Analyzer
TKN	10 mL- Influent 20 mL- Effluent	No	5 % Sulfuric acid solution (H_2SO_4)	4500-N_{Org} D. Block Digestion and Flow Injection Analysis	Lachat QuikChem 8000 Flow Injection Analyzer
TP	10 mL- Influent 20 mL- Effluent	No	5 % Sulfuric acid solution (H_2SO_4)	4500-P H. Manual Digestion Method	Lachat QuikChem 8000 Flow Injection Analyzer
TSS/VSS	10 mL	Yes (with 1.2 µm filter)	Analyzed directly after sampling	2540D. Total Suspended Solids / 2540 E. Fixed and Volatile Solids	

*: COD_{FF} (flocculated filtered COD) sample preparation was completed by adopting the Mamais *et al.* (1993) methodology. The first step was flocculation of 100 mL influent wastewater samples with 1 mL of 100 g/L $ZnSO_4$. Then, the pH of the mixed sample was adjusted to approximately 10.5 with 6 M sodium hydroxide solution. After a few minutes of settling, the final step was filtration with 0.45 µm membrane filter.

4.2.3 Process start-up

The bench scale system was seeded on 18th February, 2010 with mixed liquor from the full scale BNR WWTP at Salmon Arm, British Columbia. The Salmon Arm treatment plant employs a trickling filter and a suspended growth system combination for ammonium and phosphorus removal respectively. The volume of mixed liquor transported to UBC was sufficient to fill the reactors to their designed HRT (Table 4.1) levels. The MBNR systems were operated in continuous mode from day 1 with municipal wastewater providing the required nutrients. pH, DO and flow rates were closely monitored from the beginning. Initially, the systems shown in Figure 4.1 were six stage processes with a second aerobic tank between the post-anoxic reactor and the membrane tank. After review of the literature, it was decided that the 2nd aerobic tanks would not contribute to LoT nutrient removal and they were removed on 10th May, 2010. In addition, there were many modifications in the process recycle rate, the VFA addition to the anaerobic tank and the membrane tank sizing during the period between 18th February, 2010 and 10th May, 2010. Since these modifications were expected to impact steady state operation, the results for that period are not discussed here. The two systems were reset by stoppage of wasting on 11th May, 2010 and hence, became the de facto operating day 1 of this project. Wasting did not begin again until day 45. The intention was to operate a high MLSS steady state system, considered typical for MBR configurations.

4.2.4 Recycle rates and nutrient supplementation

Determination of recycle rate is a very important design consideration for MBNR system nutrient removal objectives. For better solids distribution in the reactors and reduced solids load on the membrane tank, anoxic (IR1), aerobic (IR2) and returned activated sludge (IR3) recycle rates were initially kept high, with values of 2Q, 3Q and 4Q respectively. On the other hand, higher recycle rates increase the probability of elevated nitrate concentrations in the anaerobic tank which could inhibit EBPR activity.

For that reason, nitrate was monitored in the anaerobic and pre-anoxic reactors with the results shown in Figure 4.2 and Figure 4.3 respectively.

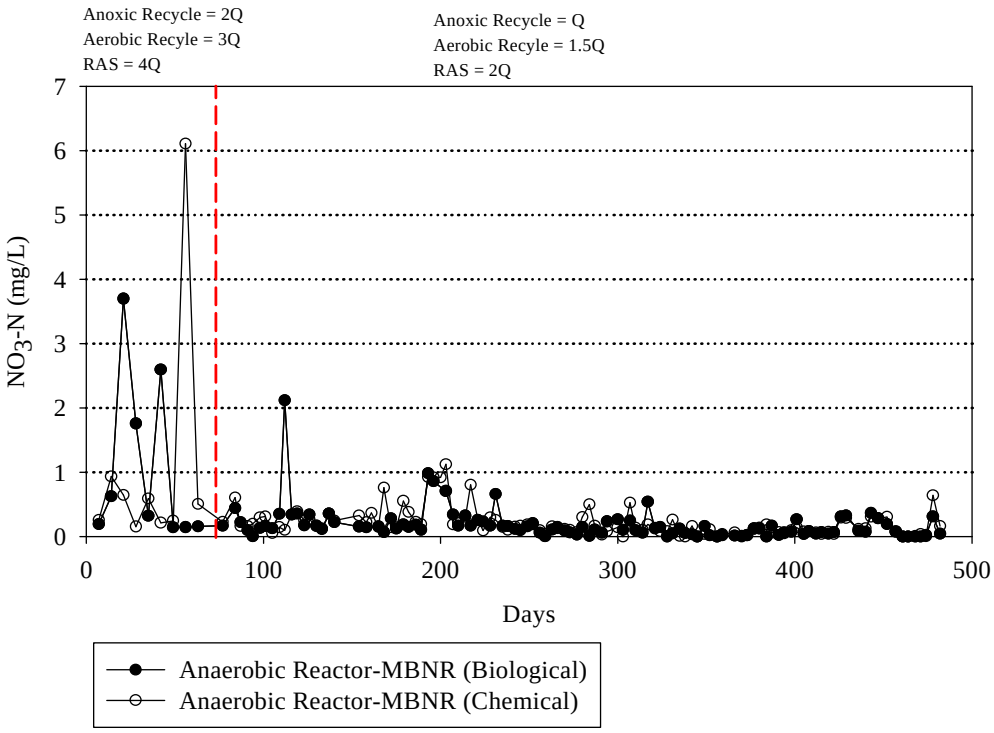


Figure 4.2 NO_3-N profiles in anaerobic reactor of parallel MBNR systems

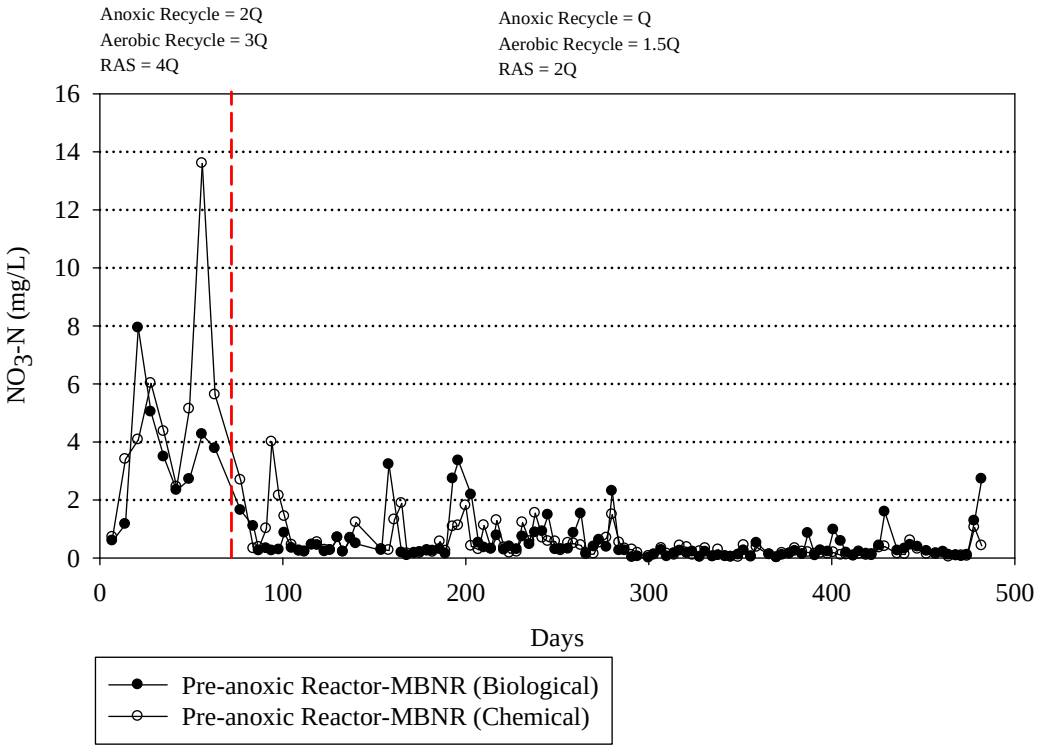


Figure 4.3 NO_3-N profiles in pre-anoxic reactors of parallel MBNR systems

The figures clearly demonstrate the presence of nitrate in both zones until day 72, which indicates that the systems were not optimized for EBPR activity. This led to the decrease in all the recycle rates by 50 percent on Day 73. The reduced recycle rates were maintained for the remainder of the studies. The modification resulted in very low and stable nitrate concentrations in both reactors thereafter.

The parallel MBNR systems were supplemented with methanol (external carbon) and acetate (VFA), vital nutrients for enhanced denitrification and biological phosphorus removal respectively. Post-anoxic reactor methanol dosing began on day 80 and the initial target concentration after addition was 24 mg/L. The selection of the initial methanol supplementation concentration was based on measured average permeate nitrate concentrations of the parallel MBNR systems during first 79 days of operation (pre-methanol dosing period). As described in **Section 5.3.3.**, the average permeate nitrate concentrations for that period were 11.3 and 13 mg/L for the MBNR (Biological) and MBNR (Chemical) systems respectively. To begin with, an approximate methanol dosing

to permeate nitrate ratio of 2 was chosen and was increased in a step-wise manner to 3 (methanol dosing of 36 mg/L), 4 (methanol dosing of 48 mg/L) and finally 6 (methanol dosing of 72 mg/L). Acetate supplementation was utilized from day 1 so that EBPR activity would not be affected by *carbon-limited* conditions. Initially, acetate was added to the mixed liquor directly to produce a nominal initial concentration of 80 mg COD /L in the anaerobic reactor of the parallel MBNR systems. Selection of acetate dosing concentration was done with the singular objective of promoting EBPR activity in a VFA-rich environment. However, past pilot research work at UBC has demonstrated excellent EBPR with only 40 mg COD/L of acetate added to the anaerobic zone (Monti, 2006). Although the previous study was carried out with a modified UCT system, the wastewater characteristics were comparable to those of the present study. From day 73 onwards, acetate addition was reduced to 40 mg COD/L in both systems. Alum was the preferred metal salt for the MBNR system shown in Figure 4.1b. For this project, it was postulated that a functional EBPR process would be necessary to analyze the impact of alum in the MBNR (chemical) system. Accordingly, addition of alum did not start until day 226. The initial membrane tank alum dosage was 20 mg/L and was increased in a step-wise mode until effluent TP ~ 0.1 mg/L was achieved. Figure 4.4 and Table 4.5 illustrate the different phases and concentrations of the MBNR system operation in terms of acetate, methanol and alum supplementation respectively. Nitrogen and phosphorus removal performance of the parallel MBNR systems will be discussed with respect to each phase of operation.

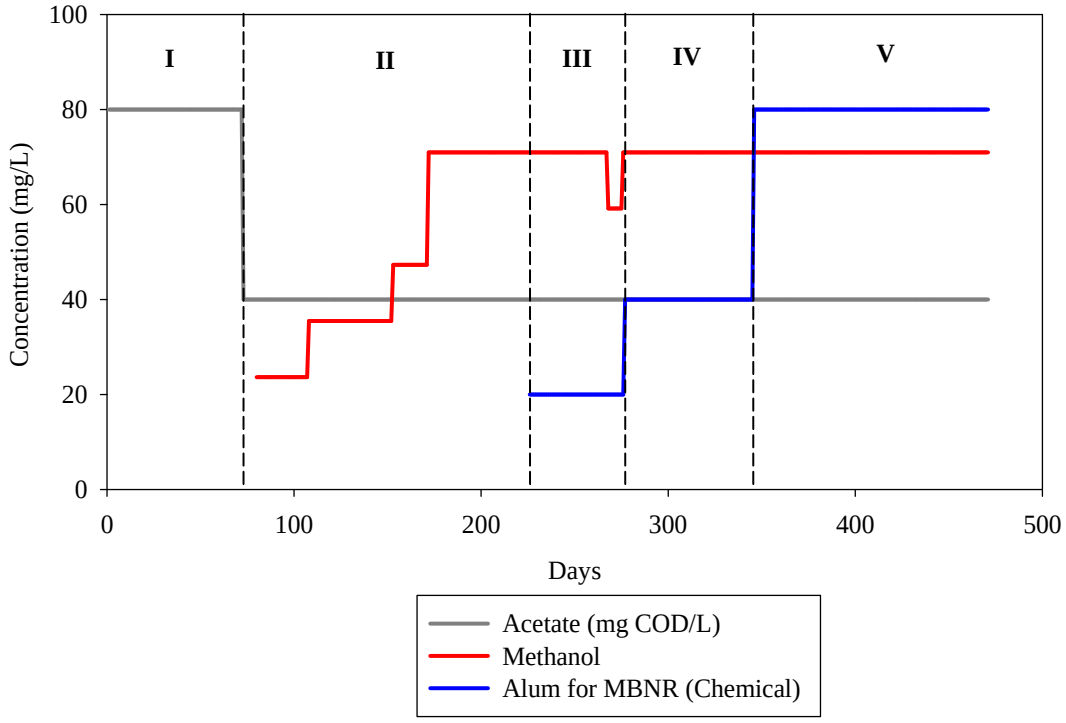


Figure 4.4 Different phases of MBNR operation w.r.t. acetate (anaerobic reactor), methanol (post-anoxic reactor) and alum (membrane tank) dosing

Table 4.5 Dosing set-points for acetate, methanol and alum supplementation during different phases of MBNR operation

Parameter	Days	Recycle Flow	Acetate (mg/L)	Methanol (mg/L)	Alum (mg/L) in MBNR (Chemical)
Phase I	1-73	Anoxic = 2Q Aerobic = 3Q RAS = 4Q	80	0	0
Phase II	74-225	Anoxic = Q Aerobic = 1.5Q RAS = 2Q	40	24-72	0
Phase III	226-276	Anoxic = Q Aerobic = 1.5Q RAS = 2Q	40	72	20
Phase IV	277-345	Anoxic = Q Aerobic = 1.5Q RAS = 2Q	40	72	40
Phase V	346-480	Anoxic = Q Aerobic = 1.5Q RAS = 2Q	40	72	80

4.3 Off-line Batch Tests

The relationship between enhanced biological phosphorus removal and chemical phosphorus removal was also investigated by conducting off-line batch tests in the laboratory. Mixed liquor from the aeration tanks of the parallel MBNR systems was subjected to sequential anaerobic-aerobic conditions during the tests. The first tests were conducted in Phase II of the program when the MBNR (Chemical) system was not supplemented with alum. Further tests were conducted in Phase III, IV and V to evaluate EBPR under a step-wise increase in alum addition. Three batch tests were conducted in each phase to document the EBPR activity with time. The batch test set-up is shown in Figure 4.5 and the methodology is described below.

4.3.1 Methodology

The batch test set-up was comprised of a 1.0 L Erlenmeyer flask, magnetic stirring bar, a rubber stopper seal with septa, pH probe, sampling tube and nitrogen-filled balloon. A nitrogen-filled balloon was used to compensate for the volumes of sampled liquid and to prevent oxygen intrusion to maintain anaerobic conditions (Comeau, 1989). The test temperature and pH were constant at 20 ^{0}C and 7.0 respectively. Depending on the measured mixed liquor pH value, either 0.1 N HCl or 0.1 N NaOH was added to maintain the set-point pH. A VWR portable probe was used for mixed liquor pH and temperature measurement. An initial non-aerated period of 2-4 hours was maintained for endogenous denitrification (with mixing). The duration of the non-aerated period was dependent on the initial nitrate concentration in the mixed liquor. The objective of this procedure was to prevent any denitrification-related carbon consumption during the tests themselves. After the completion of endogenous denitrification, N_2 was introduced to the batch reactor to rapidly establish anaerobic conditions.

The anaerobic period was maintained for 2 hours. Acetate was added to the mixed liquor to produce a nominal initial concentration of 100 mg COD /L for optimal EBPR performance. The first sample was collected 1-2 minutes after substrate addition for

measuring acetate, NO_3-N, Mg^{2+}, K^+ and PO_4-P concentrations. Further samples were taken every 30 minutes. Following the anaerobic period, an aerobic phase was imposed of 3 hours duration. The N_2 gas was replaced by air sparging and the DO concentration was maintained between 2.0-3.0 mg/L during the aerobic period. Sampling parameters and frequency were similar to those of the anaerobic period.

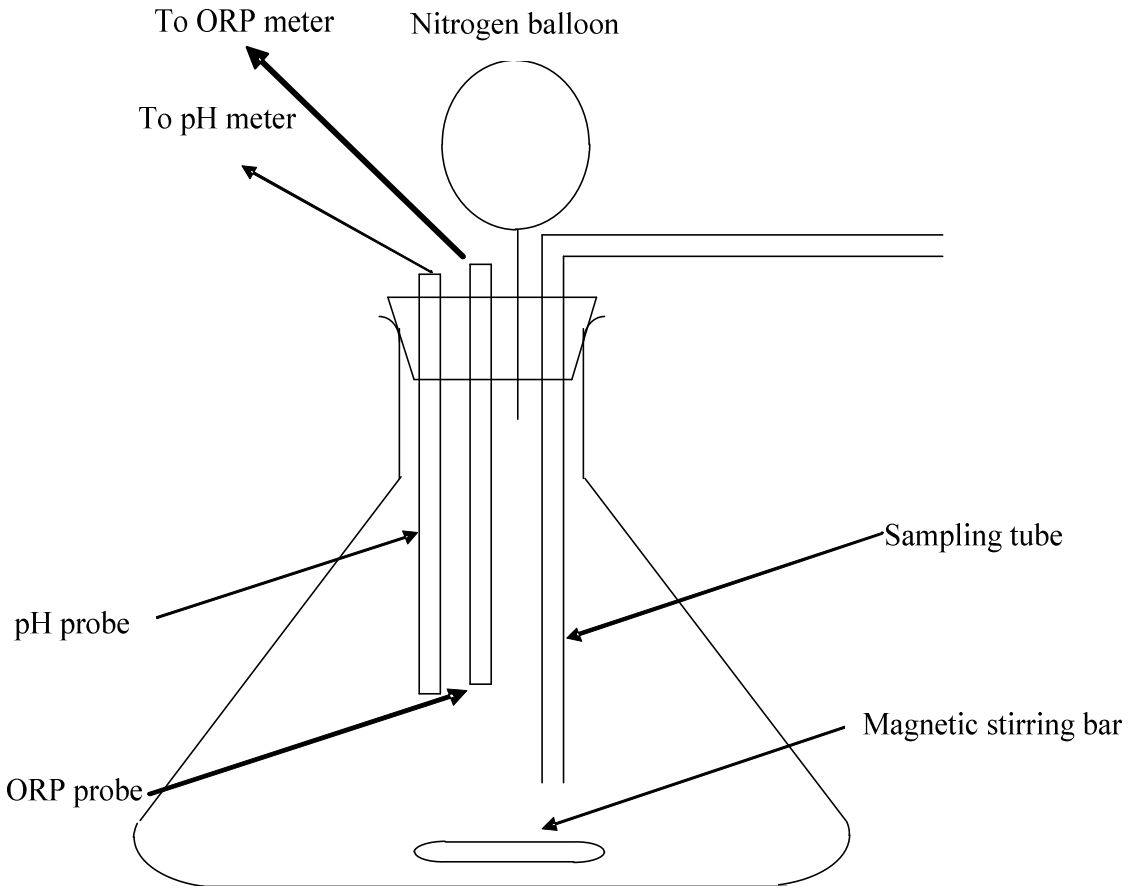


Figure 4.5 Batch reactor schematic

4.3.2 Chemical Analysis

The batch tests required determination of NO_3-N, Mg^{2+}, K^+ and PO_4-P concentrations in the liquid phase of the mixed liquor. The procedure for NO_3-N and PO_4-P analysis has been described in Table 4.4. Mg^{2+} and K^+ samples were prepared and analyzed in general accordance with 3120 B. Inductively Coupled Plasma (ICP) Method (APHA *et al.*, 2005).

A Perkin Elmer Optima 7300 DV Optimal Emission Spectrometer was used for final analysis.

4.4 MBNR System Modeling and Simulation

4.4.1 General introduction

The BioWin™ (version 3.1) simulator was used for MBNR process modeling. The simulator is based on a combined Activated Sludge/Anaerobic Digestion (ASDM) model which the developers refer to the as the *BioWin General Model* (EnviroSim Associates Ltd, Hamilton, Ontario, Canada). In total, the *General Model* has fifty state variables and sixty process expressions. The activated sludge component is primarily based on ASM1 for nitrification and denitrification and on work by Wentzel *et al.* (1989a and b) for biological phosphorus removal. The *General Model* also incorporates a fermentation process which converts readily biodegradable COD to short chain fatty acids (assuming a loss of COD from the system), hydrolysis of enmeshed slowly biodegradable COD under anoxic and anaerobic conditions and anoxic growth of PAO organisms. The rationale for the modifications and their values can be found in the article written by Barker and Dold (1997). The BioWin™ simulator also enables the user to model a process using the default ASM series models. The other important modeling options that have been integrated into the main simulator are:

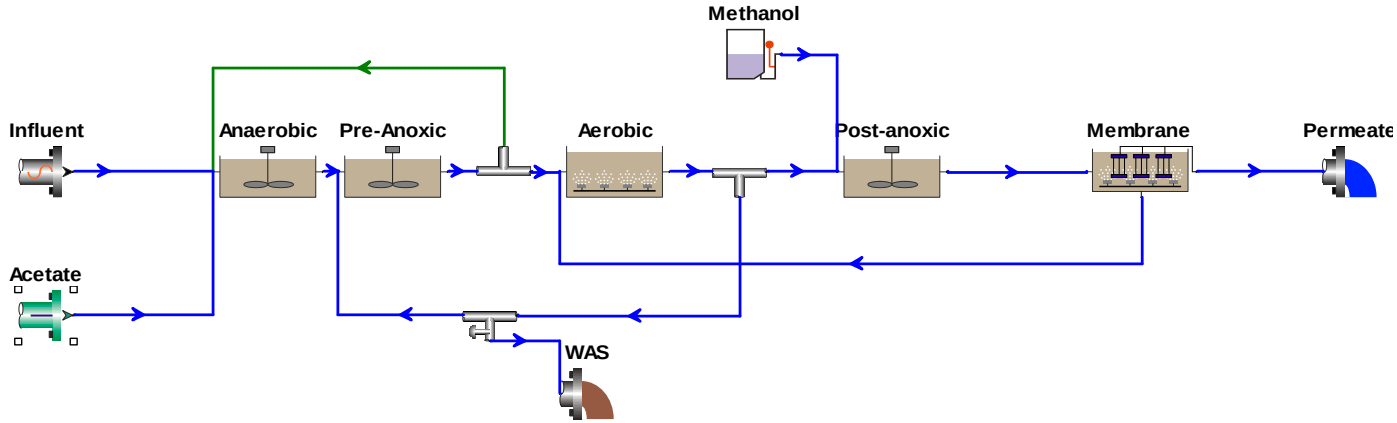
- Oxygen modeling
- pH modeling (includes the option of pH limitation on sludge kinetic equations)
- Chemical precipitation modeling for struvite, hydroxy-dicalcium-phosphate (HDP) and hydroxy-apatite (HAP)
- Chemical phosphorus precipitation modeling (options include alum or ferric)
- Settler modeling (options include modified Vesilind or double exponential)

BioWin™ can be used for both steady state and dynamic modeling of activated sludge systems. In steady state modeling, equilibrium relationships between model variables are independent of time. On the other hand, model variables are described by

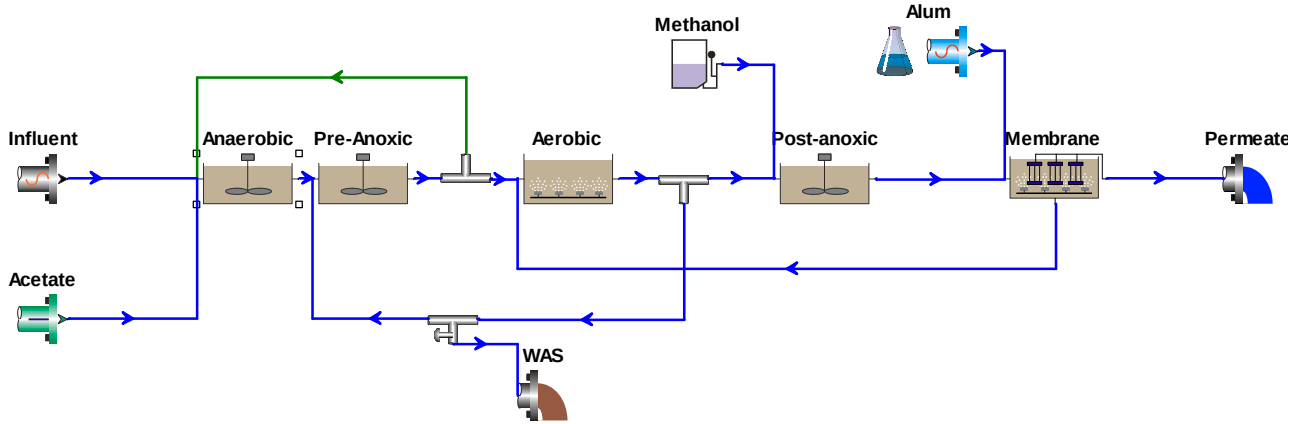
differential equations for dynamic modeling. Steady state modeling can provide vital information on design and capacity evaluation of wastewater treatment plants. Dynamic modeling is typically employed to address issues related to daily or seasonal variations in a wastewater treatment plant, for instance changing flow rate, influent concentrations, internal pumping rates or aeration patterns varying over time.

4.4.2 MBNR system configuration in BioWin™

The first step in the simulation involved setting up the parallel MBNR systems in the BioWin™ interface. This was done by inserting dimensions for each bioreactor in the simulator. Then the bioreactors were connected with pipes and splitters and provided with accurate flow rates. The *General Model* was selected in both MBNR systems for simulation of suspended/ volatile solids production, nitrogen removal and EBPR. The *Chemical Phosphorus Precipitation Model* was the addendum for simulation of alum dosing in the MBNR (Chemical) system. Figure 4.6 shows the configurations of the MBNR systems set up in the BioWin™ interface along with separate element units for influent, permeate, waste activated sludge (WAS), acetate, methanol and alum.



a. MBNR (Biological) system



b. MBNR (Chemical) system

Figure 4.6 Parallel MBNR system configuration in BioWin^{TM}

4.4.3 Simulation strategy

Different simulation strategies have been recommended in the literature for activated sludge system modeling (Cosenza *et al.*, 2013; Melcer *et al.*, 2003; Vanrolleghem *et al.*, 2003; Langergraber *et al.*, 2004). In the current project, the "Biomath-Calibration" protocol (Vanrolleghem *et al.*, 2003) was broadly adopted for simulation of the parallel MBNR systems. The four major steps of the "Biomath-Calibration" are: (1) definition of objectives, (2) comprehensive data collection and analysis from an activated sludge system, (3) steady state calibration and (4) dynamic calibration and evaluation of results. In the current project, definition of objectives and evaluation of simulation results can be found in Chapter Three and Chapter Seven respectively. This section describes methodologies used for data collection, sensitivity analysis, calibration and validation.

4.4.3.1 Data collection, analysis and steady state simulation (with default parameters)

Steady state simulation was initially carried out for the MBNR (Biological) system with default BioWin^{TM} kinetic and stoichiometric parameters. Since the MBNR (Biological) system was a reference for evaluating the influence of chemical phosphorus removal on EBPR, it was the default choice for steady state simulation and calibration. The assumption was that a calibrated MBNR (Biological) system would host the same EBPR mechanism as the MBNR (Chemical) system before the addition of alum.

The influent data collection period for the modeling task was from operating days 236 to 356. During that period, influent municipal wastewater was collected once per week from the Staging Environmental Research Centre (SERC), South Campus, University of British Columbia to maintain a relatively steady input to the model. The selection of input parameters was driven by the requirements of the BioWin^{TM} influent specifier element and their average values are summarized in Table 4.6.

Table 4.6 Input data for BioWin^{TM} influent specifier

Parameter	Number of Samples (n)	Concentration (Min.-Max.)
COD_{Total} (mg/L)	50	302 (±65) (189-483)
COD_{Sol} (mg/L)	50	122 (±34) (67-205)
COD_{FF} (mg/L)	50	84 (±24) (45-139)
BOD_{Total} (mg/L)	20	95 (±17.7) (64-129)
BOD_{Sol} (mg/L)	20	48 (±12) (23-70)
TKN (mg/L)	35	43 (±7.4) (31.5-57)
NH_4 -N (mg/L)	50	31.2 (±5.9) (20.1-44.9)
TP (mg/L)	35	4.9 (±7.4)
PO_4-P (mg/L)	50	3 (±0.7) (1.8-5.1)
VFA (mg/L)	50	19.3 (±7.7) (2.1-36.4)
Alkalinity (mg $CaCO_3$/L)	23	147 (±10.4) (128-165)
TSS_{Raw} (mg/L)	16	103 (±37.5) (40-160)
pH	67	7.6 (±0.4) (7.1- 8.6)
Ca^{2+} (mg/L)	18	19.2 (±6.8) (12- 34)
Mg^{2+} (mg/L)	18	2.1 (±0.6) (1.1- 3.2)

±: Standard Deviation
Data Period: Operating days 236 to 356

The sampling schedule and analytical procedures for the above parameters (except for alkalinity, BOD_5 and Ca^{2+}) were presented in Table 4.2 and Table 4.4 respectively. Sampling for alkalinity, BOD_5 and Ca^{2+} tests was done on Monday and Friday of each week during the data period. Alkalinity and BOD_5 were subsequently determined by using the 2320 B. Titration Method and 5210 B. 5-Day BOD Test method respectively (APHA *et al.*, 2005). The BOD samples were analyzed using an YS1 52 Dissolved Oxygen Meter. Ca^{2+} samples were prepared and analyzed in general accordance with 3120 B. Inductively Coupled Plasma (ICP) Method (APHA *et al.*, 2005). A Perkin Elmer Optima 7300 DV Optimal Emission Spectrometer was used for final analysis.

4.4.3.2 Sensitivity analysis

Preliminary steady state simulation of the MBNR (Biological) system demonstrated (results in Chapter Seven) discrepancy in suspended solids concentration and EBPR mechanism when compared to measured data from the real system. A sensitivity analysis was therefore completed to understand the importance of specific kinetic and stoichiometric parameters on suspended solids concentration and EBPR. In modeling

work, the main objective of a sensitivity analysis is to assess the influence of specific parameters on the model outputs. A kinetic or stoichiometric model parameter is classified as highly sensitive if a small change in its value can cause a large change in model prediction (Liwarska-Bizukojc and Biernacki, 2010). On the contrary, a low sensitivity parameter can be varied significantly without much impact on model output. In the current project, the selection of modeling parameters for sensitivity analysis was based on information available in current MBNR modeling literature. Modeling parameter selection rationale and results of sensitivity analysis is described in detail in Chapter Seven.

4.4.3.3 Calibration of model with steady state simulation

Once the most important parameters were identified in sensitivity analysis, their values were adjusted to calibrate a steady state model against measured data. The first step of calibration required the identification of kinetic and stoichiometric parameters that significantly influenced suspended solids concentration in the simulated system. The calibration of suspended solids concentration is typically the first step, which is then followed by calibration of nitrogen removal (nitrification and denitrification) and EBPR in modeling of activated sludge systems (Brdjanovic *et al.*, 2000; Meijer *et al.*, 2001; Hulsbeek *et al.*, 2002; Petersen *et al.*, 2002). Since nitrogen removal model predictions matched very well with MBNR (Biological) system measured data with default nitrification and denitrification kinetic and stoichiometric parameter values, the second step was calibration of kinetic and stoichiometric parameters relevant to the EBPR mechanism. Detailed data on the outcome of steady state simulation (with calibrated model parameters) can be found in Chapter Seven.

4.4.3.4 Validation of model by dynamic simulation of MBNR (Biological) system

After successful calibration of the model under steady state conditions, a dynamic simulation of MBNR (Biological) system was conducted for operating days 359 to 449. The motivation behind this exercise was to evaluate predictive capability under

moderately dynamic influent conditions. Detailed data on the outcome of dynamic simulation of the MBNR (Biological) system can be found in Chapter Seven.

4.4.3.5 Dynamic modeling of MBNR (Chemical) system

Finally, dynamic simulation of the MBNR (Chemical) system was conducted to investigate the impact of step wise increments of alum concentrations on EBPR activity. This exercise also included a comparative analysis of suspended solids production, nitrification and denitrification as predicted by the model relative to measured data. The simulation period was between operating days 226 to 470. Discussion on the potential negative influence of alum on EBPR as predicted by the model versus MBNR (Chemical) system measured data is detailed in Chapter Seven.

4.5 Method Development for DON Measurement

Method development for reliable DON measurement was one of the main requirements for understanding effluent total nitrogen speciation. The two key needs of a method were the removal of nitrate to the background levels (by anion exchange resin) and conversion of residual DON to nitrate (by persulfate digestion). The adequacies of the above methods were investigated by quality control (QC) experiments. Final nitrate analysis did not pose a big challenge as it could be done by any of the methods described in Standard Methods. Once the methodology was fully developed, samples were collected from the reactors and permeate of parallel MBNR systems for DON profiling.

4.5.1 Batch anion exchange resin method

Removal of nitrate by an anion exchange resin method was put forward by Crumption *et al.* (1992) and their methodology is documented in **Section 2.3.4.2**. For the present project, a batch method was developed to remove residual nitrate from the effluent samples of the parallel MBNR systems. The batch method offered some key advantages, i.e. many samples could be processed simultaneously, very little expert training was

required for the experiments and it was much less expensive than the burette column method of Sattayatewa and Pagilla (2008). The step by step by procedure for the batch anion exchange resin test is described in the next paragraph.

The first step involved the determination of the weight of ion exchange resin required for different ranges of NO_x-N concentrations. After experiments with standards of known nitrate concentrations and permeates from both MBNR systems, it was concluded that 0.75 g and 1.25 g of dry ion exchange resin could extract NO_x-N in the concentration range of 0 – 5 mg/L and 5 – 10 mg/L respectively (detailed results is discussed in Chapter Eight). During the experiments, depending on the sample being analyzed, a fixed weight of dry ion exchange resin (Acros Organics Dowex 1X8 50-100 mesh; 3.2 meq/dry g total capacity) (0.75 or 1.25 g) was carefully poured into the bottom of a 50 mL clean centrifuge tube. In addition, 5 mL of distilled water was transferred to the centrifuge tube to ensure that resin sticking to the side of the tube was rinsed to the bottom of the tube for soaking. A tube cap was screwed on securely and the tube was stored upright in a rack overnight at room temperature. Mixing was not required for the stored aliquots of resin. These above steps were replicated with multiple centrifuge tubes for simultaneous anion exchange experiments. Subsequently, 10 mL of sample was filtered (0.45 µm) and poured into a graduated cylinder. Process sampling was done in triplicates and multiple samples were prepared at the same time with designated graduated cylinders. The next step was acidification of the samples (target pH < 2), achieved by the addition of three drops of 3N HCl to each graduated cylinder. The samples were then poured into their dedicated resin-water-containing centrifuge tubes and the tubes were placed into a rotating mixer. The rotating mixer was operated at 10 RPM for 1 hour. After switching off the mixer, approximately 0.65 mL of 0.5 N NaOH was added to each sample for neutralization. The mixer was then switched on again for a minute to mix and neutralize the samples. Aliquots of 5 mL of each neutralized supernatant samples (without any resin) were carefully poured into 10 mL test tubes or sample bottles. Finally, the samples were stored in refrigerator at 4 ^{0}C for processing with the persulfate digestion method. Figure 4.7 illustrates a typical set-up with centrifuge tubes (with soaked resin and samples) in a rotating mixer.

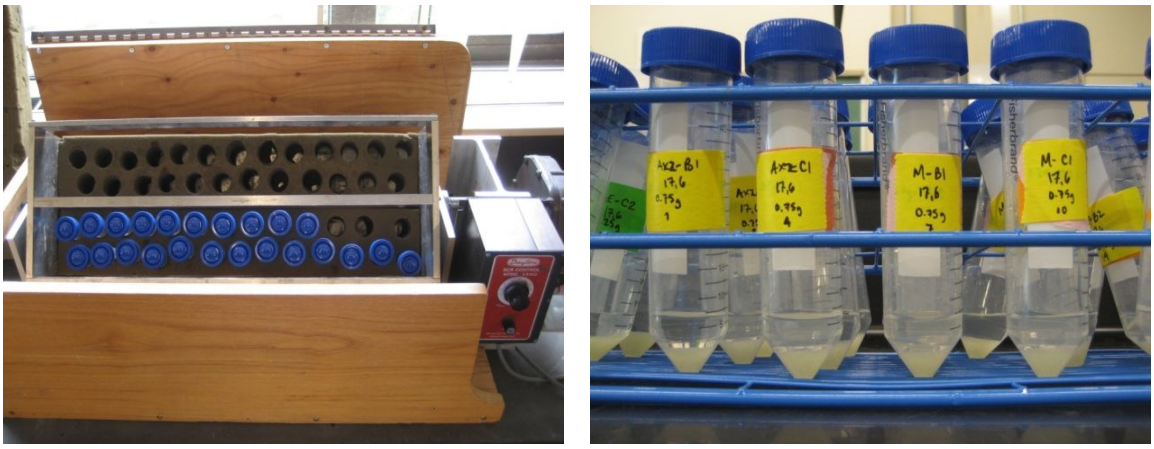


Figure 4.7 Rotating mixer with centrifuge tubes

The anion exchange resin quality control (QC) tests were completed with standards of known nitrate concentration for analyzing removal consistency and with ammonium and urea (representing DON) for recovery consistency. It was hypothesized that no ammonium or urea would be removed during the nitrate removal phase of the DON analysis. The test results are discussed in Chapter Eight.

4.5.2 Persulfate digestion method

The persulfate digestion method was used to convert all non-adsorbed forms of nitrogen to nitrate. In the present project, the digestion utilized the 4500-N C. Persulfate Method (APHA *et al.*, 2005). The method required alkaline oxidation of samples at temperatures in the range from 100 to 110 ^{0}C. Temperatures in this range accelerate persulfate ($K_2S_2O_8$) auto decomposition, thus generating the O_2 needed for the oxidation of N.

Persulfate digestion QC tests were undertaken to study the conversion consistency of standards with known concentrations of ammonium, urea and glutamic acid to nitrate. In addition, recovery tests were conducted on standards containing known concentrations of nitrate. The results are discussed in the Chapter Eight.

4.5.3 Nitrate analysis

Nitrate was determined by using 4500-NO_3^-F. Automated Cadmium Reduction Method (APHA *et al.*, 2005) and the sampling procedure is detailed in Table 4.4.

4.5.4 Sampling for DON profiling

Sampling for DON was completed during Phase V of the experimental program. For permeate, sampling began on operating day 403 and was finished on operating day 464. The permeate DON measurement was expected to provide information on the range of concentrations in the parallel MBNR systems and the impact of up to 80 mg/L of alum addition on DON removal. Reactor DON sampling was conducted between operating days 405 and 457. Sample volume was typically 50 mL for both permeate and reactor samples. The only difference was that filtration (0.45 µm) was required for reactor samples.

5 Performance of Parallel MBNR Systems Targeting LoT Nutrient Removal

5.1 Introduction

Two parallel modified Bardenpho MBNR systems were operated with the goal of accomplishing permeate TN $\leq$ 3 mg/L and TP $\leq$ 0.1 mg/L. Alum dosing for enhanced phosphorus removal was the only difference between the two systems. During continuous operation over a period of 478 days, the parallel MBNR systems were evaluated for COD removal, nitrification, denitrification, EBPR and chemical phosphorus removal performance. These data were then used to assess the relationship between the simultaneously occurring EBPR and chemical P removal mechanisms in the MBNR (Chemical) system. An important objective of the continuous flow system operation was to determine the significance of external dosing of alum and methanol in the realization of LoT phosphorus and nitrogen targets, respectively. This chapter provides an estimate of their stoichiometric requirements.

5.2 Influent Wastewater Characterization

The parallel laboratory scale MBNR systems were operated for approximately sixteen months with municipal wastewater from the SERC at the University of British Columbia. The average influent wastewater characteristics for that period are summarized in Table 5.1.

Table 5.1 Influent wastewater characteristics

Parameter	Concentration (Min.-Max.)
COD_{Total} (mg/L)	290 (±85) (125-584)
COD_{Sol} (mg/L)	117 (±37) (40-231)
COD_{FF} (mg/L)	89 (±32) (28-217)
TKN (mg/L)	39 (±10.2) (15-69)
NH_4 -N (mg/L)	33 (±6.7) (19-57)
TP (mg/L)	4.5 (±1.2) (1.6-9.4)
PO_4-P (mg/L)	3.4 (±1.0) (1.5-7.4)
VFA (mg/L)	26 (±18.7) (0-144)
TSS_{Raw} (mg/L)	140 (±76) (10-380)
pH	7.4 (±0.4) (6.1- 9.0)

± : Standard Deviation
Data collected between May 10, 2010 and August 31, 2011.

5.3 Process Performance

5.3.1 COD profiling

Measurement of influent and permeate COD was undertaken to investigate the carbon removal efficiency in the parallel MBNR systems and the stoichiometric suitability of the wastewater for enhanced denitrification and biological phosphorus removal. Influent characterization was conducted for total COD, soluble COD and COD_{FF} concentrations and the results are shown in Figure 5.1. The average values for the study period along with standard deviations are presented in Table 5.1. The influent total COD concentration was observed to be variable with the highest average value of 316 mg/L occurring during Phase II and the lowest average value of 235 mg/L in Phase V. From Figure 5.1, it can also be seen that COD_{Sol} and COD_{FF} data are not available for the period between day 100 and 200. This occurred as a result of a systematic error in which filter paper with a pore size of 1.2 µm was used instead of 0.45 µm during this period.

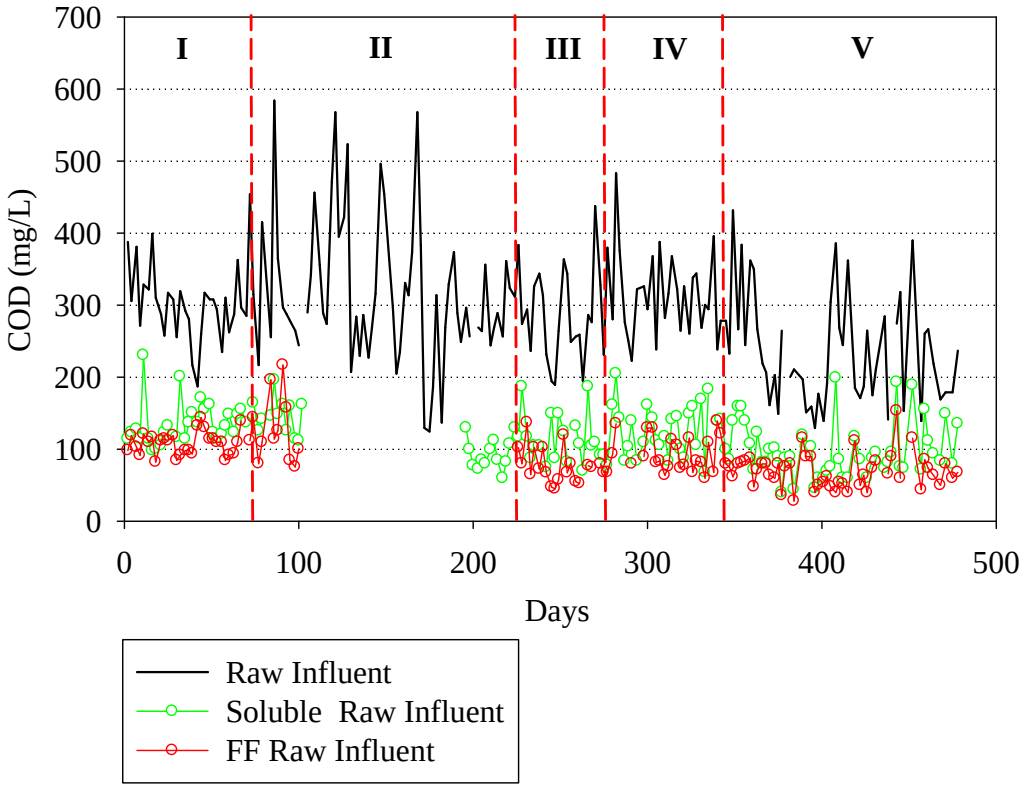


Figure 5.1 Measured raw influent COD concentrations

Influent particulate COD and readily biodegradable COD (COD_{Rb}) concentrations were estimated in the study and their values are shown in Figure 5.2. Particulate COD was determined by subtracting soluble COD from total COD values. The particulate COD value ranged between 30 mg/L and 288 mg/L, with an average concentration of 164 mg/L during the study period. Depending on biodegradability, the wide range for particulate COD was expected to significantly impact MBNR system suspended solids concentrations. The COD_{Rb} measurement was based on the theory that the influent soluble non-biodegradable COD fraction passes through the MBNR systems without being produced or utilized in the system. Therefore, the difference between COD_{FF} and permeate total COD was, in principle, the readily biodegradable fraction. The COD_{Rb} values (Figure 5.2) were calculated by using permeate total COD values from the MBNR (Biological) system. The COD_{Rb} values ranged between 13 mg/L to 195 mg/L, with an

average concentration of 64 mg/L during the study period. COD_{Rb} is a crucial influent wastewater component for achieving successful biological phosphorus removal.

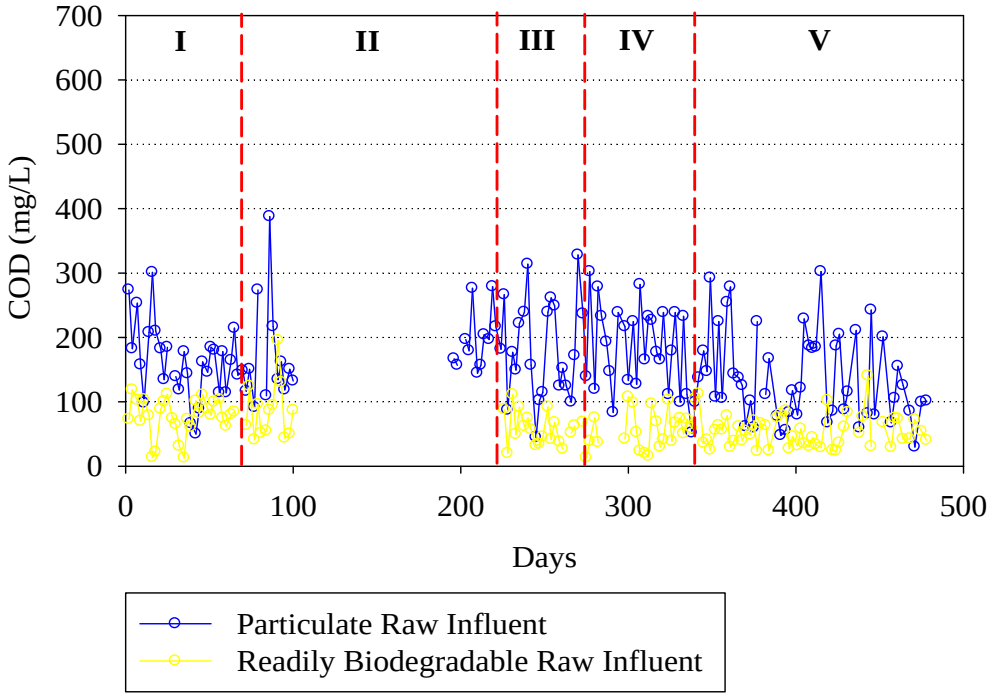


Figure 5.2 Estimated raw influent COD concentrations

COD removal in the parallel MBNR systems is illustrated in Figure 5.3. Treatment performance was very similar in both systems, with average removal efficiencies of 91 percent calculated for both systems. Average permeate total COD concentrations of 24 and 25 mg/L were observed for the MBNR (Biological) and MBNR (Chemical) systems, respectively. In addition, permeate total COD concentration was steady in all five phases of the operation in the parallel systems. High COD removal efficiencies were expected, as the MBNR systems were operated with relatively long HRTs and SRTs under ambient room temperature conditions.

Some studies have demonstrated superior and consistent COD removal with the addition of alum in MBR systems (Fleischer *et al.*, 2005; Holbrook *et al.*, 2004; Lee *et al.*, 2001). Lee *et al.* (2001) hypothesized that coagulation reduces the concentration of soluble organics in alum-supplemented sludge and as a result, lower COD concentration

is observed in the permeate. Holbrook *et al.* (2004) found that protein and polysaccharide concentrations were lower during a period of alum addition when compared to a period without alum addition. However, no enhanced COD removal was observed in the MBNR (Chemical) system (Figure 5.4), even with the highest alum doses applied. This observation could be attributed to the role of alum in the aggregation of organic particles of specific particle sizes and the pore size of the membrane filter. Holbrook *et al.* (2004) observed in their study that alum-induced aggregation rates for larger particle sizes (15 and 7.5 µm) were higher than those for 3.5 µm particles. Fan *et al.* (2007) reported that different alum dosages had greater coagulation impact on particle sizes > 10 µm in their lab scale submerged MBR system. Since alum does not coagulate particles smaller than 0.04 µm (nominal pore size of ZW1 membrane), therefore, in the present study, it is not surprising that reduced permeate COD was not observed for the MBNR (Chemical) system. This is in agreement with the data presented in Figure 5.4.

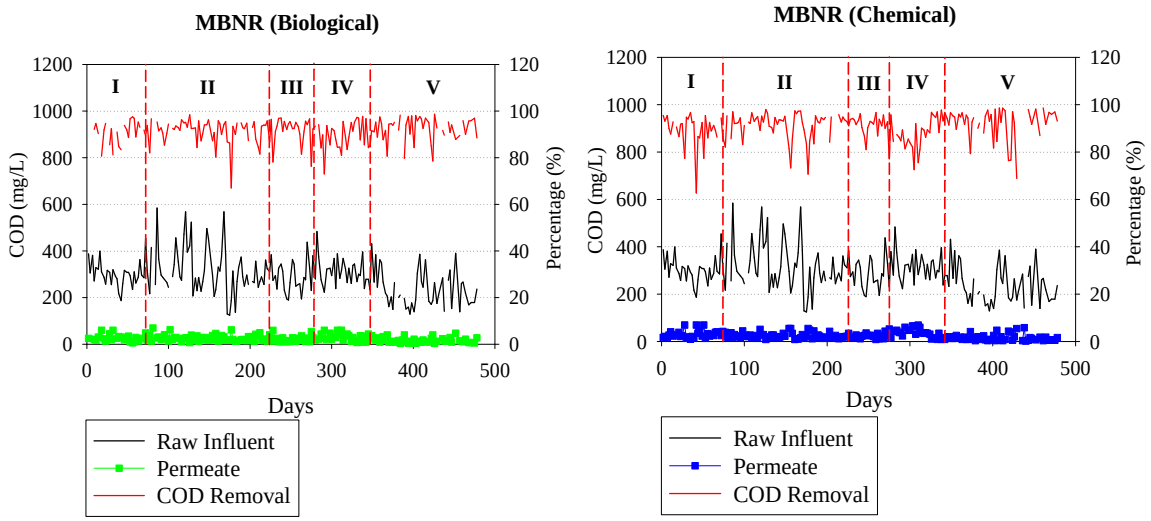


Figure 5.3 COD concentrations and removal efficiencies in parallel MBNR systems

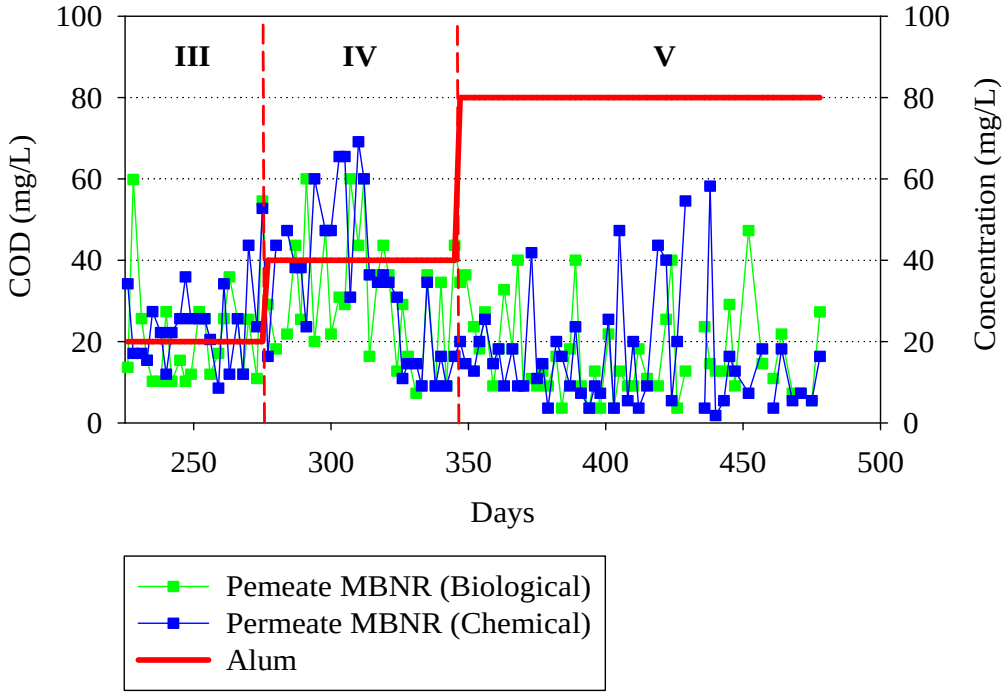


Figure 5.4 Permeate COD concentrations with and without alum addition

5.3.2 Nitrification

The parallel MBNR systems nitrification performance was assessed by measuring influent and permeate NH_4-N concentrations and the results are presented in Figure 5.5. From the figure, it can be concluded that NH_4-N removal efficiency was close to 100 percent in all five phases of operation for both systems. The MBNR (Biological) system permeate NH_4-N concentration was higher than 1 mg/L only once, on day 363. This datum was not an outlier because reactor scan data (Figure 5.6) for that day also showed incomplete nitrification in the aerobic tank. The two key parameters, i.e. process temperature and SRT were constant throughout the study period and cannot be the reasons for failure. Previous research work in SERC has reported low alkalinity in the influent stream and sodium bicarbonate supplementation was a requirement for successful nitrification (Monti, 2006). However, sodium bicarbonate was not added in the current project as enhanced methylotrophic denitrification can theoretically recover any loss in alkalinity due to nitrification.

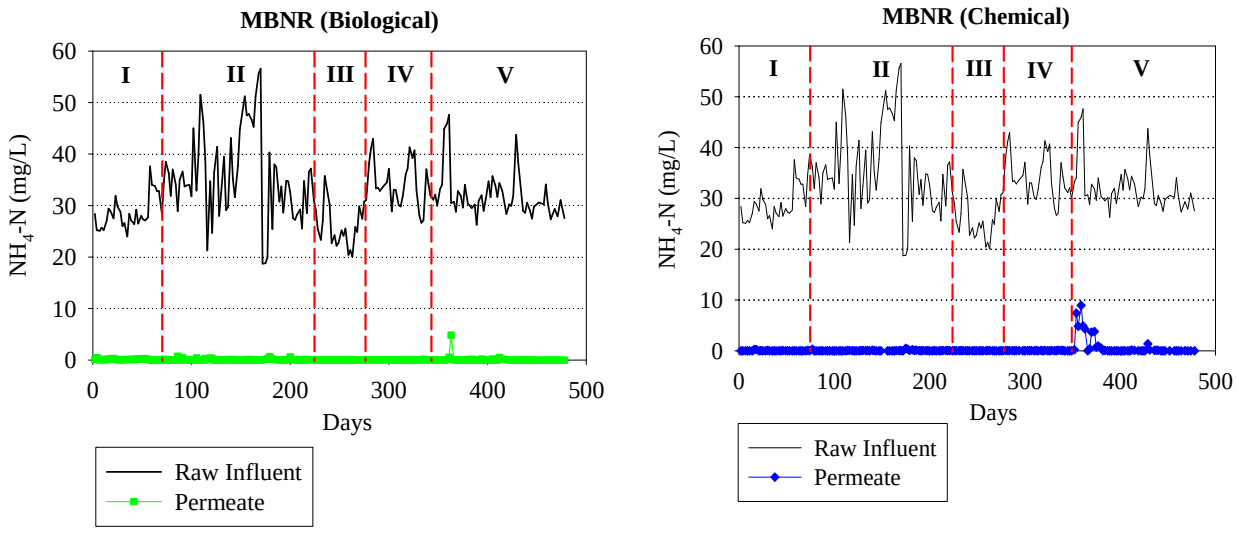


Figure 5.5 Influent and effluent NH_4-N concentrations in parallel MBNR systems

Although NH_4-N removal efficiencies were similarly high in the MBNR (Chemical) system, unusual permeate NH_4-N concentrations were observed between day 354 and day 380, at the beginning of Phase V of the operation (Figure 5.5). As described above, low influent alkalinity could have contributed to incomplete nitrification during that period. However, the extended period of failure, as compared to the parallel MBNR (Biological) system, required focus on the potential impact of alum addition on nitrification. Alum dosage was increased from 40 mg/L to 80 mg/L on day 346 of the operation. The role of alum in nitrification inhibition has been reported in the literature (Lee *et al.*, 2001; Liu *et al.*, 2011). Also, it is known that 1 mg/L of alum consumes 0.5 mg/L of alkalinity (as $CaCO_3$) in water (Fleischer *et al.*, 2005). Therefore, a combination of low influent alkalinity and increased alum addition might have inhibited nitrification efficiency of the MBNR (Chemical) system between day 354 and day 380 of the operation. Nonetheless, swift recovery and consistent performance was observed thereafter as far as nitrification was concerned.

NH_4-N was also measured in the individual reactors of the two MBNR systems to track removal performance and to improve understanding of individual reactor performance. The MBNR (Biological) and MBNR (Chemical) NH_4-N scan results are

summarized in Figure 5.6 and Figure 5.7, respectively. The profiles are very similar for the entire study period with the only exception being very small periods of failure in Phase V of the operation. As expected, the internal recycles caused dilution of NH_4-N in the anaerobic and pre-anoxic reactors of the parallel MBNR systems. The scan data also illustrate that the majority of the NH_4-N was removed in the aerobic reactors. The post-anoxic reactor NH_4-N profiles were very similar to those of the aerobic reactor. There were signs of nitrification in the membrane tank, as residual NH_4-N from the aerobic reactor was reduced to below the detection limit. One of the key advantages of the reactor scan was validating whether higher permeate NH_4-N concentration was a result of sampling and analysis error, or due to reactor performance. In the MBNR (Biological) system, the breakthrough on day 363 can be attributed to incomplete nitrification in the aerobic reactor (Figure 5.6). Similarly, Figure 5.7 demonstrates the presence of elevated concentrations of NH_4-N in the aerobic reactor of the MBNR (Chemical) system between operation day 354 and day 380.

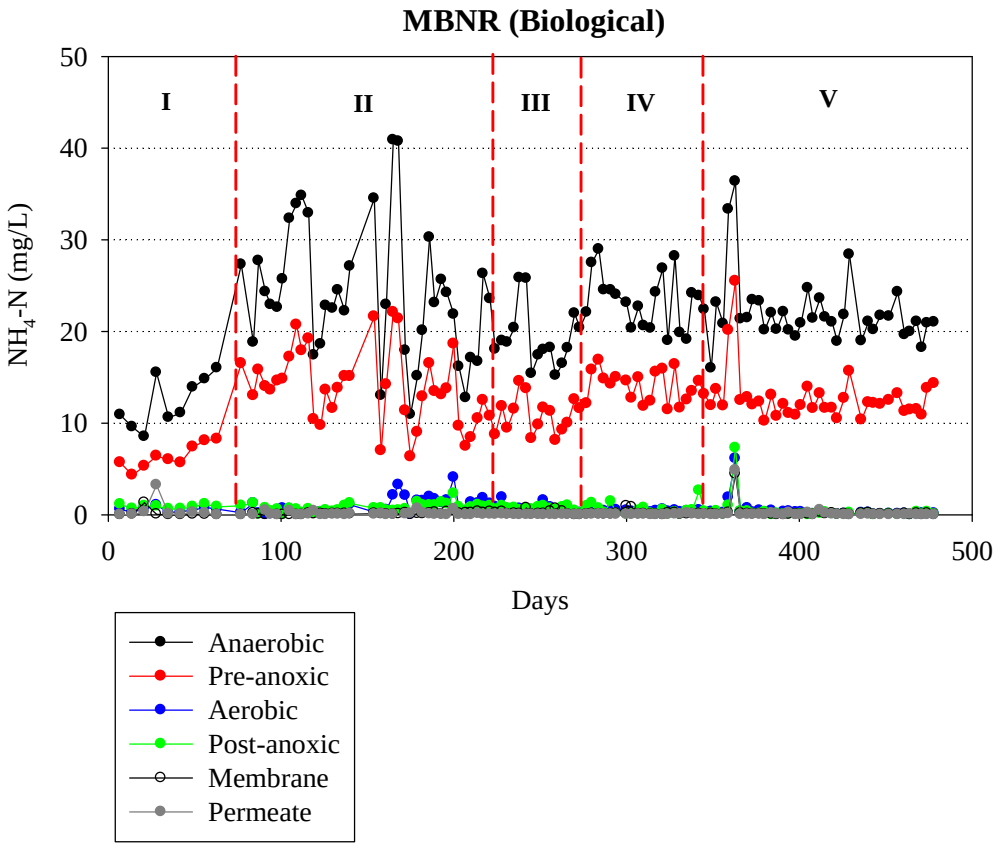


Figure 5.6 NH_4-N reactor scan data for MBNR (Biological) system

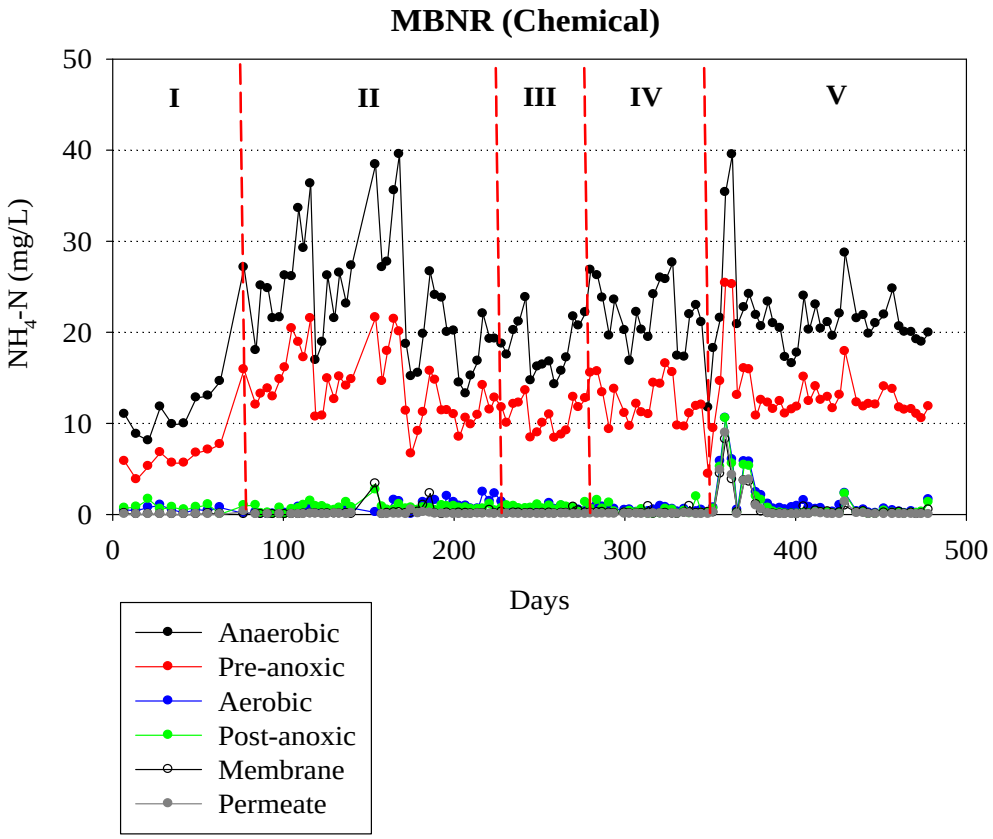


Figure 5.7 NH_4-N reactor scan data for MBNR (Chemical) system

5.3.3 Denitrification

Dentrification performance of the parallel MBNR systems is shown in Figure 5.8. Influent nitrate concentration was close to zero, which is the case with most municipal wastewaters. The effluent nitrate profile was always dependent on the extent of methanol addition. In Phase I, i.e. without methanol addition, the average permeate NO_3-N concentration was 11.3 and 13 mg/L for the MBNR (Biological) and MBNR (Chemical) systems respectively. The average influent total COD/TKN ratio was 12 for the present study, which was higher than the recommended ratios of 6 (Barnard, 1988) or 8.6 (Ekama *et al.*, 1984) for excellent denitrification performance. It could therefore be said that denitrification performance was not optimized in the two MBNR systems. Two possible reasons can be offered for the sub-optimum performance. First, the aerobic recycle was set at a relatively low rate for creating favorable conditions for PAOs in the upstream reactors. This decision might have caused underloading of the pre-anoxic reactor.

Second, fermentation of influent COD_{Rb} to VFA and the subsequent use by PAOs in the anaerobic zone most probably reduced the available COD in the pre-anoxic zone.

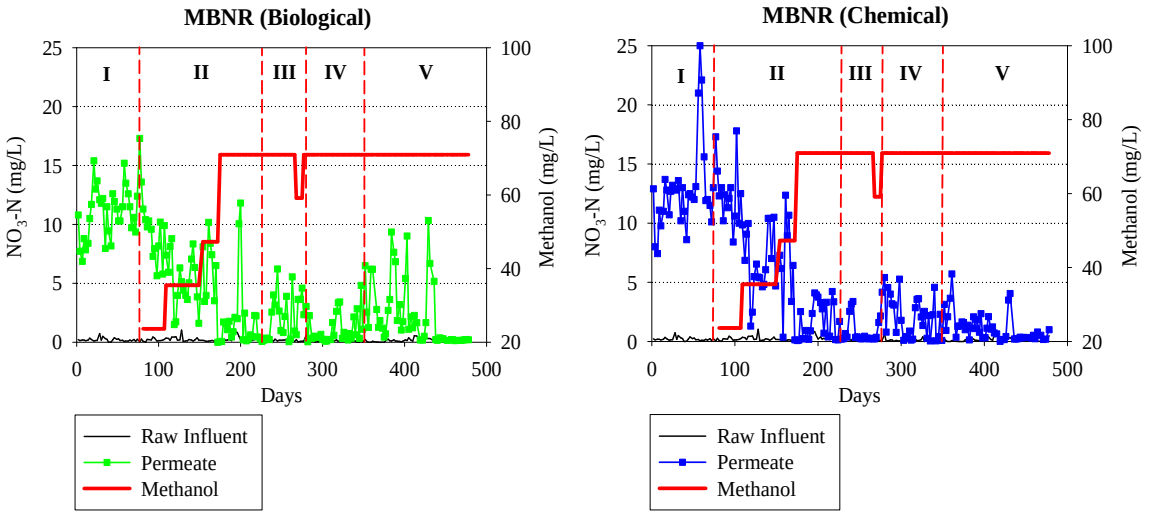


Figure 5.8 Influent and effluent NO_3-N concentrations in parallel MBNR systems

Methanol supplementation improved denitrification performance considerably in the parallel MBNR systems (Figure 5.8). To obtain a better understanding of the impact of different dosages of methanol addition, average permeate NO_3-N concentrations along with minimum, maximum and standard deviation values were calculated and these are summarized in Table 5.2. The table demonstrates the impact of increased methanol addition on the reduction of residual nitrate in the permeate to very low concentrations. With 72 mg/L of methanol supplementation, average permeate NO_3-N concentrations of 2.0 mg/L and 1.4 mg/L were observed in the MBNR (Biological) and MBNR (Chemical) systems, respectively. In fact, permeate NO_3-N concentration was below the detection limit a number of times in both systems. The data in Figure 5.8 demonstrate the capability of both MBNR systems in removing nitrate to extremely low levels in the effluents. Another observation from Figure 5.8 is the difference in variability of effluent NO_3-N concentration in the parallel systems. NO_3-N concentration was consistently below 5 mg/L in the MBNR (Chemical) system permeate during operation with 72 mg/L of methanol supplementation (Figure 5.8). On the other hand, the MBNR (Biological) permeate NO_3-N concentration was variable and was particularly high at some times

during Phase V of operation (Figure 5.8). It is important to understand why the system denitrification was not optimal, even with very high methanol supplementation. Although methanol was added to the post-anoxic reactor of both processes, foam was observed only in the MBNR (Biological) system. The problem was addressed by opening the reactor on a daily basis and mixing the foam into the mixed liquor vigorously. However, this task could not be maintained consistently during the periods of failure. Since methanol was added from the top of the post-anoxic reactor, it is thought that foam prevented the effective mixing of methanol with the mixed liquor in the post-anoxic reactor. As a result, nitrate concentration was high in the permeate. Data for the subsequent days indicated that methanol-induced denitrification recovered quickly and was optimal with the resumption of foam mixing.

Table 5.2 Average permeate NO_3-N concentration for different methanol dosages

Methanol Concentration (mg/L)	**Sample Size**	**MBNR (Biological)**		**MBNR (Chemical)**	
		Average (mg NO_3-N /L) (Min.-Max.)	**mg methanol/ mg NO_3-N**	**Average (mg NO_3-N /L) (Min.-Max.)**	**mg methanol/ mg NO_3-N**
0	34	**11.3** (±2.4) (6.9-17.3)		**13.0** (±3.8) (7.4-26.5)	
24	12	**8.9** (±1.9) (5.6-11.3)	9.9	**12.0** (±2.3) (8.4-11.3)	23.7
36	18	**5.2** (±2.3) (1.5-8.8)	5.8	**6.7** (±2.8) (1.3-10.5)	5.6
48	9	**5.7** (±3.2) (0.0-10.2)	8.6	**6.2** (±4.3) (0.1-12.3)	7.1
72	119	**2.0** (±2.5) (0.0-11.8)	7.6	**1.4** (±1.5) (0.0-5.7)	6.1

± : Standard Deviation

The efficiency of post-anoxic denitrification was evaluated by estimating the mg of methanol added/ mg NO_3-N removed ratio in the present study (Table 5.2). For this calculation, it was assumed that the difference between average permeate NO_3-N concentration for the period without methanol addition and the average permeate NO_3-N concentration for a specific methanol addition rate was solely achieved by methylotrophic denitrifiers. The ratios are illustrated in Table 5.2. The values in the table were higher than the range of 3 to 3.5 suggested by McCarty *et al.* (1969) in their work. Philips *et al.* (2010) suggested that inefficient denitrification can occur due to poor contact between bacteria, substrate and nitrate and air entrapment in the floc of the anoxic reactor. For the

present project, the main objective of the mixer in the post-anoxic reactor was to keep the solids in suspension. It is unknown whether the mixing was ideal for methanol-induced denitrification. Also, since the post-anoxic reactor was placed after the aerobic reactor, it received mixed liquor with elevated dissolved oxygen from the membrane tank. Therefore, the presence of oxygen in the floc could have been a potential factor leading to a methanol dosage requirement.

The reactor NO_3-N profiles for the parallel MBNR systems are shown in Figure 5.9 and Figure 5.10. Although the anaerobic NO_3-N concentration was variable during Phase I, very little nitrate was observed for rest of the study period. The observed low NO_3-N concentration in the anaerobic reactor was crucial for EBPR viability. Similarly, the pre-anoxic NO_3-N concentration was usually < 0.5 mg/L in both MBNR systems. Figure 5.9 also confirms that nitrification in the aerobic reactor caused high NO_3-N concentrations. The profiles were almost identical in the parallel MBNR systems, with NO_3-N concentrations in the range of 5-10 mg/L. It is essential to note that the nitrification-related absolute NO_3-N concentrations in the aerobic reactor were diluted by flow (Q) from the pre-anoxic reactor and the recycle flow (2Q) from the membrane tank. A review of the data in Figure 5.9 and Figure 5.10 confirmed low NO_3-N concentrations in the two reactors and hence, diluted aerobic NO_3-N values.

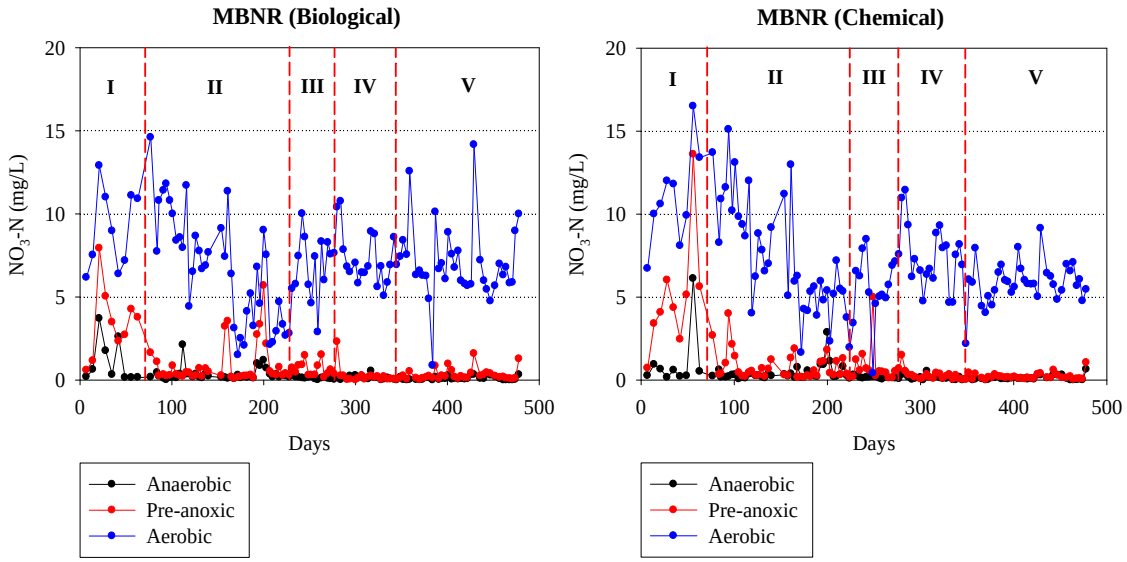


Figure 5.9 NO_3-N data for anaerobic, pre-anoxic and aerobic reactors of the parallel MBNR systems

The post-anoxic NO_3-N data in Figure 5.10 definitively illustrated the influence of methanol in enhanced denitrification. In fact, the post-anoxic NO_3-N concentrations were close to zero in the two MBNR systems during periods of operation. On the other hand, the NO_3-N profile in Figure 5.10 further confirmed the earlier observation of foam-related methylotrophic denitrification failure in the MBNR (Biological) system. NO_3-N data for the membrane tank were very similar and sometimes, higher than in the post-anoxic reactor. The higher NO_3-N value was not entirely unexpected and can be attributed to endogenous decay and nitrification activity in the membrane tank (WEF MOP 36, 2011).

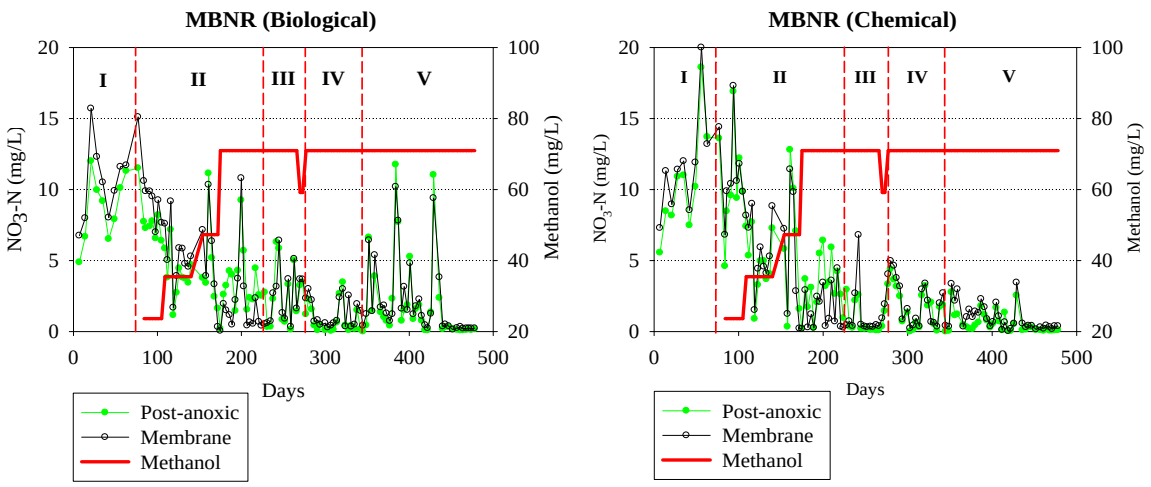


Figure 5.10 NO_3-N data for post-anoxic and membrane reactors of the parallel MBNR systems

5.3.4 Phosphorus removal

The phosphorus removal performance was investigated in the context of understanding the capabilities of the parallel MBNR systems and the relationship between simultaneous biological and chemical phosphorus removal mechanisms. As mentioned before, EBPR and simultaneous EBPR-chemical phosphorus removal mechanisms were fostered in the MBNR (Biological) and MBNR (Chemical) systems respectively.

5.3.4.1 MBNR (Biological) system

A phosphorus removal performance summary for the MBNR (Biological) system is shown in Figure 5.11. The average permeate PO_4-P concentration and removal efficiency were 2 mg/L and 41 percent respectively, for the entire study period. Clearly, EBPR performance was variable in the different phases of operation in the MBNR (Biological) system. The performance was also reflected in PO_4-P concentration profiling (Figure 5.12) and PO_4-P release/uptake profiling (Figure 5.13) of each reactor. PO_4-P release/uptake in Figure 5.13 was calculated by mass balance in each reactor. If the difference between inflow and outflow of soluble PO_4-P was negative, phosphorus

release was occurring in the reactor. On the other hand, uptake was the prevalent mechanism if the difference between inflow and outflow soluble PO_4-P was positive.

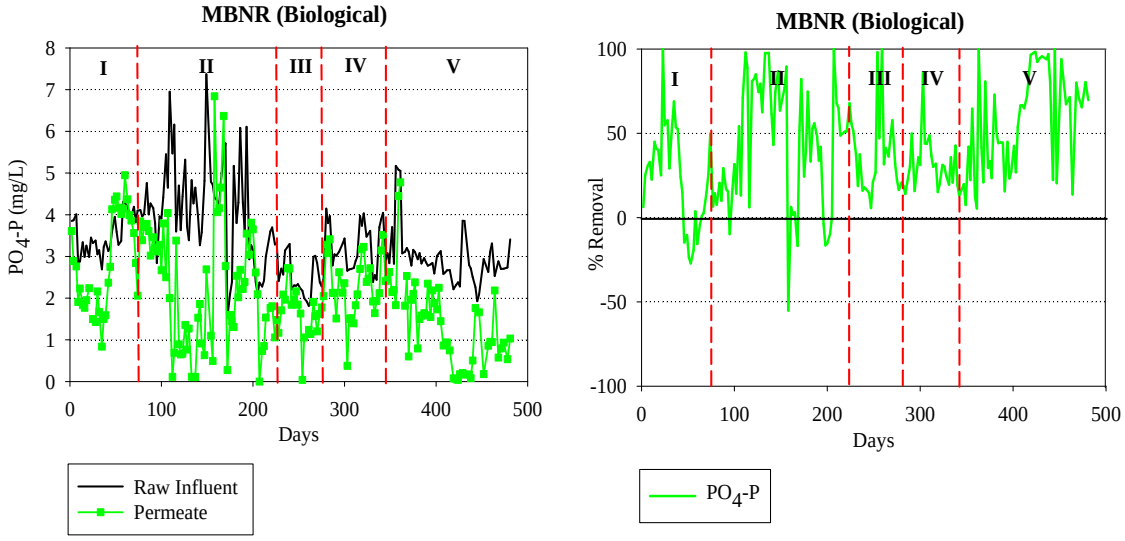


Figure 5.11 PO_4-P removal in the MBNR (Biological) system

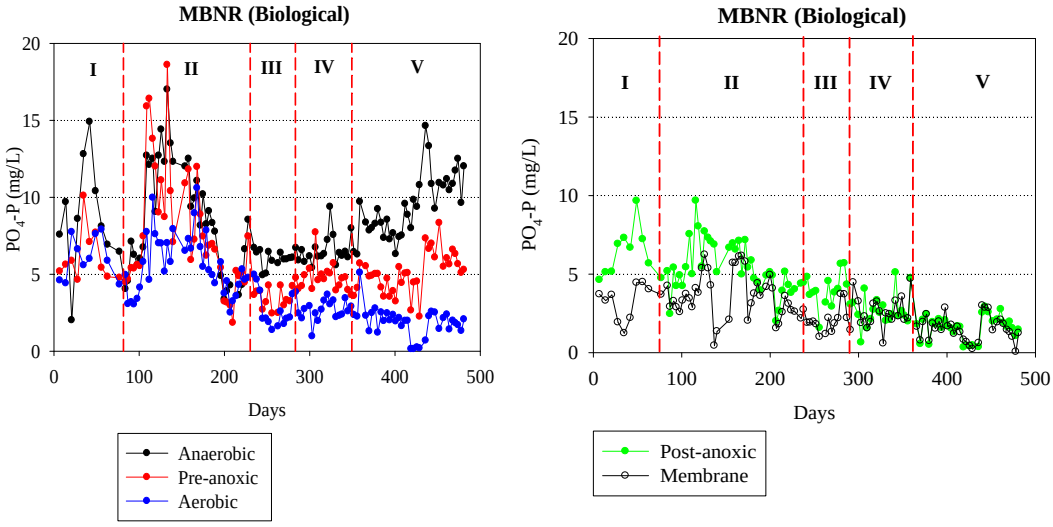


Figure 5.12 Reactor PO_4-P profile in the MBNR (Biological) system

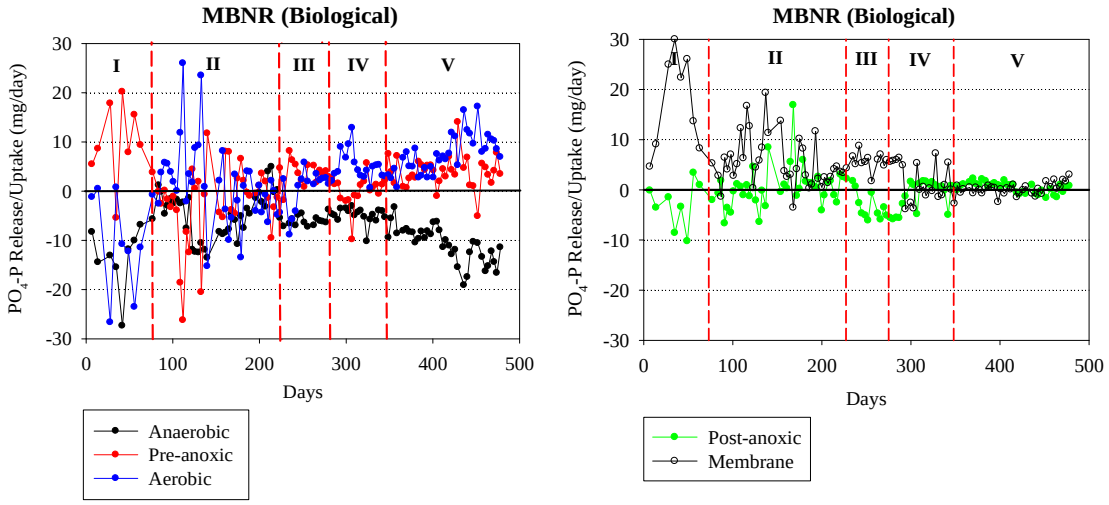


Figure 5.13 Reactor PO_4-P release (-)/uptake (+) profile in the MBNR (Biological) system

During the first 44 days of operation (no excess sludge wasting), both PO_4-P removal and release in the anaerobic reactor improved gradually in the MBNR (Biological) system, as shown in Figure 5.12 and Figure 5.13. However, the PO_4-P release/uptake profiles in the other reactors (Figure 5.13) showed that the classic EBPR mechanism was not established in the MBNR (Biological) system. In the aerobic reactor, unexpected PO_4-P release was observed, while significant PO_4-P uptake occurred in the pre-anoxic reactor and the membrane tank. Additionally, PO_4-P release was observed in the post-anoxic reactor. Although uptake of some PO_4-P was expected in the pre-anoxic reactor and the membrane tank, usually P uptake takes place in the aerobic reactor of all well-functioning EBPR processes. Interestingly, once sludge wasting was resumed on day 45, there was an immediate decline in PO_4-P release in the anaerobic zone, as well as in the P uptake that occurred in the pre-anoxic reactor and the membrane tank. This was also reflected in elevated permeate PO_4-P concentrations (Figure 5.12). It can be argued that the MBNR (Biological) system was not operating at steady state for the first 95 days due to the absence of sludge wasting for 44 days, the modification of the MBNR recycling rates (Refer Materials and Methods Chapter), a change in acetate supplementation, the augmentation of methanol addition and the presence of NO_3-N in the anaerobic reactor. As a result, the initiation of EBPR mechanism was negatively impacted in the MBNR (Biological) system.

After approximately 2 SRTs, the PO_4-P concentrations steadily increased in both the anaerobic and pre-anoxic reactors until operating day 140, at which time these concentrations decreased unexpectedly until day 221. Between operating days 95 and 140, the pre-anoxic PO_4-P concentration was sometimes higher than that observed in the anaerobic reactor (Figure 5.12). Figure 5.13 furthermore confirms higher PO_4-P release in the pre-anoxic reactor during that period. The absence of nitrate in the pre-anoxic reactor (Figure 5.9) and very high VFA in the raw influent and in the anaerobic zone (Figure 5.19) might have caused unusually high PO_4-P concentrations in the pre-anoxic reactor. The most probable explanation is that the pre-anoxic zone behaved like an anaerobic zone in the absence of nitrate (underloading in pre-anoxic zone) and caused further phosphorus release in the presence of abundant VFAs. In addition to the unexpectedly high PO_4-P release in the pre-anoxic reactor, both uptake and release were observed in the aerobic reactor between operating days 95 and 140 (Figure 5.13). On the other hand, PO_4-P uptake was fairly consistent in the membrane tank, as illustrated in Figure 5.13. Depending on the PO_4-P uptake performance in the membrane tank, a low permeate PO_4-P concentration was achieved intermittently in the MBNR (Biological) system between operating days 95 and 140 (Figure 5.12).

Aerobic PO_4-P release in the MBNR (Biological) system was a cause of concern and the reason for this was further investigated. Various researchers have observed aerobic PO_4-P release in their experimental work and this was often attributed to the presence of acetate in the aerobic tank (Bradjanovic *et al.*, 1998; Guisasola *et al.*, 2004; Randall and Chaplin, 1997). Different theories have been offered regarding the impact of aerobic zone acetate on EBPR performance. While Bradjanovic *et al.* (1998) and Guisasola *et al.* (2004) suggested that ATP generation due to oxidative phosphorylation under aerobic conditions is supplemented by PO_4-P release, Randall and Chaplin (1997) proposed that the presence of acetate under aerobic conditions promoted excessive growth of filamentous bacteria that resulted in washout of PAOs. In the current study, uncharacteristically high VFA concentrations observed in the influent between days 95 and 170 might have progressed to the aerobic reactor and caused P release (Figure 5.19). All three literature studies predicted failure of EBPR in such a scenario. That was exactly

what happened in the present study, as the anaerobic PO_4-P release deteriorated and complete failure was observed between days 196 and 221 (Figure 5.13).

The variability of influent VFA concentrations and the steady state modeling task required modification in the wastewater collection schedule from twice per week to once per week on day 193 of operation. The other known change in the process was an increment in methanol dosing to 72 mg/L on operating day 175. Thereafter, the system recovered and EBPR performance was steady for the rest of the study period. The PO_4-P profiles in Figure 5.12 resembled those of a functioning EBPR from day 224 until the end of the study. The average effluent PO_4-P concentration and removal efficiency were 1.7 mg/L and 46 percent, respectively, between day 224 until the end of operation. This was a marked improvement as compared to the EBPR performance in the first 223 days of operation (average effluent PO_4-P concentration and removal were 2.48 mg/L and 36 percent respectively). More importantly, Figure 5.13 provides evidence of major PO_4-P release and uptake taking place in the anaerobic and the aerobic reactors, respectively. Furthermore, consistent PO_4-P uptake was observed in the pre-anoxic reactor while no uptake or release was observed in the post-anoxic reactor and membrane tank after day 224. This period included a phase, between days 415 and 443, during which the system effluent PO_4-P concentration was less than 0.5 mg/L, indicating excellent EBPR. On average, 87 percent phosphorus removal was observed during that period. It is not clear why the MBNR (Biological) system EBPR performed so well during that period. Nonetheless, performance could not be maintained and the permeate phosphorus values increased to approximately 1 mg/L for rest of the study.

A review of the reactor and effluent PO_4-P profiles illustrates that performance was most consistent from day 224 until the last day of operation. Moreover, there were no modifications in the process recycle rates, operation or methanol addition during that period. Hence, it can be concluded that the EBPR performance from day 224 until the last day of operation is the best that could be achieved in the MBNR (Biological) system. Evaluation of the MBNR (Biological) system phosphorus removal performance during that period and the potential factors associated with it was expected to provide valuable

information from the stand point of the classic EBPR mechanism. The important factors could have been either the influent wastewater characteristics or the process operating conditions.

One of the important parameters for successful EBPR operation is the availability of influent COD in adequate concentrations in the anaerobic zone. The average COD/TP ratio was 70 in the current project, more than the recommended value of 40 for reducing phosphorus to less than 1 mg/L in the effluent (Dr. James L. Barnard, pers. comm.). Additionally, the average COD_{Rb}/ TP ratio was 12.5 in the present study. A COD_{Rb}/TP ratio in the range of 10 to 16 is typically sufficient (Dr. James L. Barnard, pers. comm.) to meet an effluent TP goal of 1 mg/L in wastewater treatment plants. Moreover, in the present study, to avoid large impacts from variation in influent wastewater characteristics, the MBNR (Biological) system was always supplemented with acetate, added directly to the anaerobic reactor. As mentioned before, acetate was the chosen external VFA for the initial 268 days of operation. However, continued inefficient EBPR performance prompted replacement of acetate by a mixture of acetate and propionate (4:1 ratio) in the parallel MBNR systems for the rest of the study period. This step was taken based on the finding that PAOs have competitive advantage over GAOs when supplemented with a solution of acetic and propionic acid (Dr. James L. Barnard, pers. comm.). However, the EBPR performance did not show any noticeable improvement as a result of the modification in external VFA composition.

The other two parameters that could have influenced EBPR activity were temperature and pH. Since the MBNR (Biological) system was operated at a constant temperature of 20 ^{0}C, no major influence was expected with respect to variation in EBPR performance. pH is another key parameter, as it has been found to impact anaerobic phosphorus release (Filipe *et al.*, 2001a; Kuba *et al.*, 1997a; Liu *et al.*, 1996; Smolders *et al.*, 1994a; WEF and ASCE, 2006) as well as aerobic uptake efficiencies (Filipe *et al.*, 2001a). From the studies mentioned above, a pH of 7.0 to 8.0 is the recommended range for optimum anaerobic phosphorus release efficiency. As far as P uptake by PAOs in the aerobic reactor is concerned, Filipe *et al.* (2001a) observed in batch studies that efficiency was

essentially the same at pH 7.0 and 7.5, but decreased greatly at pH 6.5. The pH profiles of the influent, anaerobic and aerobic reactors of MBNR (Biological) system are shown in Figure 5.14. pH was consistently above 7.0 in the anaerobic reactor except for the last two months of operation. It can, therefore, be assumed that the observed anaerobic pH did not have a significant effect on phosphorus release efficiency. On the other hand, the pH of the aerobic reactor varied from the lowest value of 5.8 to the highest value of 8.5 during the study (Figure 5.14). Influent wastewater variations and nitrification were the two major factors controlling pH in the aerobic reactor of the MBNR (Biological) system. The average pH was 7.1 between day 1 to day 224, while it was 6.6 for the rest of the study period. The lower aerobic pH coincided with the steadiest, yet non-optimal, biological phosphorus removal observed in the MBNR (Biological) system. Moreover, as shown in Figure 5.12, EBPR performance was mostly limited by the uptake capability of PAOs in the aerobic reactor. Hence, it could be concluded that low aerobic pH might be one of the major reasons for inefficient EBPR in the MBNR (Biological) system.

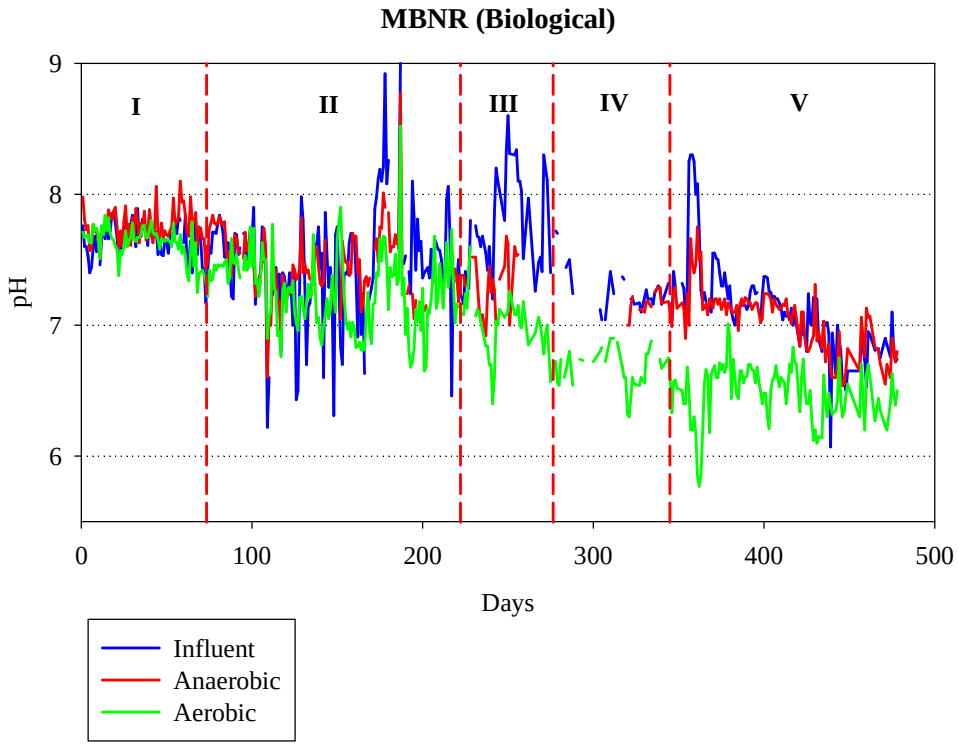


Figure 5.14 pH profile of influent, anaerobic and aerobic reactors of the MBNR (Biological) system

The presence of nitrate and oxygen in an EBPR anaerobic zone can severely inhibit EBPR activity in BNR systems. In the present study, the anaerobic reactor NO_3-N concentration in the MBNR (Biological) system was below the detection limit except for the initial days of operation (refer Figure 5.9). Similarly, the DO concentration was always observed to be zero from intermittent DO measurements in the anaerobic tank (data not shown here). The impact of the above two parameters on EBPR performance was, therefore, concluded to be negligible.

Hydraulic retention time (HRT), which remained constant in the MBNR (Biological) system, has been studied extensively to evaluate its impact on EBPR efficiency. Anaerobic HRT is particularly important, as adequate time is required for the formation of PHAs. Researchers have recommended that an anaerobic hydraulic residence time (HRT) of 0.25 to 1.0 hour to be sufficient to induce the target metabolisms (Barnard, 1984; Barnard and Fothergill, 1998; Grady *et al.*, 1999). Coats *et al.* (2011) further noted that anaerobic HRT in the range of 1 to 3 hours would enrich the mixed liquor with PAOs and enable successful operation. In the context of present study, the set anaerobic HRT of 1.5 hours was in the recommended range and was not expected to inhibit phosphorus release. Aerobic HRT is generally set for achieving nitrification requirements. However, a very short HRT may impede phosphorus uptake efficiency in EBPR systems. Neethling *et al.* (2005) reported that an aerobic HRT to anaerobic HRT ratio between 3 and 4 was optimal for phosphorus removal. In the MBNR (Biological) system, aerobic HRT to anaerobic HRT ratio was maintained at 4 throughout the present study.

The MBNR (Biological) system was operated with a relatively long SRT of 25 days, generally not applied in conventional activated sludge systems. Long SRT has been reported to degrade effluent quality because endogenous biomass activity prompts secondary phosphorus release. Nonetheless, Lesjean *et al.* (2002) operated pilot MBR systems with an SRT of 26 days and accomplished 99.3 percent and 99.2 percent TP removal in pre-denitrification and post-denitrification modes respectively. Ersu *et al.* (2010) evaluated MBR system TP removal performance with different SRTs of 10, 25, 50

and 75 days. These authors reported the highest TP removal efficiency of 80 percent at a 50 day SRT and the lowest removal efficiency of 60 percent at a 75 day SRT. The results from these studies suggest that low TP removal efficiency in the MBNR (Biological) system was unlikely caused by the applied SRT.

Internal recirculation rates in the MBNR (Biological) system did impact the mixed liquor suspended solids distribution, particularly in the upstream anaerobic reactor. In contrast to conventional activated sludge systems in which biomass is more evenly distributed throughout the system, the filtration barrier used in MBRs produces suspended solids accumulation in the downstream membrane tank. The specification of recirculation rates is, therefore, very important in MBR systems as far as maintaining upstream anaerobic reactor solids inventories and phosphorus release is concerned (Crawford *et al.*, 2006; Erdal *et al.*, 2010). In the present study, Figure 5.15 shows reactor suspended solids distribution profiles for the MBNR (Biological) system. The fractional suspended solids distributions were calculated as follows.

$$\%\,Solids\quad TSS\quad Rea\quad o\ ol\quad e\ \sum^{5}\quad TSS\quad Rea\quad o\ ol\quad e\ 100 \qquad (9)$$

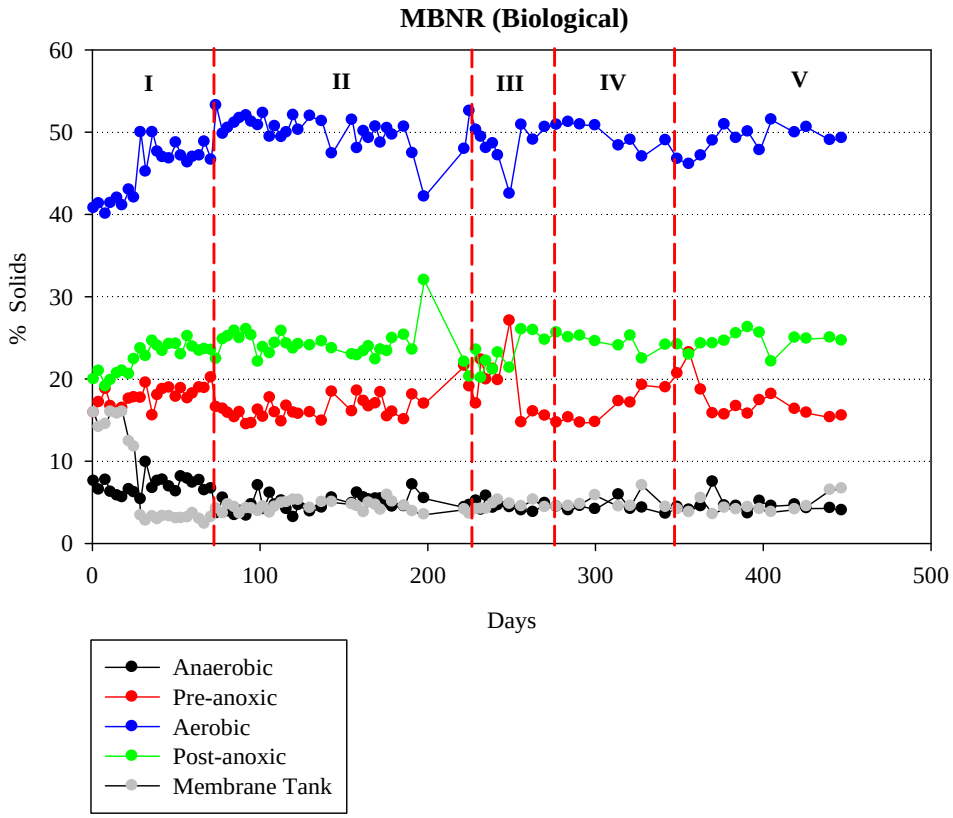


Figure 5.15 Reactor suspended solids distribution in MBNR (Biological) system

From Figure 5.15, suspended solids distribution among the anaerobic, pre-anoxic, aerobic, post-anoxic and membrane zone was approximately 5 percent, 17 percent, 49 percent, 24 percent and 5 percent respectively. It is interesting to note that the anaerobic zone suspended solids fraction was very low relative to those of the other reactors. Though in-depth studies have not been conducted regarding the ideal anaerobic solids fraction requirement, data are available for a few full scale MBR plants with comparable process configurations. Daigger *et al.* (2009) reported that anaerobic solids fraction was 10 percent in the Traverse City modified VIP MBR plant and 13 percent in Broad Run modified Bardenpho MBR Water Reclamation Plant (WRP). Both the Traverse City and Broad Run plants primarily employed EBPR to meet effluent TP concentration of 0.5 mg/L and 0.1 mg/L respectively. In comparison, the anaerobic fraction of the total biomass (in other words, anaerobic SRT) was significantly lower in the present MBNR (Biological) system. Grady *et al.* (1999) reported that low anaerobic SRT may have an adverse affect on EBPR performance. It is therefore postulated that a low anaerobic

suspended solids fraction played a key role in limiting the efficiency of EBPR in the MBNR (Biological) system. Nevertheless, detailed investigation is still needed to improve understanding of the relationship between anaerobic solids fraction and EBPR performance.

5.3.4.2 MBNR (Chemical) system

MBNR (Chemical) system phosphorus removal performance is summarized in Figure 5.16. This system was operated for biological P removal only, for the first 225 days of operation (Phase I and Phase II), after which time alum was incrementally supplemented for the rest of the study. During EBPR-only operation, the average effluent PO_4-P concentration was 2.8 mg/L and removal efficiency was 27 percent. It can be argued that performance was non-optimal during that period (Figure 5.16). In fact, the MBNR (Chemical) system EBPR performance mimicked that of the MBNR (Biological) system during the first 225 days of operation (Figure 5.17 and Figure 5.18). The reasons for the poor EBPR efficiency were discussed above and the focus of this section will be on the period of operation with simultaneous biological and chemical phosphorus removal mechanisms.

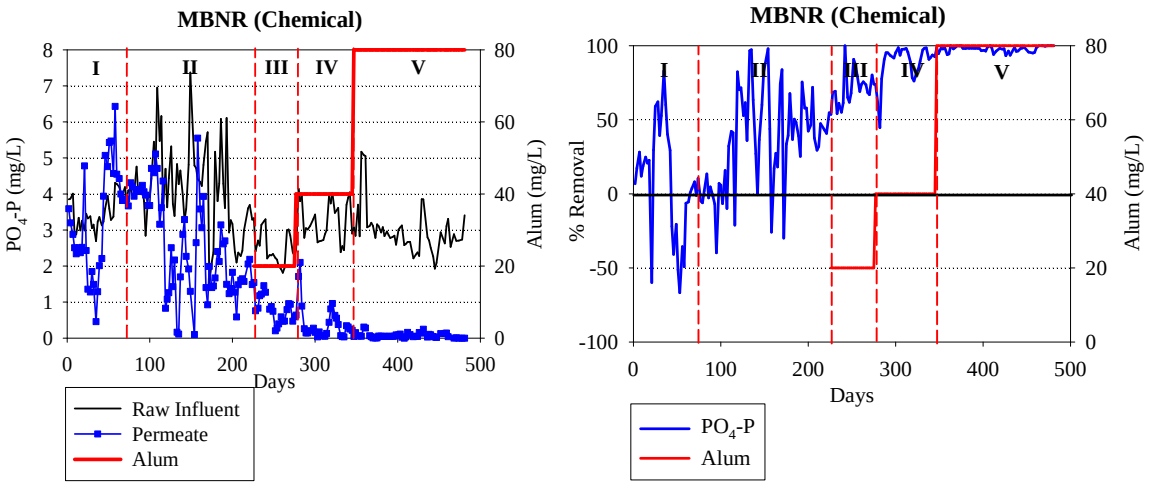


Figure 5.16 PO_4-P removal in the MBNR (Chemical) system

There were two clear objectives of alum addition in the MBNR (Chemical) system: (1) accomplishing effluent TP ≤ 0.1 mg/L and (2) analyzing the relationship of chemical P removal to the existing EBPR mechanism. Initially, a low dosage of alum was added to the membrane tank mixed liquor to realize a nominal initial concentration 20 mg/L, for 2 SRTs (Phase III). PO_4-P removal performance immediately improved with an average effluent concentration of 0.80 mg/L and removal efficiency of 71 percent (Figure 5.16). Of course, the total observed P removal was attributable to combined EBPR and chemical phosphorus removal mechanisms. Although the MBNR system did not recover completely from the previous high influent VFA period (Figure 5.19), the existence of EBPR was clearly evident as phosphorus release occurred in the anaerobic reactor in Phase III of operation (Figure 5.18). Phosphorus release was also observed in the aerobic and post-anoxic reactors while uptake took place in the pre-anoxic reactor and the membrane tank. A key conclusion from Phase III was that alum supplementation offered value in stabilizing overall phosphorus removal while EBPR was not optimal in the MBNR system. The PO_4-P profile in Phase IV, as demonstrated in Figure 5.17 and Figure 5.18, provided evidence that functional EBPR (anaerobic release and aerobic uptake) was starting to take place in the MBNR (Chemical) system. Therefore, 20 mg/L of alum supplementation did not seem to inhibit EBPR activity in the MBNR (Chemical) system. This observation was further corroborated by batch studies in which the mixed liquor EBPR potential of the MBNR (Biological) system was found to be comparable to that of the MBNR (Chemical) system. The results of the batch studies are discussed in **Section 6.3**.

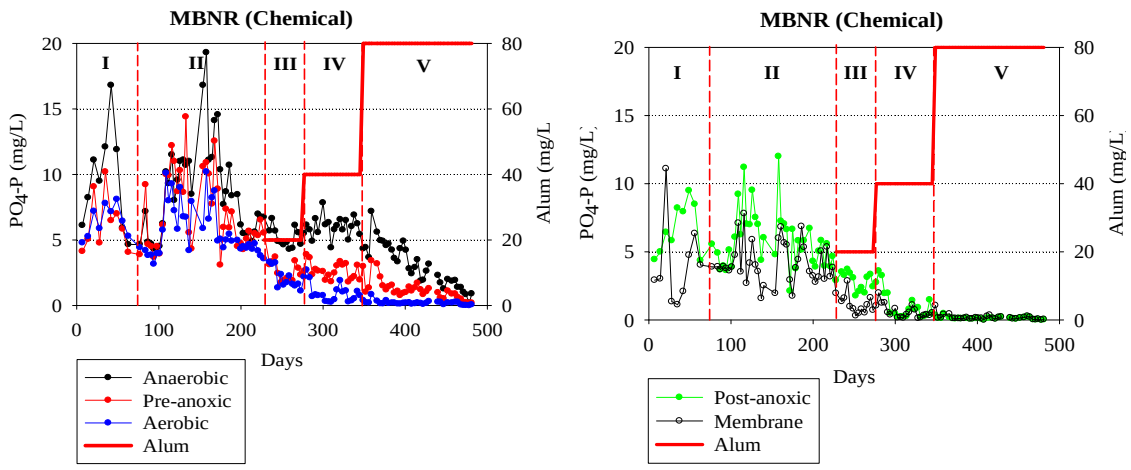


Figure 5.17 Reactor PO_4-P profile in the MBNR (Chemical) system

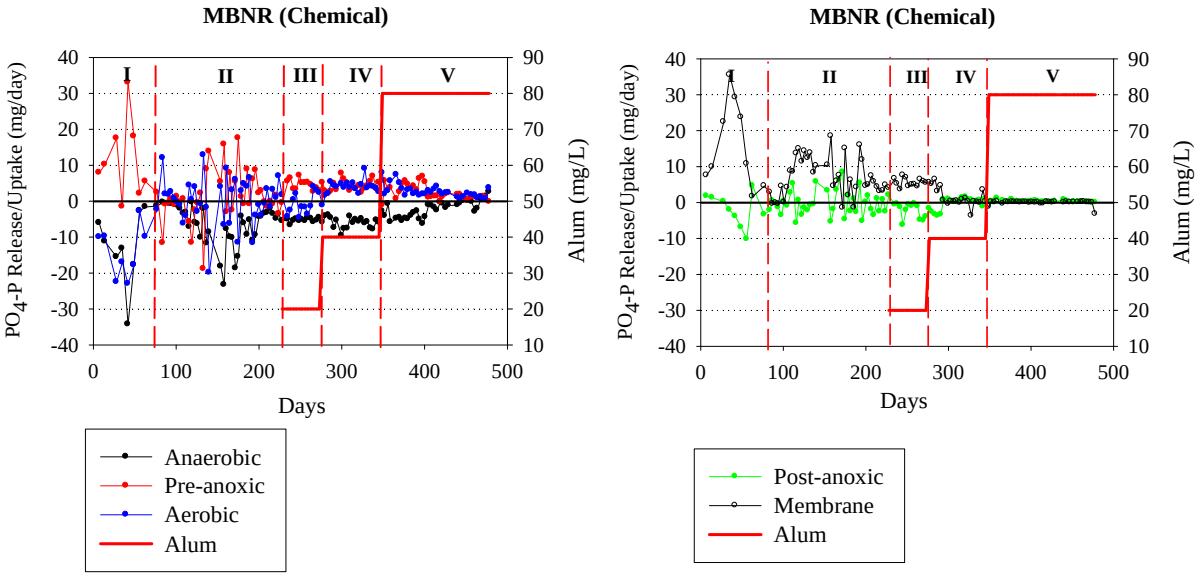


Figure 5.18 Reactor PO_4-P release (-)/uptake (+) profile in the MBNR (Chemical) system

Alum dosage concentration was increased to 40 mg/L on operating day 277 in the MBNR (Chemical) system and this dosage was maintained for 70 days (Phase IV). During that period, the average effluent phosphorus concentration and removal efficiency that could be attained were 0.42 mg/L and 89 percent respectively. At the same time, EBPR performance stabilized with consistent phosphorus release and uptake in the anaerobic and aerobic reactors respectively (Figure 5.18). Figure 5.18 further illustrates that phosphorus uptake occurred in the pre-anoxic reactor while all activities stopped in the post-anoxic reactor and the membrane tank. Interestingly, PO_4-P concentration

gradually decreased in all the reactors except the anaerobic reactor during the period of 40 mg/L of alum supplementation (Figure 5.17). It is postulated that as alum reacted with phosphorus to form precipitated compounds, the soluble phosphorus concentration became progressively lower in the MBNR (Chemical) system. As far as reaction in the anaerobic reactor is concerned, it is unclear whether biological phosphorus release and alum-induced phosphorus uptake was occurring simultaneously. The average PO_4-P concentration in the MBNR (Chemical) anaerobic reactor was 6 mg/L between operating days 277 and 346, whereas it was 6.45 mg/L in the MBNR (Biological) anaerobic reactor during the same period. The comparable data in both systems suggest that insignificant chemical phosphorus removal was taking place in the anaerobic reactor. It can be concluded that both alum and EBPR were responsible for the low PO_4-P concentration in the MBNR (Chemical) system effluent in Phase IV of operation. Regarding inhibition of EBPR at the increased alum concentration of 40 mg/L, there was no evidence of it in the MBNR (Chemical) system reactor PO_4-P scan profile (Figure 5.17), the PO_4-P release (-)/uptake (+) profile (Figure 5.18) or the batch test results (**Section 6.4**) in Phase IV of operation. The conclusion that could be derived is that sufficient phosphorus was available in the MBNR (Chemical) system for both chemical phosphorus removal and EBPR without interference. As mentioned earlier, the EBPR performance was not efficient in the parallel MBNR systems. For that reason, more phosphorus was available for alum complexation. This scenario might have been different with a higher functioning EBPR system. It is also important to mention that 20 mg/L and 40 mg/L of alum addition were continued only for a short period of time. In a hypothetical situation, if the MBNR (Chemical) system had been supplemented with either of the above dosages for longer periods, the impact on EBPR might have been different. This is because the alum inventory would have steadily increased in the system such that eventually, there would have been substantial free alum available for complex formation. If both PAOs and alum targeted the same soluble phosphorus, chemical phosphorus removal would have been expected to be the dominant removal mechanism due to faster reaction rates.

Alum dosage was doubled to 80 mg/L on day 347 and this level of addition was continued until the end of the study (Phase V). The average effluent PO_4-P concentration

was 0.07 mg/L and removal efficiency was 98 percent during that period (Figure 5.16). In fact, there were periods in which effluent PO_4-P concentration was below the detection limit (detection limit was 0.01 mg/L) for the MBNR (Chemical) system. It seems that the high alum dosage produced extremely low effluent PO_4-P concentrations along with consistent removal, as demonstrated in Figure 5.16. On the other hand, the EBPR mechanism gradually declined until it was almost negligible in the MBNR (Chemical) system (Figure 5.17 and Figure 5.18). Figure 5.18 also shows that both anaerobic release and aerobic uptake mechanisms were affected. The inhibition of EBPR was most likely caused by competition for soluble phosphorus with alum. Without phosphorus, the aerobic growth of PAOs stopped and hence, their concentration slowly decreased in the MBNR (Chemical) system mixed liquor. Results from batch studies during Phase V confirmed the observations from the continuous flow system (**Section 6.5**). The overall phosphorus removal occurring in the MBNR (Chemical) system during the period of 80 mg/L dosing was initially attributable to simultaneous biological and chemical removal, and then subsequently to chemical phosphorus removal only for the rest of period.

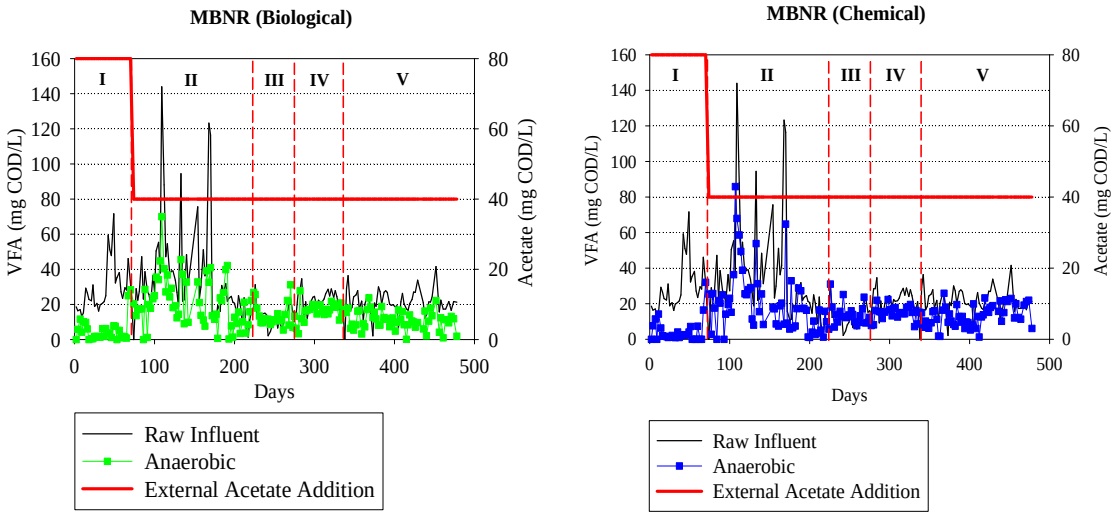


Figure 5.19 Raw influent and anaerobic VFA profile of the parallel MBNR systems

Although chemical phosphorus removal helped to achieve the low observed effluent PO_4-P concentrations, further analysis is required to understand the efficiency of treatment. Figure 5.20 summarizes the mole Al (added)/mole TP (removed) and mg alum

(added) /mg TP (removed) ratios observed in the MBNR (Chemical) system. The average molar Al/TP ratios were 0.6 (Phase III), 0.8 (Phase IV) and 1.9 (Phase V). The value of 1.9 is comparable to the data published by Daigger *et al.* (2009), who reported that an Al/TP molar dose between 1.0 and 2.5 at the Broad Run water reclamation plant (WRP) achieved less than 0.05 mg/L TP in the effluent. The Broad Run MBNR WRP was configured to produce effluent TN < 3 mg/L and TP < 0.1 mg/L. The plant design incorporated simultaneous biological and chemical phosphorus removal. Nonetheless, it is postulated that alum dosage could have been further optimized in the present MBNR (Chemical) system. In the present system, alum was directly added to the membrane tank. High shearing conditions in the tank most probably hindered flocculation of aluminum precipitates and microbial floc. As a result, more alum may have been added than was required to reduce effluent TP concentrations. In full scale plants, therefore, it has been recommended to add alum in the mixed liquor pipe between the bioreactor and membrane tank. In a recent development, Liu *et al.* (2011) added a small alum contact tank (10 minute HRT) to optimize chemical phosphorus removal in a pilot UCT MBR process.

Figure 5.20 also illustrates that specific phosphorus removal (mg alum added/mg TP removed) decreased with an increase in alum dosing. This observation has been documented in other studies (Szabó *et al.*, 2008) and the rationale for it can be found elsewhere (Maurer and Boller, 1999). A high alum requirement would be a significant cost driver for wastewater treatment plants targeting LoT TP goals. For plants designed for simultaneous phosphorus removal mechanisms, an optimally functioning EBPR capability is, therefore, a crucial requirement. Once it is achieved in an MBNR system, the alum requirement would be minimized with no affect on final effluent TP concentrations.

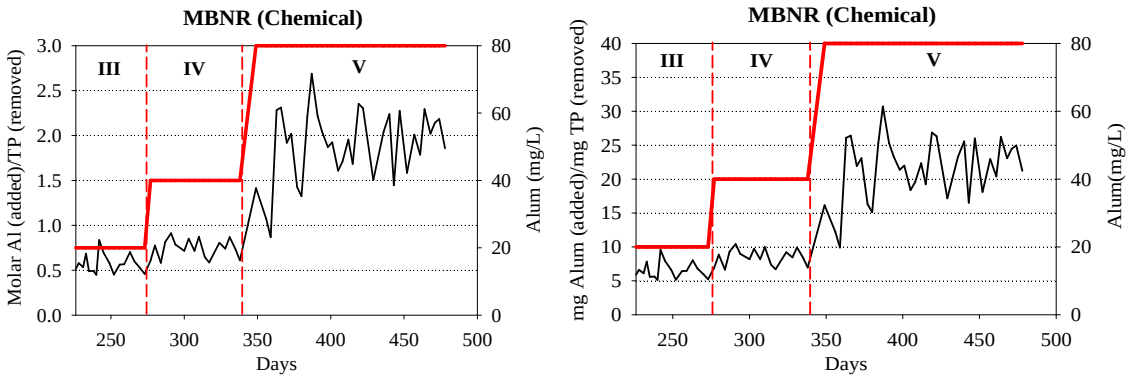


Figure 5.20 Alum-induced phosphorus removal in MBNR (Chemical) system

The preceding discussion of the phosphorus removal performance of the MBNR (Chemical) system provides important insight into the relationship between the simultaneous biological and chemical phosphorus removal mechanisms. It seems that both mechanisms can co-exist if sufficient phosphorus is available in soluble form. Once there is competition for phosphorus, it is most likely that chemical phosphorus removal would become the dominant mechanism. As a result, EBPR would be inhibited and the system would evolve to become a chemical phosphorus removal process only. For successful operation of simultaneous phosphorus removal, the alum dosage requirement could be calculated by evaluating P removal performance of the designed EBPR system with respect to effluent discharge limits. The case of LoT phosphorus removal is very important. It is highly unlikely that engineers would design a standalone EBPR system when the objective is ≤ 1 mg/L TP in the effluent. They would probably opt for either a combined simultaneous phosphorus removal system (e.g. Broad Run WRP) or a chemical removal system (e.g. Spokane WRF) (Daigger *et al.*, 2009). In case of a combined system targeting LoT phosphorus removal, the contribution of EBPR in TP removal should be evaluated first for the chosen configuration. This could be achieved by using either ASM-based simulators or pilot operation with the same influent wastewater, or the combination of both. A thorough investigation of EBPR removal potential should be conducted as PAOs are susceptible to dynamic influent conditions and seasonal changes. Following that, the stoichiometric alum requirement could be calculated. During operation of the plant, anaerobic phosphorus release and aerobic uptake data should be profiled regularly, to monitor the impact of chemical phosphorus removal. However, sometimes, unexpected

suboptimal EBPR might be encountered. If so, higher alum dosing would be needed to meet effluent discharge criteria. In such a scenario, the potential inhibition of EBPR and the capability of the system in a subsequent revival of optimum EBPR and the timeframe for it, are not very well understood. Further research is needed in this direction, to assess the recovery of a chemical phosphorus removal-dominated system to that of a functioning EBPR system, with reduction or complete stoppage of alum addition. Additionally, the timeframe (i.e. number of SRTs) for such a conversion would provide important information on the persistence effect of metal salt in the mixed liquor.

5.4 Suspended Solids Data

Suspended solids concentration was related to the design SRT of the parallel MBNR systems. Based on the selected SRT of 25 days, mixed liquor wasting was done on a daily basis to maintain steady state conditions in the two systems. Mixed liquor wasting was calculated in the present study by the following formula:

$$W \quad \frac{\sum_{ea} \qquad\qquad\qquad\qquad e}{} \tag{10}$$

where,

= Mixed liquor wasting rate (L/day)

= Reactor volume (L)

S = Reactor suspended solids concentration (mg/L)

= Solids retention time (SRT) (days)

= Flow rate (L/day)

S = Effluent suspended solids concentration (mg/L)

S = Suspended solids concentration from the wasting reactor (aerobic) (mg/L)

Total suspended solids (TSS) data for the MBNR (Biological) and MBNR (Chemical) systems are shown in Figure 5.21 and Figure 5.22, respectively. In addition, individual reactor average TSS and standard deviation values are summarized in Table 5.3. The data

show that TSS values were comparable in both systems when alum was not being added to either. With alum addition, TSS gradually increased in the MBNR (Chemical) system (Figure 5.22). Although some alum was wasted during normal suspended solids wasting, the high final dosage (80 mg/L) of alum applied contributed significantly to the final MLSS concentration in the MBNR (Chemical) system.

Volatile suspended solids (VSS) to TSS percentage ratio profiles of the parallel systems are shown in Figure 5.23 and Figure 5.24 respectively. Also, individual reactor average % VSS/TSS ratio, along with standard deviation values, is summarized in Table 5.4. The data demonstrate that the %VSS/TSS ratio was higher than typically observed in other activated sludge systems. It was most probably due to efficient settling of influent wastewater particulate material at different stages before it entered the bioreactors: (1) influent wastewater was collected after a preliminary settling tank at the SERC, University of British Columbia, (2) further settling took place in the transport containers and (3) finally, daily settling was observed in the influent holding tank. All of these resulted in loss of significant fraction of the particulate solids. Figure 5.24 and Table 5.4 also illustrate the expected decline of % VSS/TSS in the presence of alum.

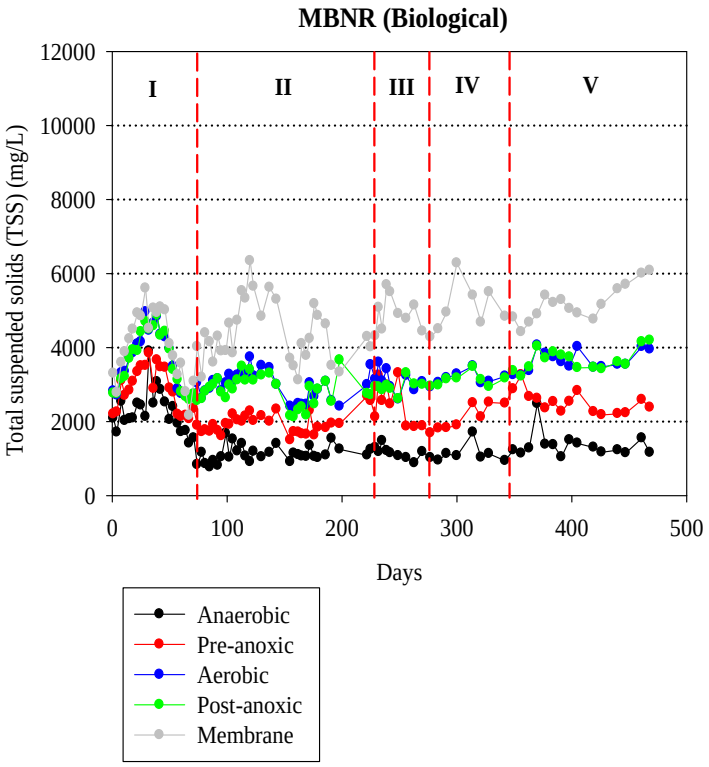


Figure 5.21 TSS concentration in MBNR (Biological) system

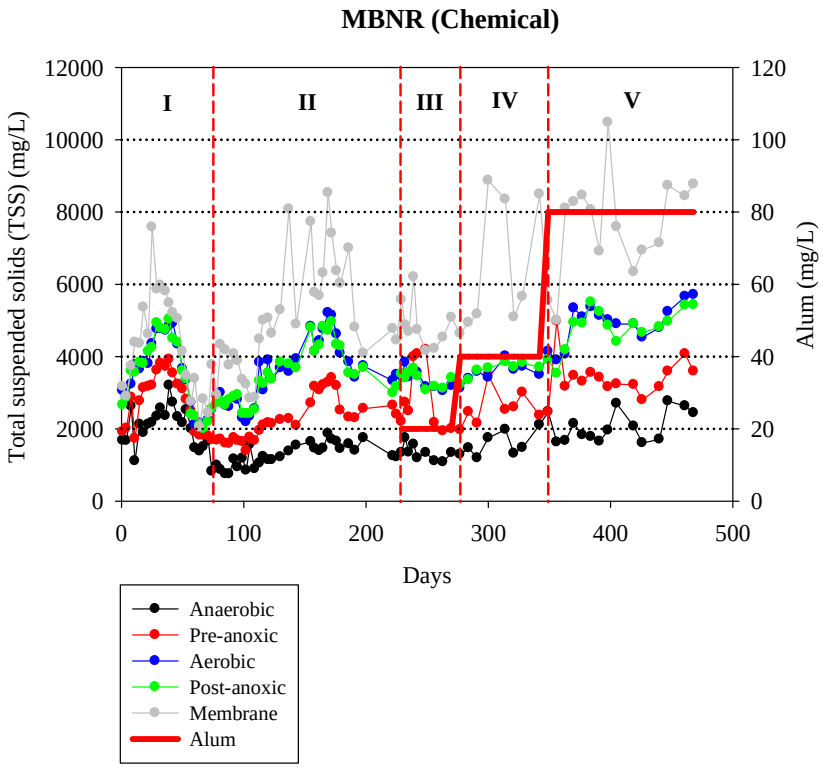


Figure 5.22 TSS concentration in MBNR (Chemical) system

Table 5.3 Average TSS values in MBNR (Biological) and MBNR (Chemical) system

	Average TSS (mg/L)				
	MBNR (Biological)	**MBNR (Chemical) Alum = 0 mg/L)**	**MBNR (Chemical) Alum = 20 mg/L**	**MBNR (Chemical) Alum = 40 mg/L**	**MBNR (Chemical) Alum = 80 mg/L**
Anaerobic	1452 (±135)	1594 (±568)	1342 (±214)	1574 (±338)	2071 (±425)
Pre-anoxic	2355 (±559)	2468 (±705)	2865 (±947)	2574 (±483)	3413 (±569)
Aerobic	3290 (±571)	3549 (±894)	3384 (±262)	3548 (±262)	4913 (±554)
Post-anoxic	3230 (±603)	3505 (±872)	3375 (±209)	3620 (±223)	4780 (±568)
Membrane	4564 (±858)	4734 (±1528)	4896 (±651)	6402 (±1824)	7653 (±1388)

±: Standard Deviation (SD)

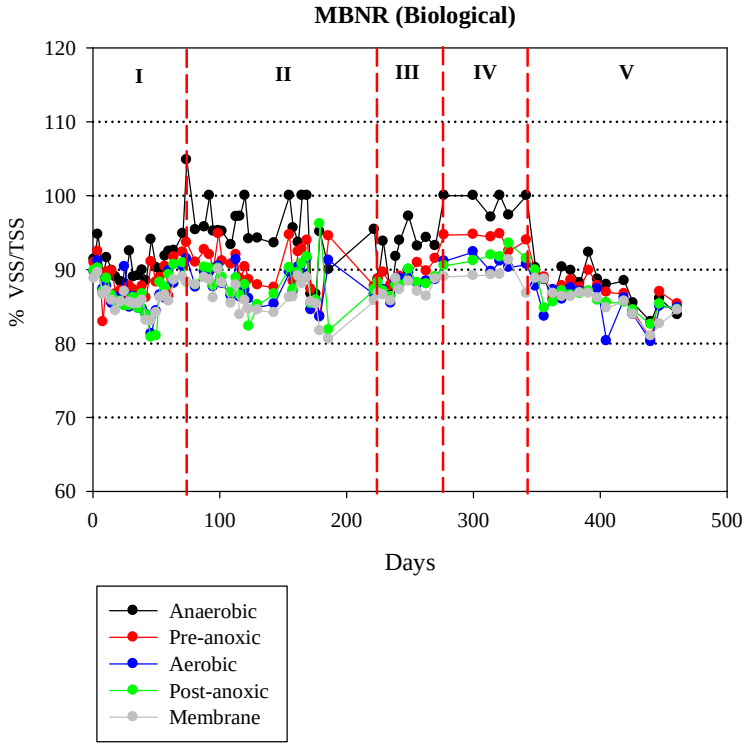


Figure 5.23 % VSS/TSS ratio in MBNR (Biological) system

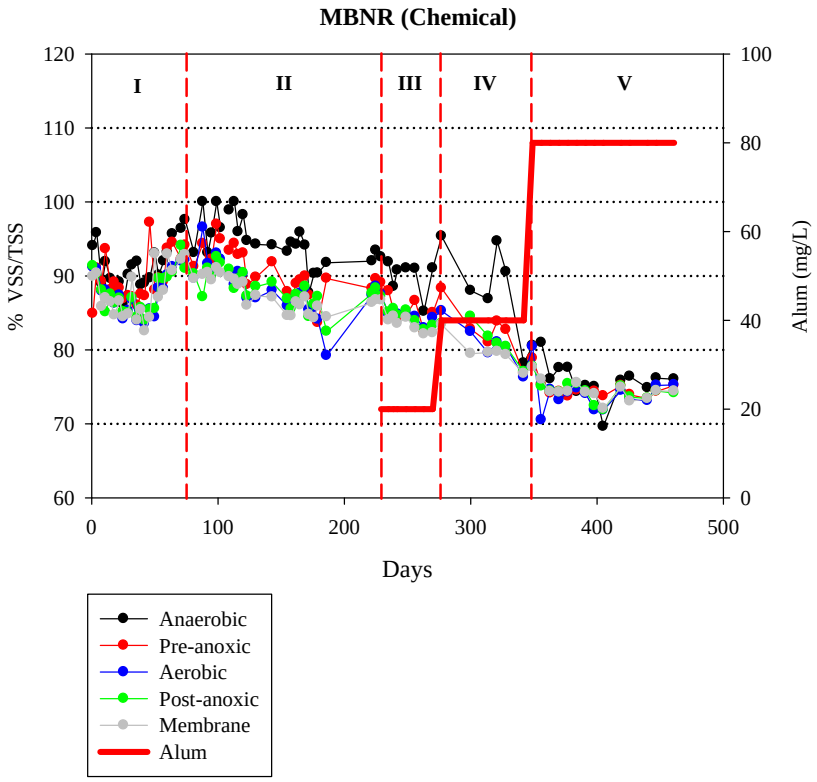


Figure 5.24 % VSS/TSS ratio in MBNR (Chemical) system

Table 5.4 Average % VSS/TSS ratios in MBNR (Biological) and MBNR (Chemical) system

	Average % VSS/TSS ratio				
	MBNR (Biological)	**MBNR (Chemical) Alum = 0 mg/L**	**MBNR (Chemical) Alum = 20 mg/L**	**MBNR (Chemical) Alum = 40 mg/L**	**MBNR (Chemical) Alum = 80 mg/L**
Anaerobic	93 (±5)	93 (±4)	91(±3)	89 (±7)	76 (±3)
Pre-anoxic	90 (±3)	91 (±3)	86 (±2)	83 (±4)	75 (±2)
Aerobic	88 (±3)	88 (±3)	85 (±2)	81 (±3)	74 (±3)
Post-anoxic	88 (±3)	88 (±3)	85 (±2)	81 (±3)	75 (±2)
Membrane	87 (±2)	87 (±2)	84 (±2)	80 (±3)	75 (±2)

±: Standard Deviation (SD)

5.5 LoT Goal-MBNR (Biological) System

The MBNR (Biological) system effluent TP and TN (TKN + NO_3-N) profiles are shown in Figure 5.25. It is evident from the TP data that the LoT objective could not be accomplished by EBPR alone in the MBNR (Biological) system. As far as TN data are

concerned, the LoT goal of 3 mg/L was accomplished. The average effluent TN concentration was 3 mg/L with 72 mg/L of methanol supplementation in the MBNR (Biological) system. An in-depth discussion has already been presented to explain the rationale for the nitrogen and phosphorus removal performance of the MBNR (Biological) system. Nonetheless, it was thought to be imperative to determine whether LoT goal could realistically be realized in processes comparable to the MBNR (Biological) system (i.e. employ biological nitrogen and phosphorus removal principles). Lesjean *et al.* (2005) reported effluent TP and TN concentrations of 0.05 mg/L (90 percent of the time) and 5 mg/L (some periods) respectively in a pilot scale 3-stage post-denitrification MBR system. These authors attributed LoT phosphorus removal to EBPR along with phosphorus precipitation with calcium and ferric ions present in the influent wastewater (approximately 130 mg Ca/L and 10 mg Fe/L). Nitrogen removal, specifically denitrification, was excellent for some periods when N-loading was constant in the process. However, variable N-loading caused fluctuations in denitrification performance and hence, impacted TN effluent concentrations. The key conclusions from their study were that chemical precipitation of phosphorus aided EBPR in meeting the LoT TP goal, whereas the LoT TN goal was not realized. Monti (2006) achieved effluent TP in the range of 0.1-0.2 mg/L by employing EBPR in a pilot scale modified UCT-MBR configuration. Although EBPR was broadly successful in that study, the authors reported periods of failure with elevated TP concentrations in the MBR effluent. In addition, effluent TN was consistently high with an average concentration of 10 mg/L in the effluent. No external carbon was added to improve denitrification during that work. The Monti study provided further evidence of the difficulty of achieving LoT nutrient removal without external addition of carbon and metal salt.

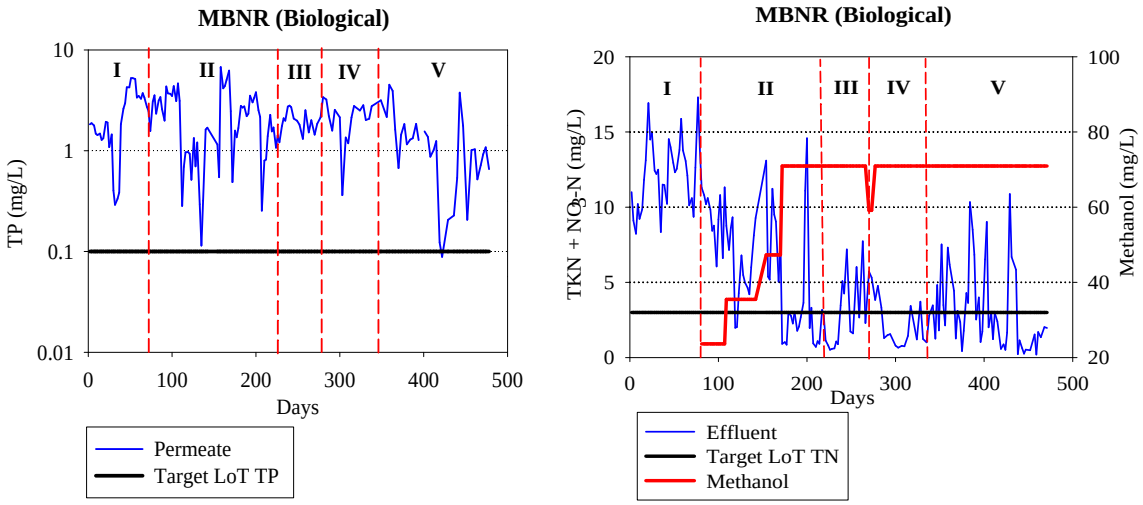


Figure 5.25 Effluent TP and TN concentrations in the MBNR (Biological) system

In conclusion, EBPR performance could hypothetically be improved in MBNR (Biological) system with pH control in the aerobic reactor and an increased suspended solids inventory in the anaerobic reactor. Similarly, resolving the issue of foam mixing in the post-anoxic reactor could help achieving LoT TN goal in the MBNR (Biological) system more consistently.

5.6 LoT Goal-MBNR (Chemical) System

The MBNR (Chemical) system effluent TP and TN (TKN + NO_3-N) profiles are shown in Figure 5.26. From the figure, it could be concluded that alum was the major driver in accomplishing extremely low effluent TP concentrations in the MBNR (Chemical) system. With the highest alum dosage of 80 mg/L, the average effluent total P concentration was 0.19 mg/L. Moreover, effluent TP was observed to be less than 0.1 mg/L on some days during that period. These data demonstrate the capability of the MBNR (Chemical) system in meeting the LoT TP goal. During the same period, effluent PO_4-P concentrations were very low with an average concentration of 0.07 mg/L, indicating that most of the total P concentration was composed of other forms of phosphorus. It is imperative to understand the other fractions of phosphorus in the LoT effluent. According to Neethling *et al.* (2007), effluent from chemical phosphorus removal processes consists primarily of ortho-phosphorus and organic phosphorus.

Organic phosphorus is refractory in nature and typically present in the range of 0.01 to 0.05 mg/L in secondary and tertiary wastewater treatment plant effluents. Furthermore, the authors commented that determination of the characteristics of refractory organic phosphorus and its treatability is a key factor in pursuing the LoT TP goal. In the present study, due to the use of membrane-based solids-liquid separation, only rDOP (refractory dissolved organic phosphorus) was present in the permeate. The average permeate rDOP concentration was 0.12 mg/L (0.19 mg/L TP -0.07 mg/L PO_4-P) in the MBNR (Chemical) system, which was higher than the range reported above (Neethling *et al.*, 2007). The value shows that removal of rDOP would be crucial in meeting the LoT TP goal. Alum has been reported to remove rDOP (Arnaldos and Pagilla, 2010). For the MBNR (Chemical) system, there could be a requirement for significantly higher alum dosing to accomplish very low rDOP concentrations.

The TN profile in Figure 5.26 demonstrates the ability of the MBNR (Chemical) system in meeting LoT goal. The average permeate TN concentration was 2.4 mg/L with 72 mg/L of methanol supplementation, which was lower than the target LoT effluent TN of 3 mg/L.

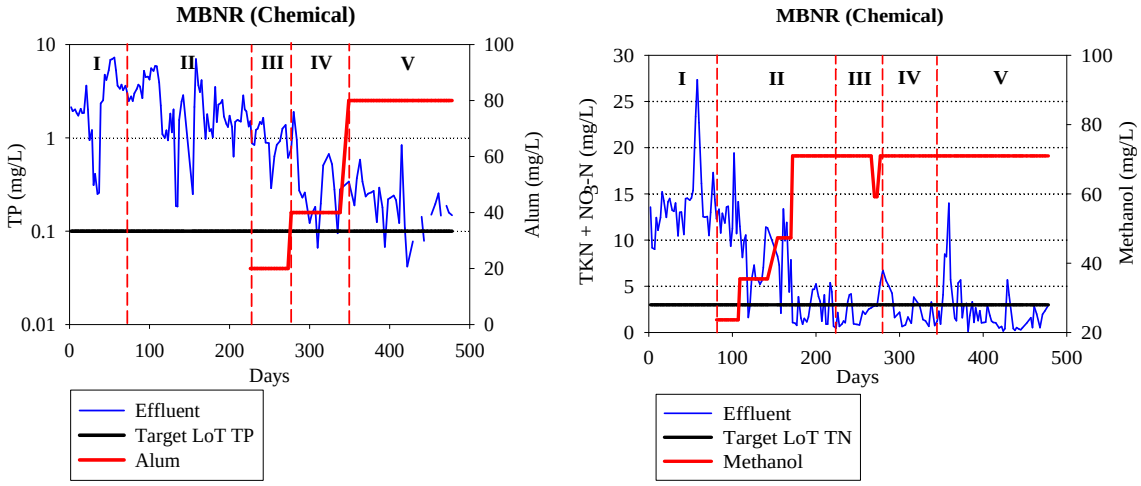


Figure 5.26 Effluent TP and TN concentrations in the MBNR (Chemical) system

The key conclusion was that the MBNR (Chemical) system had the capability of meeting the LoT TP and TN goals. Nonetheless, external addition of methanol and alum were key factors in realizing the goal for the chosen process configuration.

5.7 Conclusions

The key conclusions on performance of the parallel MBNR systems are the following.

- Permeate COD concentrations were similar in both MBNR systems with average removal efficiencies of 91 percent for the study.
- Nitrification was essentially complete in the MBNR (Biological) system. Similar performance was achieved in the MBNR (Chemical) system, except when the average permeate ammonium concentration was 3.6 mg/L between operating day 354 and 380.
- Denitrification performance was primarily driven by methanol dosing. Without methanol addition, average permeate NO_3-N concentrations were 11.3 mg/L and 13 mg/L in the MBNR (Biological) system and MBNR (Chemical) system respectively. With the highest methanol dosing applied (i.e. 72 mg/L), permeate NO_3-N concentrations were very low with average values of 2.0 mg/L and 1.4 mg/L in the MBNR (Biological) system and MBNR (Chemical) system respectively.
- The stoichiometric methanol ratio, i.e. mg methanol required / mg NO_3-N removed, was calculated to be 7.6 and 6.1 for reducing the average permeate NO_3-N concentration to 2 mg/L and 1.4 mg/L for the MBNR (Biological) system and MBNR (Chemical) system respectively.
- EBPR employed in the MBNR (Biological) system (whole study period) and MBNR (Chemical) system (first two phases of operation) accomplished an average PO_4-P removal efficiency of 41 percent and 27 percent respectively. The sub-optimum phosphorus removal performance was attributed to low observed aerobic reactor pH and low anaerobic SRT.
- Permeate PO_4-P removal steadily improved to 71 percent, 89 percent and 98 percent during the 20 mg/L, 40 mg/L and 80 mg/L alum dosing periods respectively, in the MBNR (Chemical) system.
- In the MBNR (Chemical) system, an average molar Al/TP ratio of 1.9 was required to reduce the PO_4-P concentration to 0.07 mg/L in the permeate. The ratio is in the recommended range of 1.0 to 2.5 for accomplishing < 0.05 mg/L TP in the effluent.

- Operation of the MBNR (Chemical) system demonstrated that the relationship between chemical P removal and EBPR is dynamic and is dependent on alum dosage concentration. Alum dosing up to a concentration of 40 mg/L complemented EBPR in improving permeate P removal. On the other hand, 80 mg/L of alum supplementation competed with and finally, inhibited EBPR until the MBNR (Chemical) system was converted fully to a chemical P removal system. The dynamic relationship, i.e. complimentary or competitive, needed further confirmation, particularly the related impact on EBPR kinetics. A series of sequential anaerobic-aerobic batch tests have been conducted and the results are discussed in Chapter Six.
- Alum addition did not have any influence on COD removal and denitrification in the MBNR (Chemical) system when compared with the MBNR (Biological) system. Similar conclusion can be made for nitrification except when permeate ammonium concentration was observed to be at elevated levels between operating day 354 and day 380, at the beginning of the period of 80 mg/L of alum dosing. It is thought that low influent alkalinity in combination with alum-induced alkalinity deficit conditions in the mixed liquor impeded nitrification temporarily.
- The parallel MBNR system performance data for the period without external methanol and alum dosing provided evidence of the inability of the selected modified Bardenpho process configuration (as well as internal recycle rates) in accomplishing the LoT nutrient removal goal.
- The MBNR (Biological) system average permeate TP concentration was 2.1 mg/L, demonstrating the difficulty of using only EBPR to achieve extremely low effluent TP concentrations. On the other hand, when supplemented with 72 mg/L of external methanol, the observed average permeate TN concentration was 3 mg/L, thus meeting the LoT TN effluent target.
- The MBNR (Chemical) system demonstrated LoT TP and TN treatment capability in the present study. The average permeate TP concentration was 0.19 mg/L (including periods when TP concentration was < 0.1 mg/L) with 80 mg/L of alum dosing. Similarly, an average permeate TN concentration of 2.4 mg/L was achieved with 72 mg/L of methanol dosing. The extremely low permeate TN and TP values in the

MBNR (Chemical) system established the significance of external alum and methanol requirement in realizing LoT effluent discharge goals.

6 Batch Studies for Comparative Evaluation of EBPR Kinetics and Stoichiometry of the Parallel MBNR Systems

6.1 Introduction

Batch tests were conducted on mixed liquor from the parallel MBNR systems for deeper understanding of the relationship between biological and chemical phosphorus removal at different alum dosing levels. Mixed liquor was withdrawn periodically from the aerobic reactors of the parallel MBNR systems for the tests. The tests were conducted in 1.0 L Erlenmeyer flasks and the temperature and pH were constant at 20 ^{0}C and 7.0 respectively. Before the start of the tests, a non-aerated period of 2-4 hours was maintained to remove residual nitrate present in the aerobic mixed liquor. This step was taken to avoid interference in EBPR activity test from denitrification-related VFA consumption. Following the non-aerated period, a sequential anaerobic (for 2 hours) and aerobic period (for 3 hours) were maintained to monitor phosphorus release and uptake capabilities of the mixed liquor, respectively. The batch tests were done in Phases II, III, IV and V to analyze the role of increased alum dosing rates on EBPR potential in the MBNR (Chemical) system (**APPENDIX B**). Three tests were conducted in each phase of operation. The EBPR potential of the MBNR (Chemical) system was assessed relative to the MBNR (Biological) system and conclusions were derived. Finally, the batch tests were also expected to provide key information on EBPR kinetics and stoichiometry of both systems at different stages of operation.

6.2 Batch Tests - Phase II

Batch test NO_3-N and PO_4-P profiles of the parallel MBNR systems are shown in Figure 6.1. NO_3-N was present at the beginning of the anaerobic period of all the tests. This was unexpected as a non-aerated period of 2 hours had been allowed prior to the batch test for endogenous denitrification. The presumption was that 100 percent of the nitrate would be removed during that time period. The results in Figure 6.1 indicated

otherwise and hence, the length of the non-aerated phase was increased to 4 hours in subsequent batch tests. The NO_3-N concentration was reduced to 0 mg/L at the time of collection of the first samples from the anaerobic period (i.e. after ½ hour) in all the batch tests. It is believed that acetate added at the start of anaerobic period was used for both denitrification and phosphorus release. The presence of residual acetate after ½ hour of the anaerobic period (Figure 6.2) indicated that 100 mg COD/L of acetate was sufficient for denitrification and phosphorus release. Hence, it was concluded that nitrate did not inhibit phosphorus release in the anaerobic phase of the batch tests. Nonetheless, almost all the acetate was utilized at the end of anaerobic period in five of the tests, with the only exception being Batch Test 1 with MBNR (Chemical) system mixed liquor. Also, from Figure 6.2, maximum acetate utilization took place in the first ½ hour of the anaerobic phase.

The PO_4-P profile in Figure 6.1 illustrates functioning EBPR in the parallel MBNR systems with classic anaerobic P release and aerobic P uptake. As expected, phosphorus release coincided with acetate utilization (Figure 6.2). However, EBPR potential was highly dynamic in both MBNR systems with varied phosphorus release and uptake values observed in each of the three batch tests. A key observation from Figure 6.1 was that the EBPR potential in the MBNR (Biological) system was greater than that of the MBNR (Chemical) system during that specific period of operation. It should be reiterated that there was no difference in the operation of the parallel MBNR systems during this experimental phase.

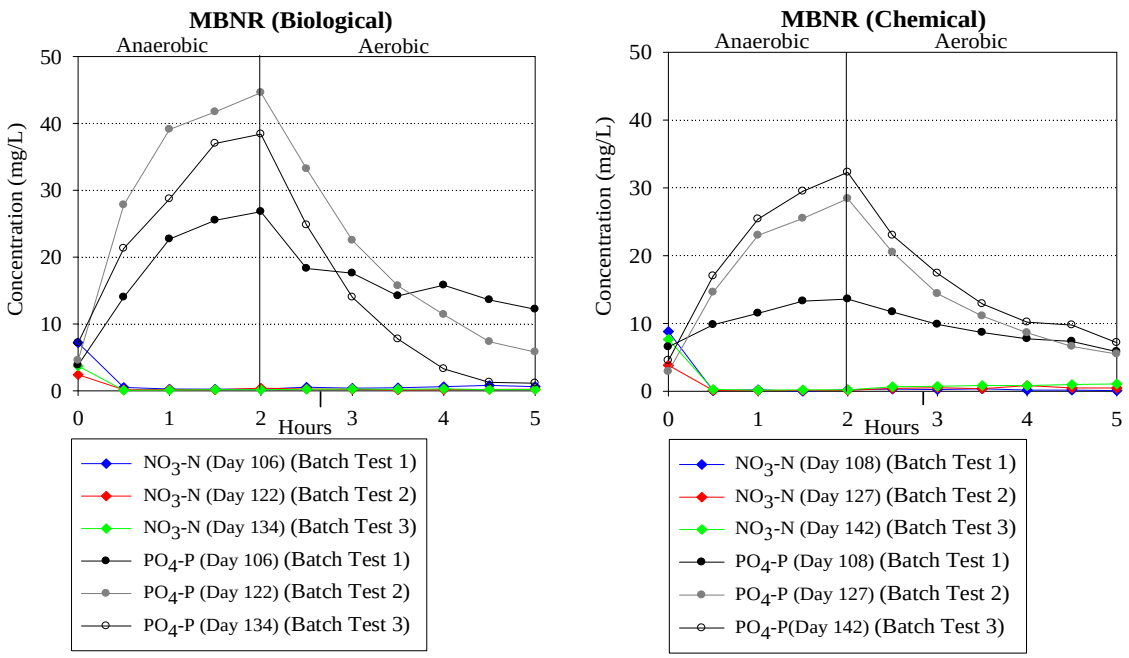


Figure 6.1 Batch test NO_3-N and PO_4-P profile of the parallel MBNR systems (Phase II)

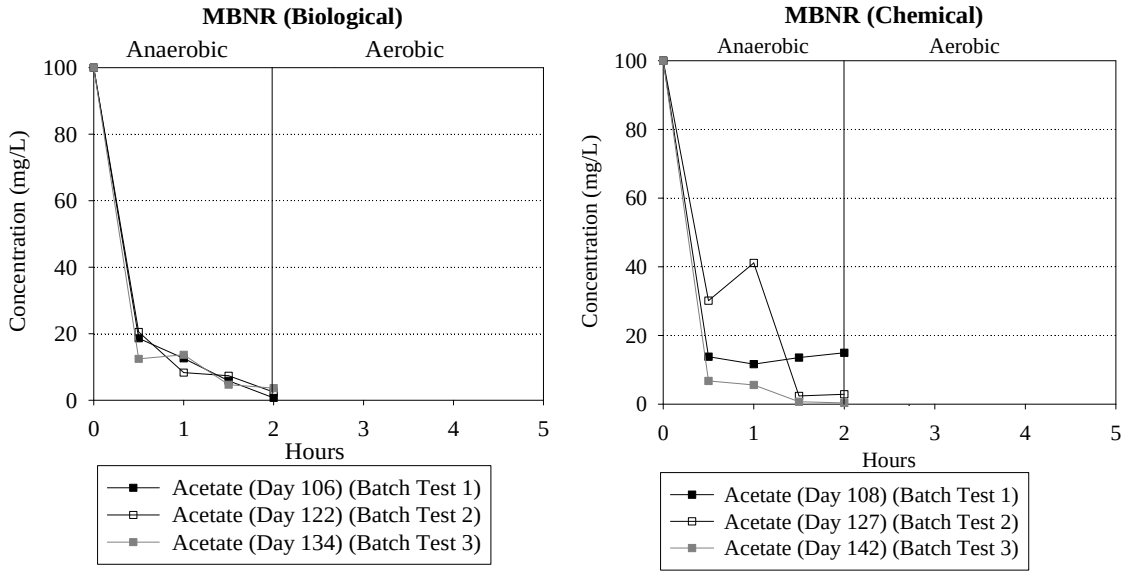


Figure 6.2 Batch test acetate profile of the parallel MBNR systems (Phase II)

EBPR kinetic and stoichiometric parameters were estimated from the batch studies. Figure 6.3 and Figure 6.4 summarize kinetic parameter values, i.e., maximum specific phosphorus release (mg P/(g VSS· hr) and uptake (mg P/(g VSS· hr) rates and the associated stoichiometric parameter values, i.e. P-released/VFAs-consumed (g P/g COD)

respectively, in the parallel MBNR systems. Both kinetic and stoichiometric values were the lowest in Batch Test 1 in both the MBNR systems. Subsequent tests resulted in higher values, although Batch Test 2 exhibited slightly higher parameter values than Batch Test 3. More importantly, the kinetic rates validate the earlier observation (Figure 6.1) of EBPR potential being significantly higher in the MBNR (Biological) system as compared to the MBNR (Chemical) system.

The stoichiometric parameter, P-released/VFAs-consumed, is typically expected to be of constant value in batch tests. This was not the case in the tests summarized in Figure 6.4. One explanation might be the occurrence of denitrification-related acetate utilization. A second factor could be presence of other microorganisms in the mixed liquor (for example, GAOs) which could utilize acetate under anaerobic conditions.

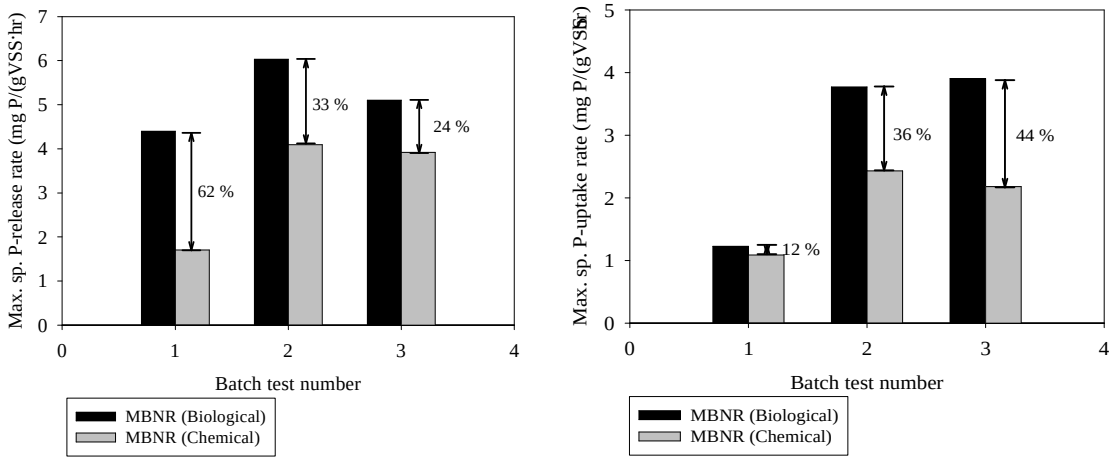


Figure 6.3 Batch test maximum specific phosphorus release and uptake profile (Phase II)

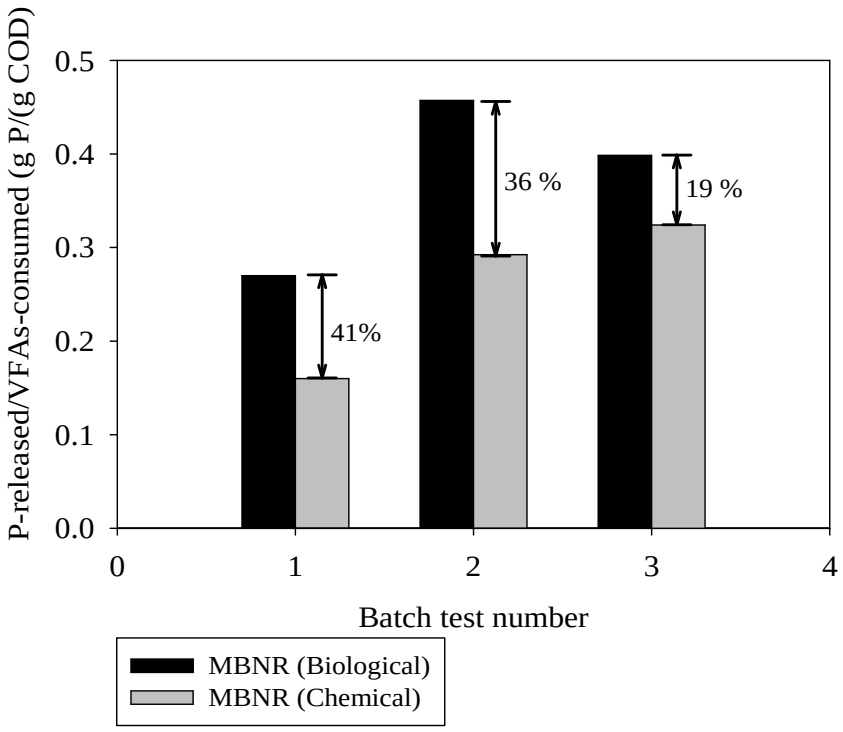


Figure 6.4 Batch test P-released/VFAs-consumed profile (Phase II)
(*VFA consumption related to denitrfication has not been subtracted)

The EBPR potential observed in the batch tests was evaluated with respect to the continuous system performance for the same period of study. Table 6.1 summarizes average anaerobic, aerobic and permeate PO_4-P concentrations of the parallel MBNR systems. The data show that anaerobic PO_4-P concentrations were higher in the MBNR (Biological) system at the times of the batch studies, whereas, aerobic and permeate PO_4-P concentration was higher in the MBNR (Chemical) system. Hence, EBPR potential differences observed in the batch studies were consistent with the phosphorus removal performances of the parallel MBNR systems.

Table 6.1 Parallel MBNR system P-profiling during the period of batch studies (Phase II)

	MBNR (Biological) system PO_4-P (mg/L) (between day 105-133)	**MBNR (Chemical) system PO_4-P (mg/L) (between day 109-140)**
Anaerobic Reactor	12.2 (±2.9)	10.1 (±1.2)
Aerobic Reactor	6.9 (±1.6)	7.5 (±1.8)
Permeate	1.4 (±1.1)	2.3 (±1.5)

±: standard deviation

6.3 Batch Tests - Phase III

NO_3-N and PO_4-P profiles from the Batch Tests 4, 5 and 6, conducted with the aerobic zone mixed liquor from the parallel MBNR systems are summarized in Figure 6.5. The measured NO_3-N concentrations were very low in both periods of all the tests. This established that a pre-test non-aerated phase of 4 hours was sufficient for removing residual nitrate present in the aerobic mixed liquor.

As far as PO_4-P profile was concerned, anaerobic P release and aerobic P uptake were observed in all the batch tests. Figure 6.5 illustrates that similar EBPR potential was observed in all three batch tests of both the MBNR (Biological) system and the MBNR (Chemical) system. Moreover, the EBPR potential of the MBNR (Chemical) system was comparable to that of the MBNR (Biological) system. The key points are that (1) EBPR potential was steady in the parallel MBNR systems in Phase III of operation (unlike Phase II) and (2) EBPR potential was not inhibited by 20 mg/L of alum supplementation in the MBNR (Chemical) system. However, PO_4-P release at the end of the 2 hour anaerobic period was lower in Batch Tests 5 and 6 (Figure 6.5) than in Batch Tests 2 and 3 (Figure 6.1) for both MBNR systems.

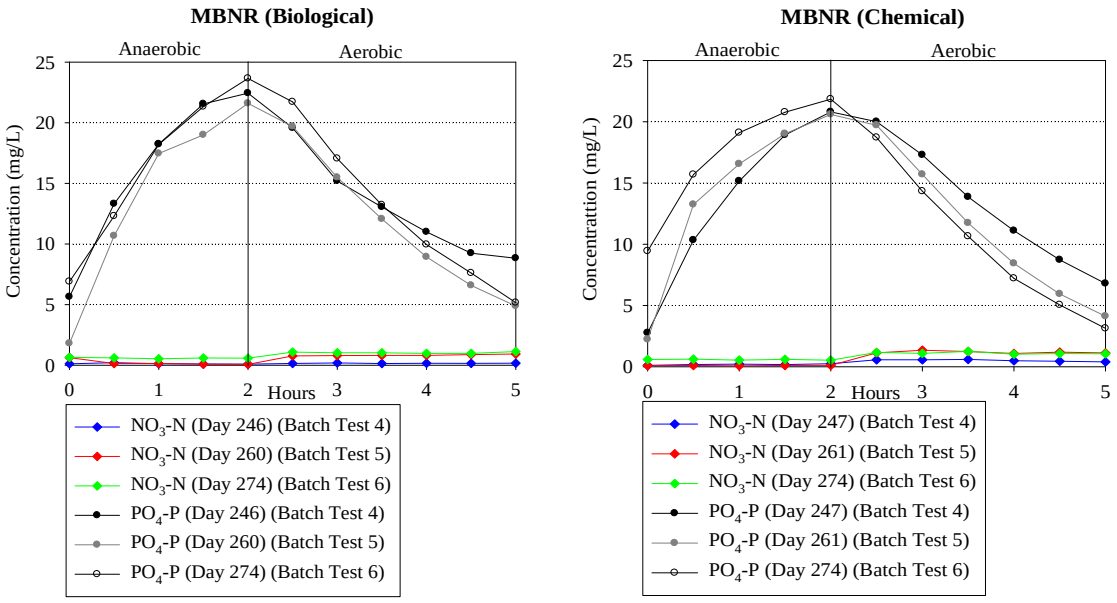


Figure 6.5 Batch test NO_3-N and PO_4-P profile of the parallel MBNR systems (Phase III)

Batch test acetate utilization profiles are shown in Figure 6.6. One set of data (batch test on day 274 with MBNR (Chemical) mixed liquor) was not reported, as samples were lost during analysis. Nevertheless, the data in Figure 6.6 illustrate that a significant amount of acetate was still present at the end of the anaerobic period. This was in contrast to the results of the Phase II batch tests (Figure 6.2) in which essentially all of the acetate had been consumed at this point. As mentioned in **Section 6.2**, the consumption of some of the acetate could be attributed to the presence of nitrate at the beginning of anaerobic phase in Phase II batch tests. In addition, it is hypothesized that a higher abundance of PAOs in the mixed liquor during Phase II of operation caused more extensive acetate uptake and hence, more phosphorus release (Figure 6.1). Nonetheless, in the Phase III tests, residual acetate was rapidly oxidized in the aerobic period as demonstrated in Figure 6.6. More acetate seemed to be utilized in the anaerobic periods of the MBNR (Biological) system batch tests than for the MBNR (Chemical) system batch tests (Figure 6.6).

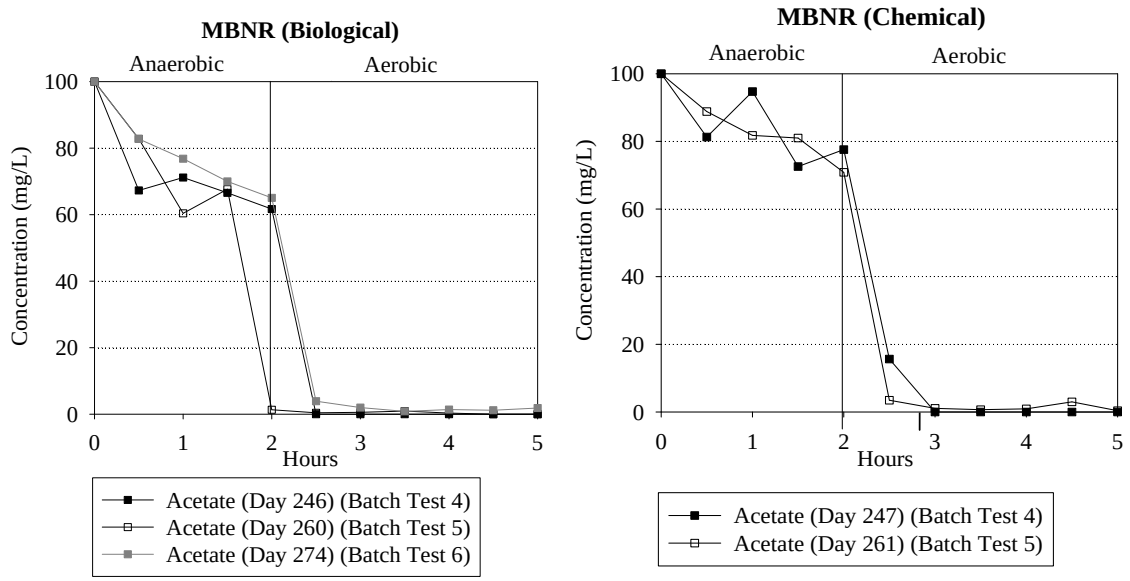


Figure 6.6 Batch test acetate profile of the parallel MBNR systems (Phase III)

EBPR potential was also assessed by profiling Mg^{+2} and K^{+1} in the anaerobic and aerobic phase of the batch tests (Figure 6.7). The samples were collected for the first two batch tests only. Nonetheless, cation concentrations mimicked the PO_4-P profiles with

release and uptake taking place in the anaerobic and aerobic periods respectively. Figure 6.7 further demonstrates that the cation profile was comparable in the two batch tests as well as in the parallel MBNR systems. This provides supplemental evidence of steady EBPR in Phase III of operation.

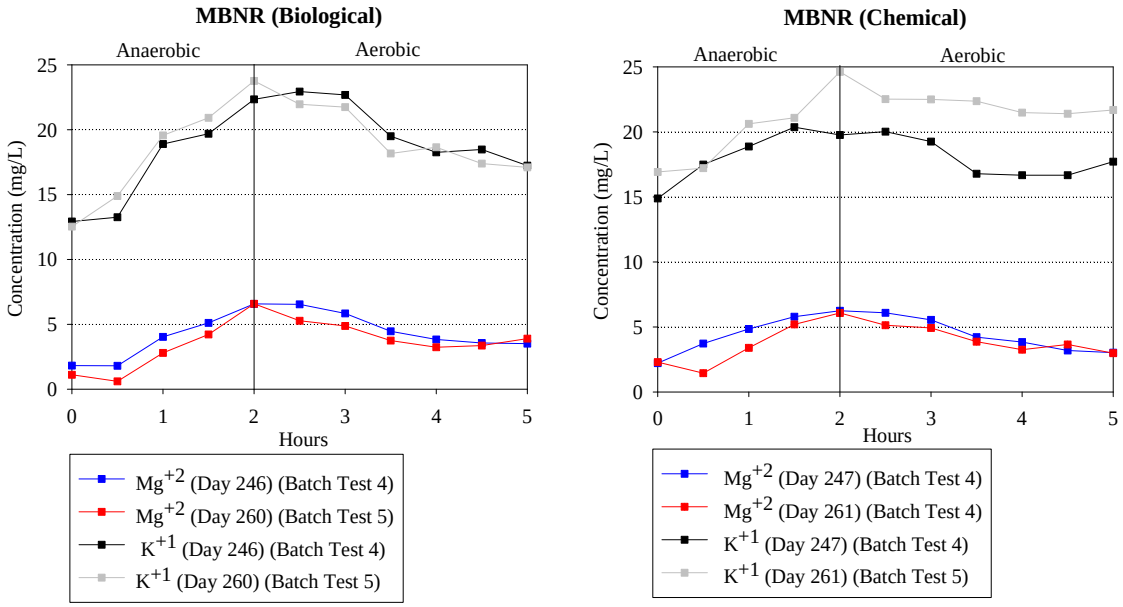


Figure 6.7 Batch test Mg^{+2} and K^{+1} profile of the parallel MBNR systems (Phase III)

EBPR kinetic and stoichiometric parameters were estimated for the Phase III batch studies. Figure 6.8 and Figure 6.9 summarize the kinetic parameter values, i.e., maximum specific phosphorus release (mg P/(g VSS·hr) and uptake (mg P/(g VSS·hr) and the values of the stoichiometric parameter, P-released/VFAs-consumed (g P/gCOD), respectively, in the parallel MBNR systems. The maximum specific phosphorus release values for both the MBNR systems were comparable in all three batch tests, although estimated values were lower in Batch Test 6 than in the previous two tests. Similarly, there was very little difference in the parallel MBNR systems as far as maximum specific phosphorus uptake data were concerned. In conclusion, the batch kinetic data provide additional confirmation of the earlier observation (refer Figure 6.5) that EBPR potential in the MBNR (Biological) system was comparable to that of the MBNR (Chemical) system during Phase III.

Interestingly, P-released/VFAs-consumed (g P/g COD) values were higher in the MBNR (Chemical) mixed liquor in batch test 4 and 5. Also, the values were different in the two batch tests for both the MBNR systems.

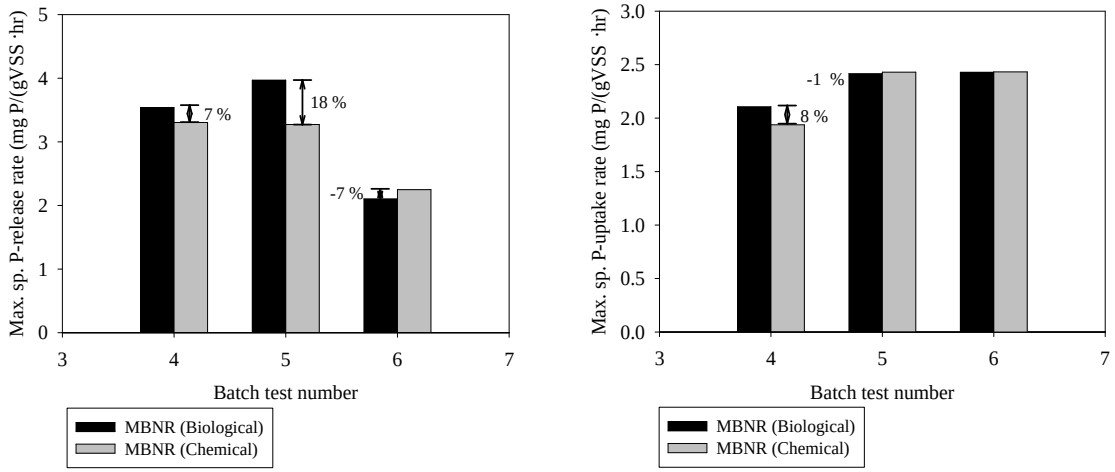


Figure 6.8 Batch test maximum specific phosphorus release and uptake profile (Phase III)

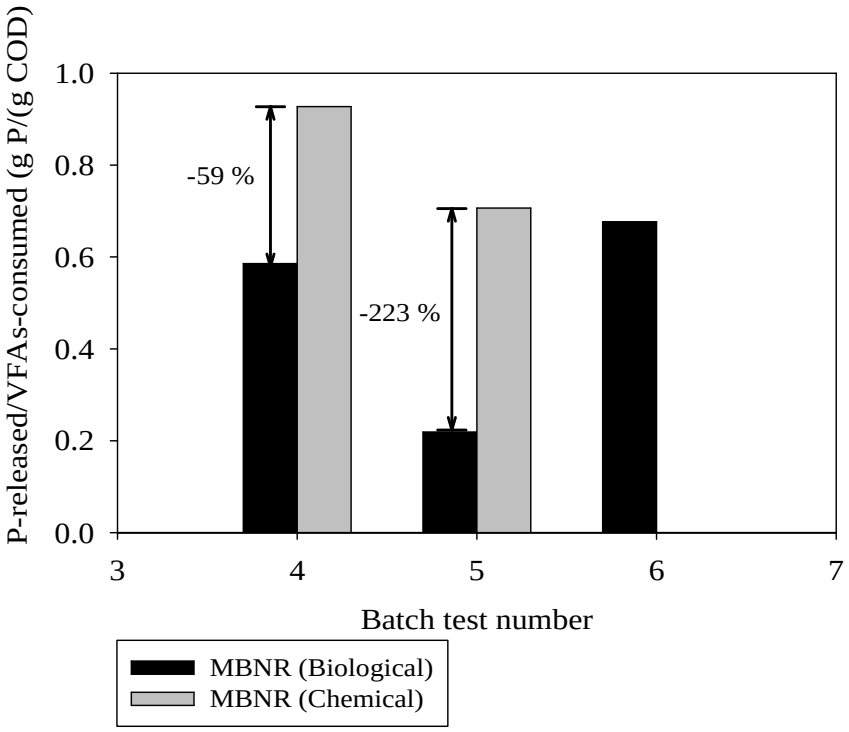


Figure 6.9 Batch test P-released/VFAs-consumed profile (Phase III)

Cation data shown in Figure 6.7 were further examined to elucidate relationships with respect to soluble orthophosphorus in the anaerobic and aerobic periods of the batch tests.

Figures 6.10 to Figure 6.13 illustrate molar K^{+1} and molar Mg^{+2} vs molar P values for the four batch tests. By examining the R^2 values of the figures, it can be said that a linear model is a reasonably good fit as far as relationship between the cations and phosphorus is concerned. This conclusion is applicable to both the anaerobic and aerobic periods of the batch tests. However, the R^2 value was lower in Batch Test 4 of the MBNR (Biological) system (Figure 6.10) and Batch Test 5 of the MBNR (Chemical) system (Figure 6.13). Another important observation was that mole K^{+1} / mole P and mole Mg^{+2} / mole P values were not constant and varied between periods, batch tests and between the parallel MBNR systems. Typically, the observed ratio was between 0.20 to 0.35 for both cations, which is in the range reported by several authors in literature (Table 6.6).

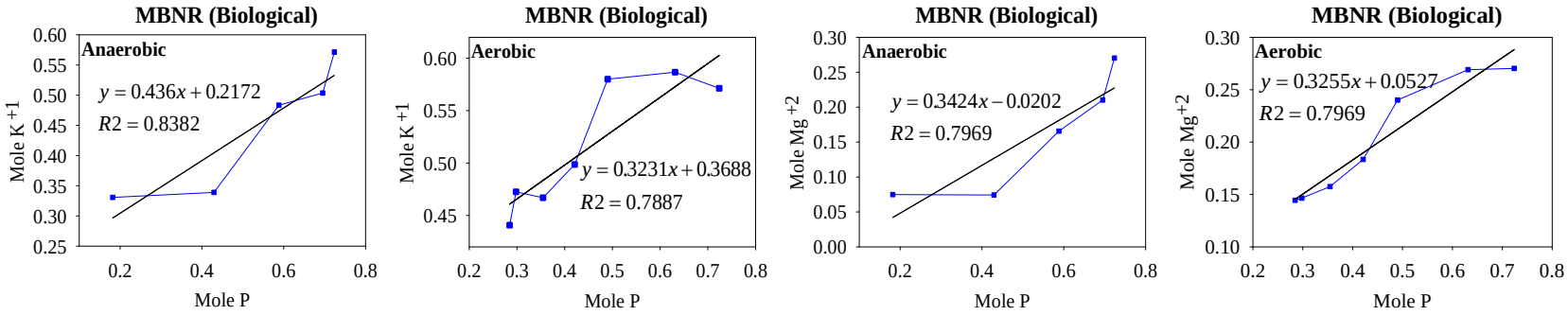


Figure 6.10 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 4 of MBNR (Biological) system (Phase III)

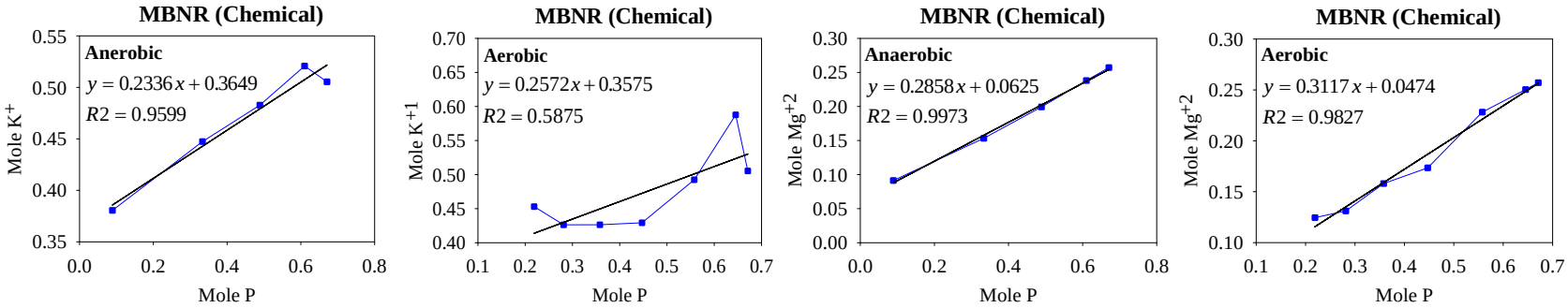


Figure 6.11 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 4 of MBNR (Chemical) system (Phase III)

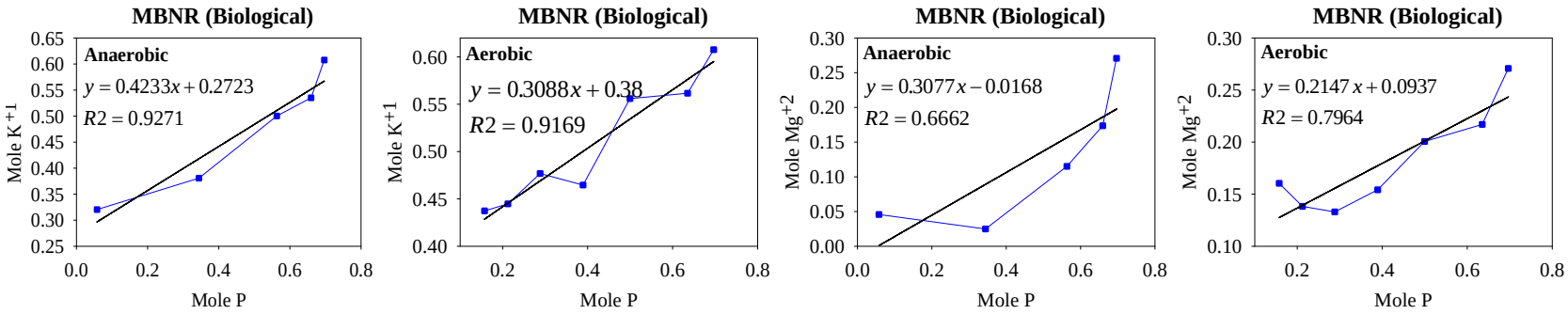


Figure 6.12 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 5 of MBNR (Biological) system (Phase III)

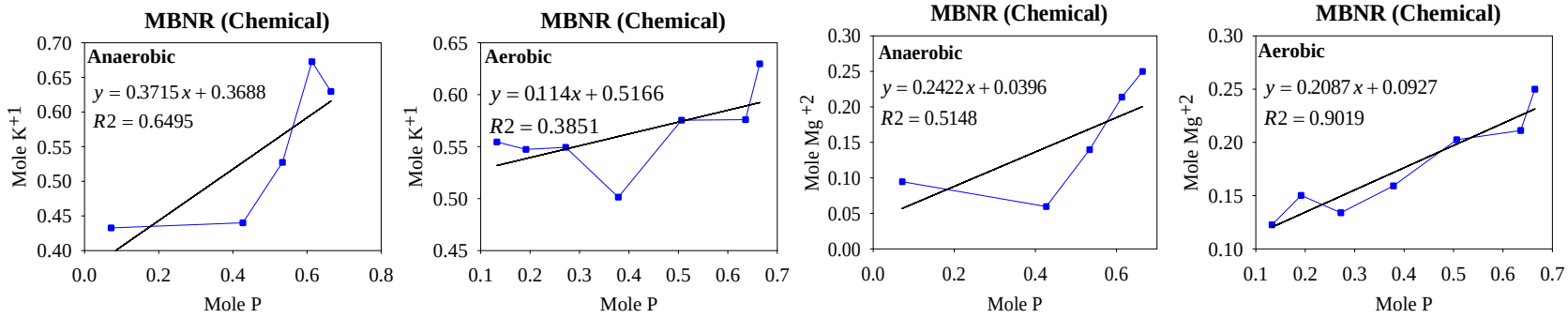


Figure 6.13 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 5 of MBNR (Chemical) system (Phase III)

Table 6.2 shows average PO_4-P concentrations in the anaerobic reactors, aerobic reactors and permeates of the parallel MBNR systems during the period of Phase III batch studies. Anaerobic PO_4-P concentrations were slightly higher in the MBNR (Biological) system whereas aerobic PO_4-P concentrations were similar in both systems between operating days 245 and 277. This observation is consistent with the batch test findings, which demonstrated comparable PO_4-P release/uptake profiles in the parallel MBNR systems. The average permeate PO_4-P concentration, on the other hand, was 1 mg/L lower in the MBNR (Chemical) system. This was attributed to the 20 mg/L of alum addition in Phase III of operation.

Table 6.2 Parallel MBNR system P-profiling during the period of batch studies (Phase III)

	MBNR (Biological) system PO_4-P (mg/L) (between day 245-277)	**MBNR (Chemical) system PO_4-P (mg/L) (between day 245-277)**
Anaerobic Reactor	5.8 (±0.5)	5.0 (±0.6)
Aerobic Reactor	2.0 (±0.4)	1.7 (±0.4)
Permeate	1.6 (±0.4)	0.6 (±0.2)

±: standard deviation

6.4 Batch Tests - Phase IV

Figure 6.14 summarizes NO_3-N and PO_4-P profiles of the batch tests 7, 8 and 9, conducted with aerobic mixed liquor from the parallel MBNR systems during Phase IV of the study. NO_3-N concentrations were extremely low in the anaerobic periods of each test. As observed earlier, a non-aerated period of 4 hours was sufficient for removal of the residual nitrate present in mixed liquor samples. However, significant NO_3-N concentrations were observed in the aerobic phases of all the batch tests. Monti (2005) reported similar observations in his batch test work. He postulated that microorganisms exposed to batch conditions for long periods of time undergo lysis and release ammonium as a by-product. The ammonium is subsequently converted to nitrate under aerobic conditions. This was most likely the case for the present batch tests, as significant NO_3-N concentrations also were observed in the aerobic phases of the batch tests conducted in Phase II (Figure 6.1) and Phase III (Figure 6.5) of operation.

The PO_4-P profiles in Figure 6.14 show anaerobic P release and aerobic P uptake in all the batch tests, thus demonstrating EBPR capability of the parallel MBNR systems. This is particularly important for the MBNR (Chemical) system as, at this time, it was being supplemented with 40 mg/L of alum. In the MBNR (Biological) system, the PO_4-P profile was slightly different in Batch Test 7 than in Batch Tests 8 and 9. On the other hand, the PO_4-P profiles were almost identical in all three MBNR (Chemical) system batch tests. For that reason, it could be assumed that the EBPR potential of the parallel MBNR systems was consistent during Phase IV. Figure 6.14 also indicates that the PO_4-P concentrations after the completion of the 2 hour anaerobic period were somewhat higher in the MBNR (Biological) system than in the MBNR (Chemical) system for Batch Tests 8 and 9. Although alum-mediated inhibition is a possibility, an accurate assessment could only be done after cation profiling along with comparative EBPR kinetic rate analysis.

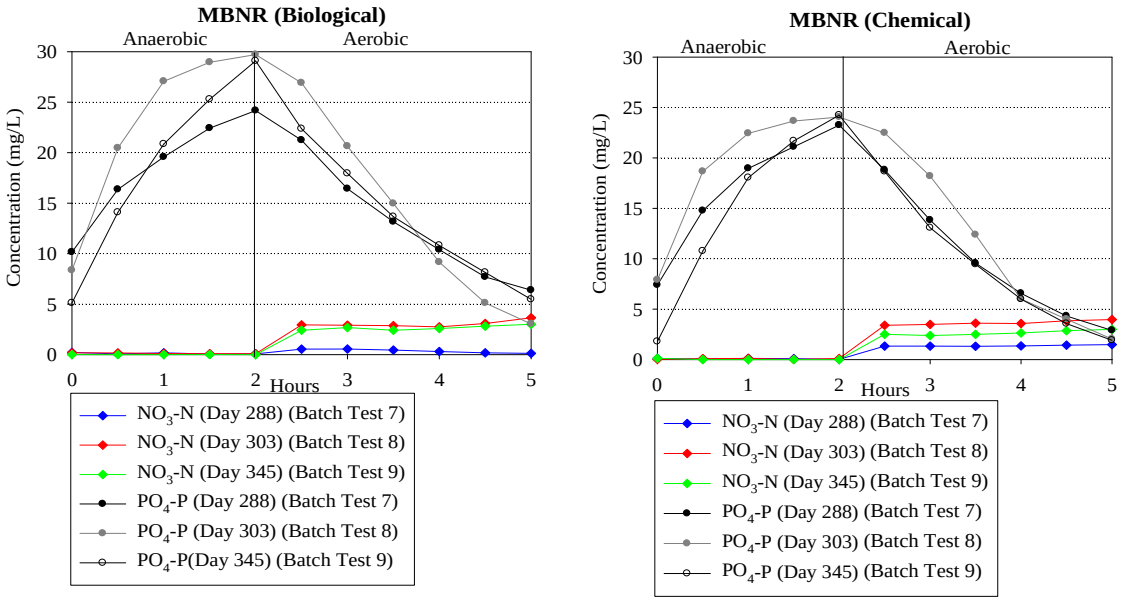


Figure 6.14 Batch test NO_3-N and PO_4-P profile of the parallel MBNR systems (Phase IV)

Acetate utilization in the Phase IV batch tests is shown in Figure 6.15. Two interesting points to note are that significant amounts of acetate remained unutilized at the end of the anaerobic phases (similar to Phase III) and each batch test exhibited a different

residual acetate after the anaerobic phase. The least acetate utilization was observed in MBNR (Biological) system Batch Test 7, while the greatest acetate utilization was observed in the MBNR (Chemical) system Batch Test 9. It was difficult to identify the cause for such a phenomenon except that the possibility of continuously dynamic mixed liquor with non-PAO cultures having the ability to utilize acetate in anaerobic conditions. Nonetheless, acetate was oxidized in the first ½ hour of the aerobic phase of all the batch tests.

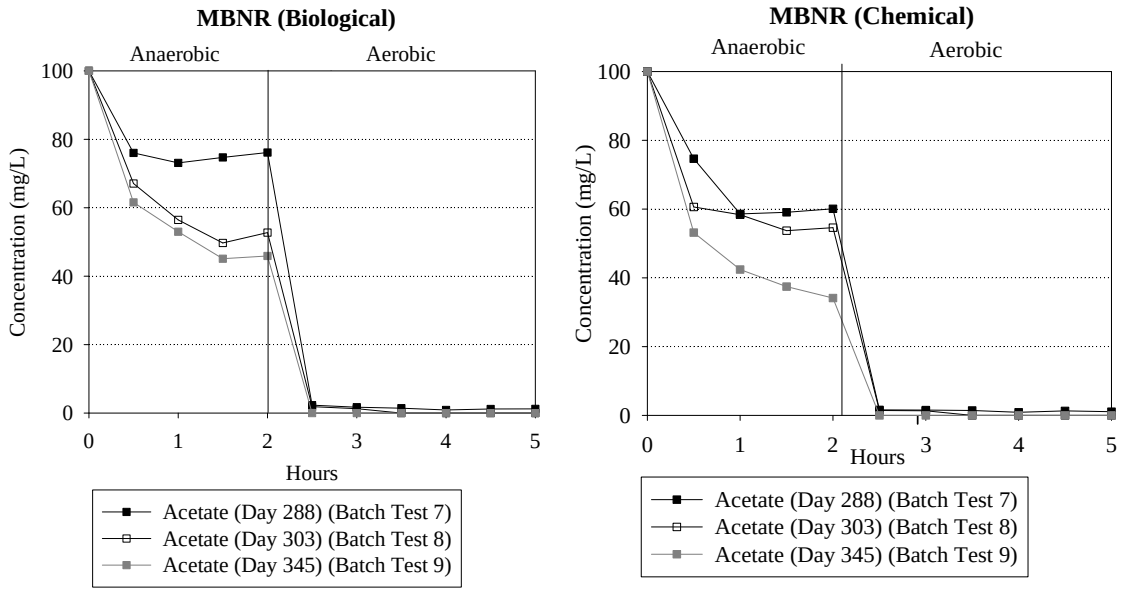


Figure 6.15 Batch test acetate profile of the parallel MBNR systems (Phase IV)

Figure 6.16 shows Mg^{+2} and K^{+1} concentrations for the batch tests conducted in Phase IV of operation. From this figure, it can be observed that cation profiles mimicked those of phosphorus, as cation release occurred in the anaerobic periods followed by uptake in the aerobic periods. Moreover, Mg^{+2} and K^{+1} profiles were very similar in all three batch tests of the MBNR (Biological) system as well as those of the MBNR (Chemical) system. This supports the earlier observation that EBPR potential was consistent in both the MBNR systems throughout Phase IV batch studies. Since the cation profiles of the MBNR (Chemical) system were also comparable to those of the MBNR (Biological) system, the likely conclusion is that 40 mg/L of alum did not inhibit EBPR during the period of Phase IV batch tests.

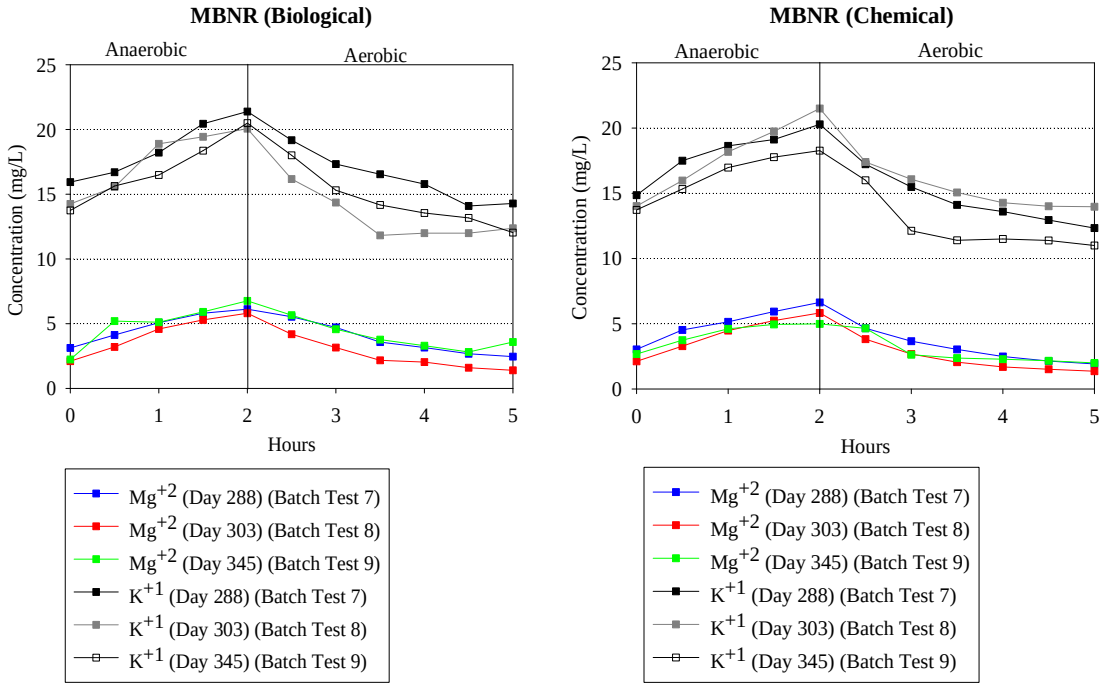


Figure 6.16 Batch test Mg^{+2} and K^{+1} profile of the parallel MBNR systems (Phase IV)

Maximum specific phosphorus release (mg P/g VSS· hr) and maximum specific phosphorus uptake (mg P/g VSS· hr) were determined for the batch studies and these are summarized in Figure 6.17. The maximum specific phosphorus release data did not suggest any specific trend with higher values in the MBNR (Chemical) mixed liquor for Batch Tests 7 and 9 and lower value for Batch Test 8. On the other hand, maximum specific phosphorus uptake was higher in the MBNR (Chemical) mixed liquor for all the batch tests. Therefore, it is absolutely evident that 40 mg/L of alum addition did not adversely influence EBPR kinetics in the MBNR (Chemical) system during the Phase IV batch studies.

The stoichiometric parameter (P-released/VFAs-consumed; g P/g COD) values for the stage IV batch tests are shown in Figure 6.18. The values were variable between each batch test for the MBNR (Biological) system as well as the MBNR (Chemical) system. Figure 6.18 also demonstrates considerably higher P-released/VFAs-consumed values for the MBNR (Biological) system in Batch Tests 7 and 9. On the contrary, a slightly lower value was observed in Batch Test 8. The variability of the stoichiometric parameter

values in the batch tests could be due to the presence of non-PAOs that could utilize acetate anaerobically.

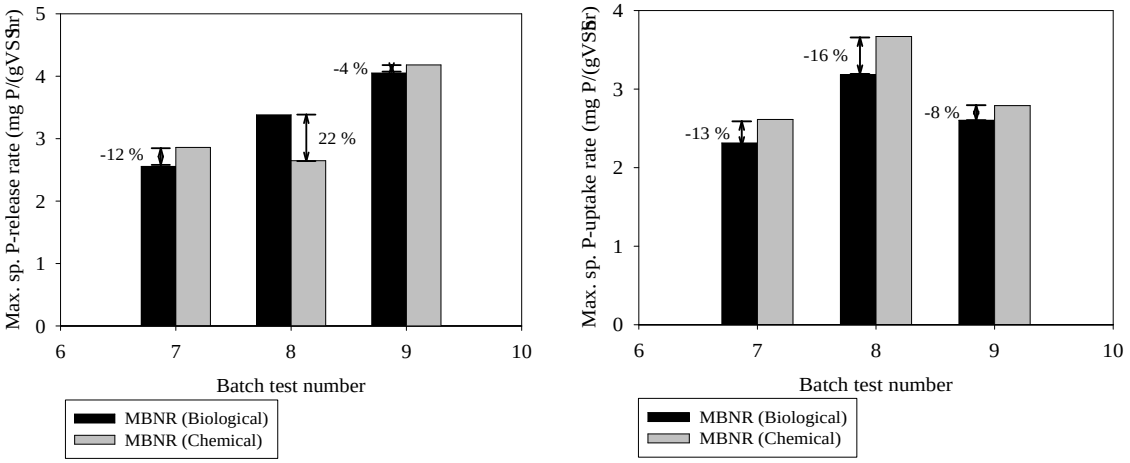


Figure 6.17 Batch test maximum specific phosphorus release and uptake profile (Phase IV)

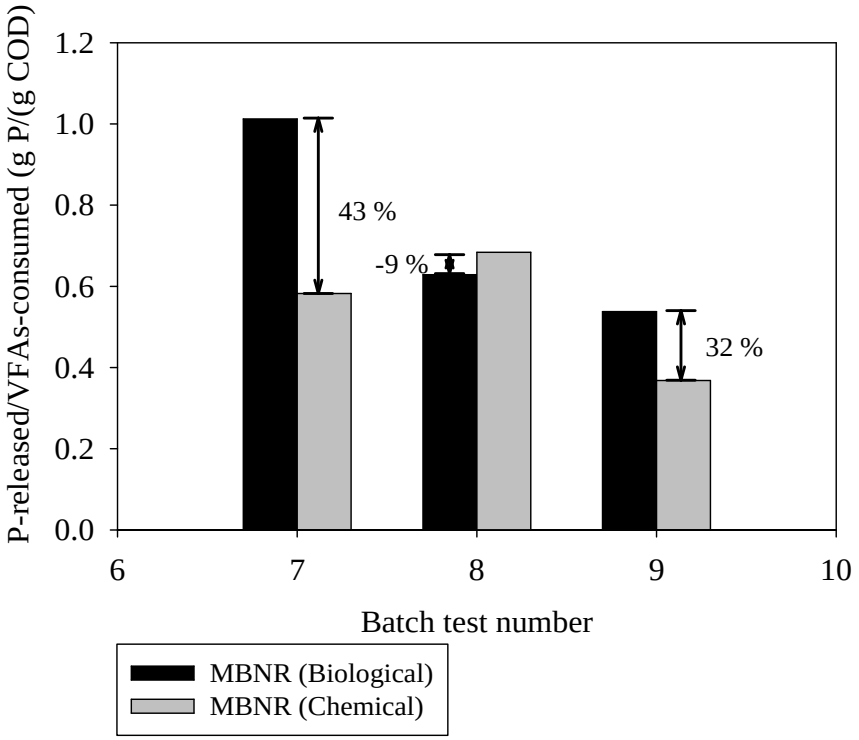


Figure 6.18 Batch test P-released/VFAs-consumed profile (Phase IV)

Molar K^{+1} and Mg^{+2} vs. molar P plots for the batch tests are illustrated in the six figures below (Figure 6.19 to Figure 6.24). The profiles include data from both the anaerobic and aerobic periods of the tests. A linear relationship exists between cation and phosphorus concentrations in both phases, although the R^2 values were different in the three batch tests. In Batch Tests 7 and 9, R^2 was mostly greater than 0.95 in the MBNR (Biological) system (Figure 6.19 and Figure 6.23) as well as in the MBNR (Chemical) system (Figure 6.20 and Figure 6.24), demonstrating excellent fit between Molar K^+ and Mg^{+2} vs. molar P. On the other hand, the R^2 value was predominantly less than 0.90 in Batch Test 8 for both the MBNR systems (Figure 6.21 and Figure 6.22). As far as mole K^{+1}/ mole P and mole Mg^{+2}/ mole P value was concerned, the typical range was between 0.20 to 0.35, with very few exceptions. Once again the observed range was in accordance with the literature values shown in Table 6.6.

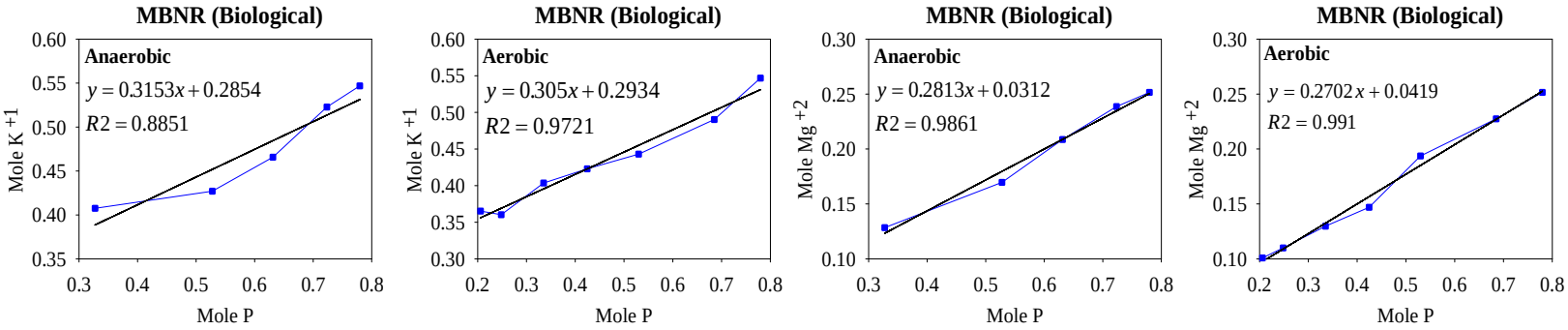


Figure 6.19 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 7 of MBNR (Biological) system (Phase IV)

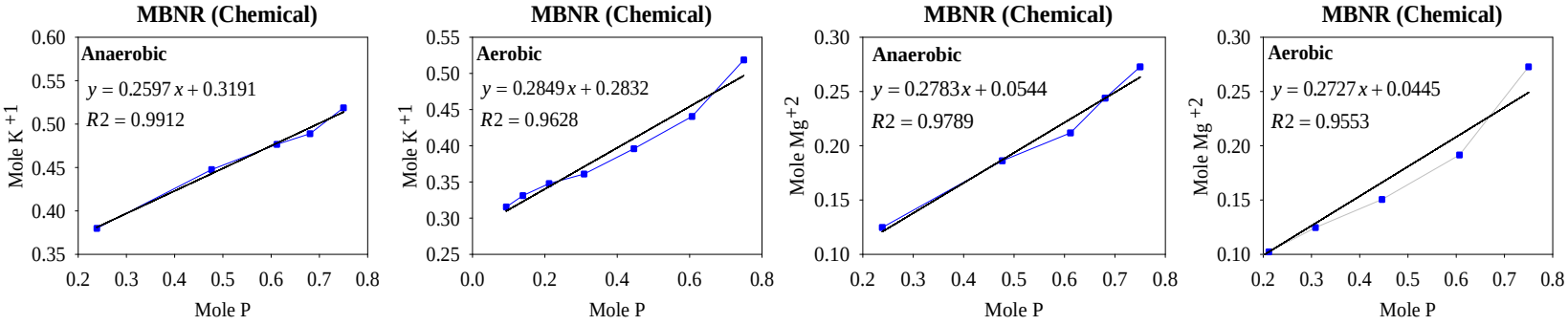


Figure 6.20 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 7 of MBNR (Chemical) system (Phase IV)

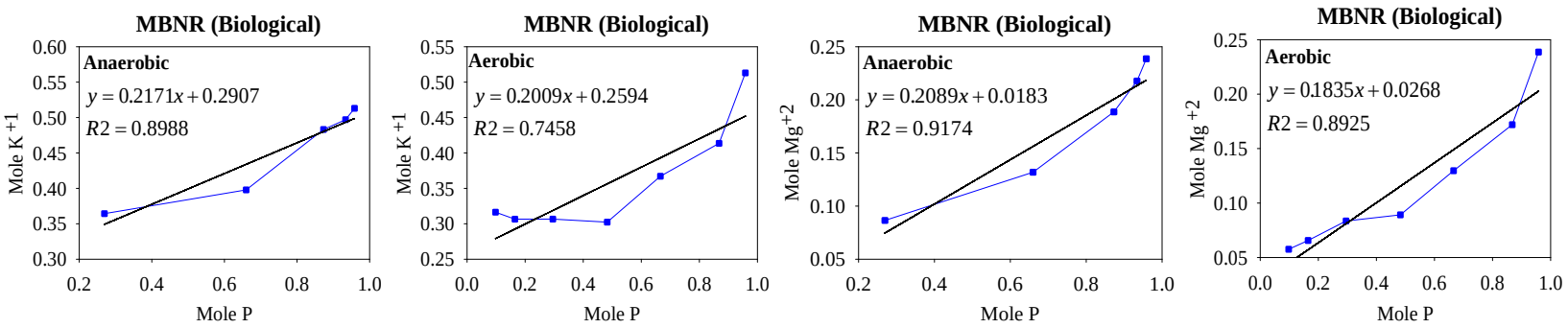


Figure 6.21 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test of MBNR (Biological) system (Phase IV)

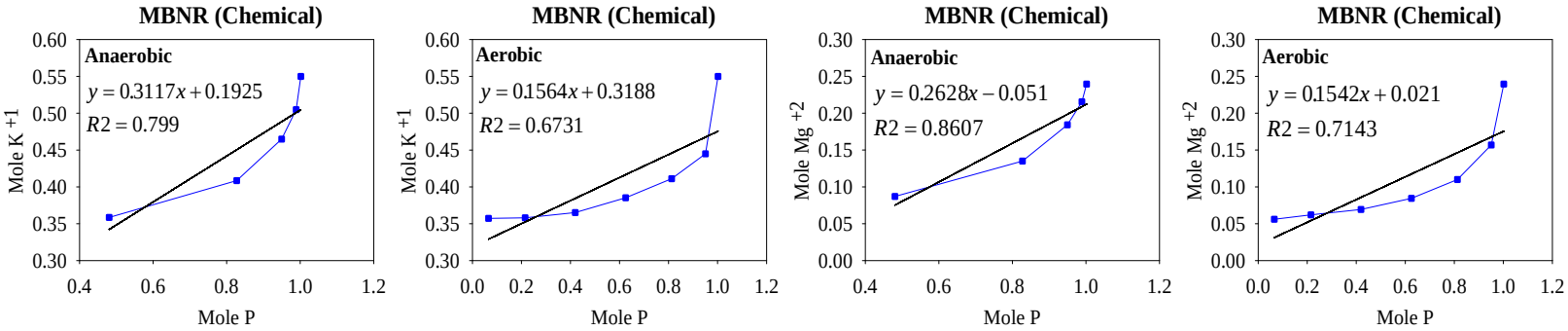


Figure 6.22 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 8 of MBNR (Chemical) system (Phase IV)

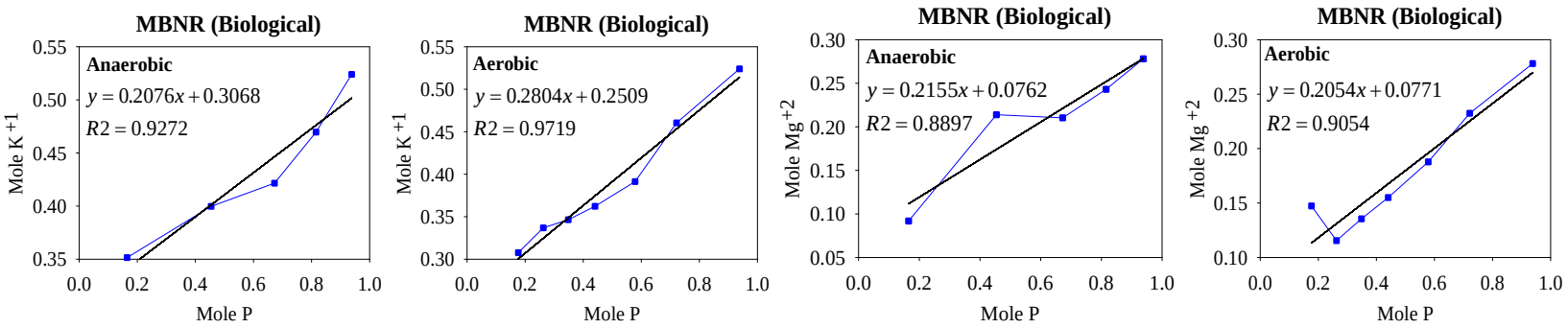


Figure 6.23 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 9 of MBNR (Biological) system (Phase IV)

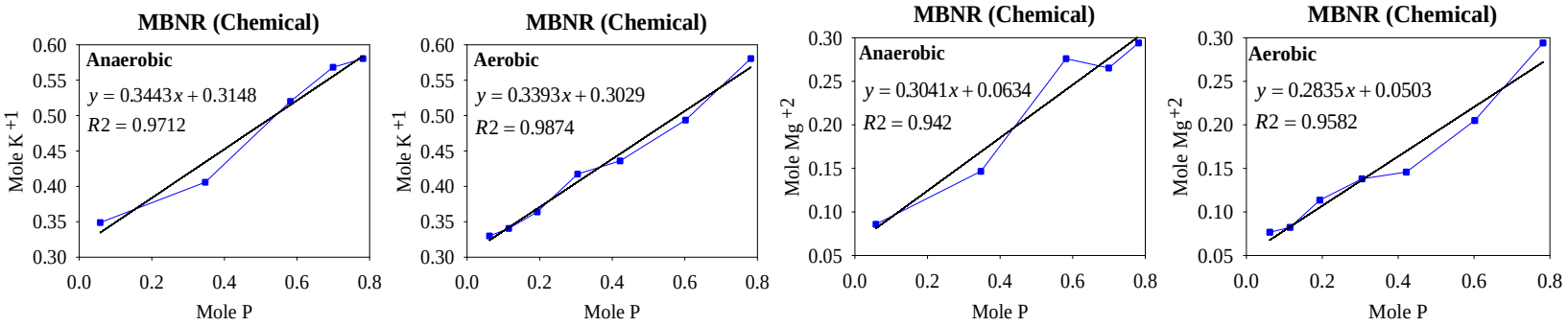


Figure 6.24 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 9 of MBNR (Chemical) system (Phase IV)

The conclusion from the Phase IV batch studies was that alum addition at the dose applied did not influence PAO kinetics. It was imperative to know whether the same trend was observed in the continuous flow system performance during that period. Table 6.3 shows average PO_4-P concentrations in the anaerobic reactors, aerobic reactors and permeates of the parallel MBNR systems. There was very little difference in PO_4-P concentration as far as anaerobic reactors were concerned. Therefore, it could be theorized that phosphorus release in the MBNR (Chemical) system was as good as that in the MBNR (Biological) system and was not inhibited by alum addition. On the other hand, the aerobic reactor and permeate PO_4-P concentrations were significantly lower in the MBNR (Chemical) system during stage IV batch studies. As discussed in **Section 5.3.4.2**, the higher removal efficiency observed was a contribution of combined chemical phosphorus removal and EBPR mechanisms.

Table 6.3 Parallel MBNR system P-profiling during the period of batch studies (Phase IV)

	MBNR (Biological) system PO_4-P (mg/L) (between day 287-345)	**MBNR (Chemical) system PO_4-P (mg/L) (between day 287-345)**
Anaerobic Reactor	6.5 (±0.9)	6.0 (±0.8)
Aerobic Reactor	2.6 (±0.7)	0.7 (±0.4)
Permeate	2.2 (±0.7)	0.3 (±0.3)

6.5 Batch Tests - Phase V

The NO_3-N and PO_4-P profiles of the batch tests 10, 11 and 12, conducted in Phase V are illustrated in Figure 6.25. As discussed previously, the NO_3-N concentrations were consistently close to zero during the 2 hours of the anaerobic period of each batch tests. In the aerobic period, elevated NO_3-N concentrations were observed in all the batch tests. This was particularly prominent in Batch Test 10 of the MBNR (Chemical) system, where approximately 5 mg/L of NO_3-N was observed in the mixed liquor (Figure 6.25). Cell lysis-related ammonium release and subsequent conversion to nitrate in the aerobic phase was the key reason for the elevated nitrate concentrations as shown in Figure 6.25.

PO_4-P profiling of the batch tests, as illustrated in Figure 6.25, yielded noteworthy results. Although anaerobic release and aerobic uptake was observed in all batch tests, it was obvious that EBPR potential was significantly lower in the MBNR (Chemical) system than in the MBNR (Biological) system. As reported in the continuous flow system performance discussion (**Section 5.3.4.2)**, inhibition of EBPR was most likely caused by alum addition. Alum dosing had been increased to 80 mg/L on day 346, which was 28 days before the first batch test of Phase V, i.e. Batch Test 10. During the intervening period, it is presumed that PAOs in the MBNR (Chemical) system could not compete with alum-based P complexation to accumulate soluble PO_4-P for their growth. As a consequence, the abundance of PAOs apparently declined in the mixed liquor as reflected in the diminishing PO_4-P profiles of Batch Tests 10 to 12 (Figure 6.25). On the other hand, the three MBNR (Biological) system batch tests exhibited much more extensive and consistent phosphorus release and uptake profiles, thus indicating steady EBPR potential in this system during the period of batch tests.

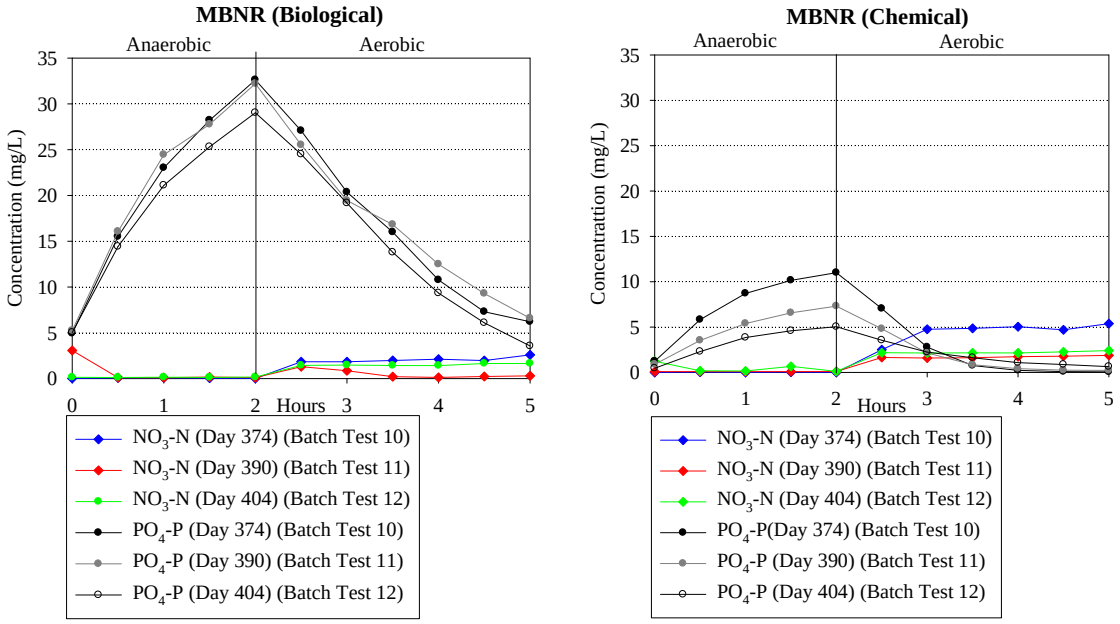


Figure 6.25 Batch test NO_3-N and PO_4-P profile of the parallel MBNR systems (Phase V)

Figure 6.26 shows acetate utilization profiles of the batch tests. Due to sample contamination, acetate concentrations could not be reported for Batch Test 12 of the

MBNR (Biological) system. Acetate utilization took place anaerobically, although it was variable in each batch test. To be specific, anaerobic utilization was greater in Batch Tests 10 and 11 of the MBNR (Biological) system than for the MBNR (Chemical) system. More extensive acetate consumption in the two batch tests was consistent with greater PO_4-P release in the MBNR (Biological) system (refer to Figure 6.25). However, the greatest anaerobic acetate utilization was observed in batch test 12 of the MBNR (Chemical) system. The puzzling fact was that the lowest PO_4-P release occurred concurrently in the same batch test (Figure 6.25). The hypothesis of non-PAOs using acetate anaerobically is the most relevant explanation for the observation. Nevertheless, acetate remaining after the anaerobic phases was oxidized completely in first ½ hour of the aerobic phases.

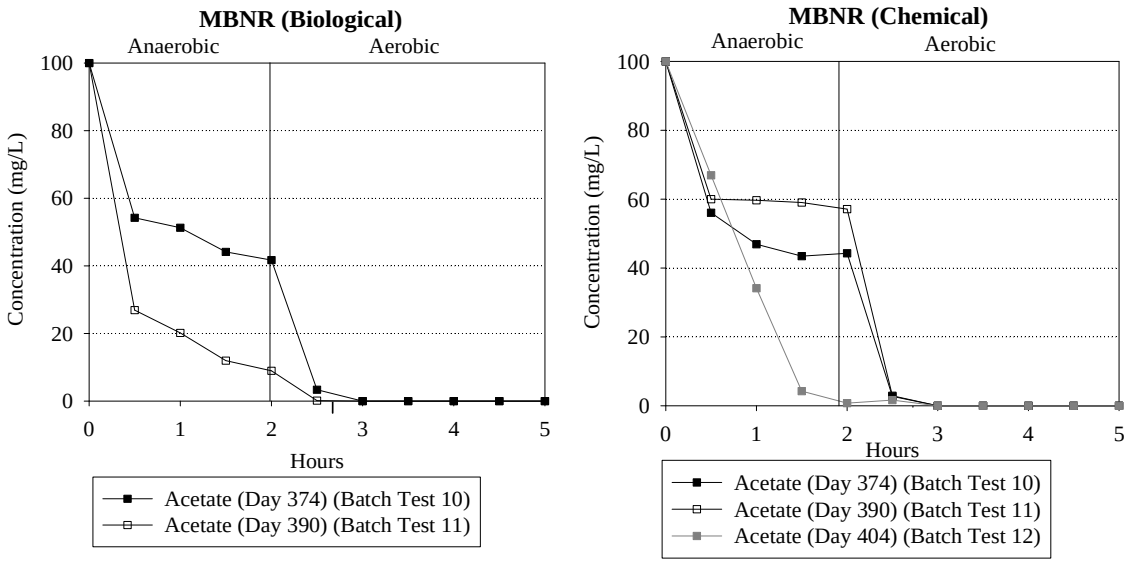


Figure 6.26 Batch test acetate profile of the parallel MBNR systems (Phase V)

K^{+1} and Mg^{+2} concentrations in Phase V batch tests are summarized in Figure 6.27. The broad conclusion from this figure is that both cations were anaerobically released and aerobically taken up in all the batch tests. In the case of the MBNR (Biological) system, identical profiles were observed for K^{+1} and Mg^{+2} in the three batch tests. This was not the case for the MBNR (Chemical) system, for which greater cation release was observed in Batch Test 10 as compared to the next two tests. Furthermore, the release and uptake was more extensive for the MBNR (Biological) system than for the MBNR (Chemical)

system. This provides additional affirmation of the adverse influence of alum addition on EBPR in the MBNR (Chemical) system.

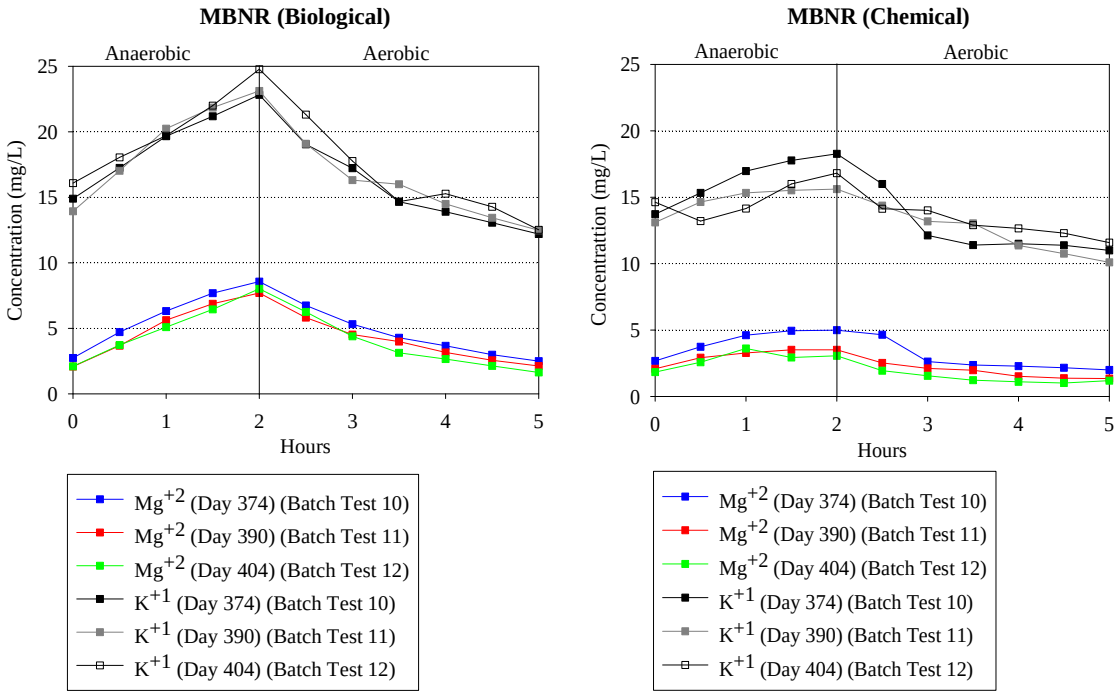


Figure 6.27 Batch test Mg^{+2} and K^{+1} profile of the parallel MBNR systems (Phase V)

Figure 6.28 shows maximum specific phosphorus release (mg P/(g VSS· hr) and maximum specific phosphorus uptake (mg P/(g VSS· hr) values for the batch studies. Maximum specific phosphorus release was steady for the MBNR (Biological) system, while it gradually decreased in the MBNR (Chemical) system. In addition, there was a significant difference between the kinetic values of the parallel MBNR systems. A similar observation was made with the maximum specific phosphorus uptake profiles. This indicates that while EBPR kinetic rates were consistent in the MBNR (Biological) system, they progressively declined in the MBNR (Chemical) system during the Phase V batch studies. The conclusion is very much in accordance with PO_4-P profile (Figure 6.25) and cation profile (Figure 6.27), which confirm alum-related inhibition of EBPR in the MBNR (Chemical) system.

Figure 6.29 shows P-released/VFAs-consumed (g P/g COD) profile for the Phase V batch tests. There was large difference between the parallel MBNR systems as far as P-released/VFAs-consumed values were concerned. Also, the values were different in each batch test. The presence of non-PAOs utilizing acetate anaerobically has been identified in the previous batch tests as a potential contributor to the variability of the stoichiometric parameter and it holds true for the Phase V batch tests.

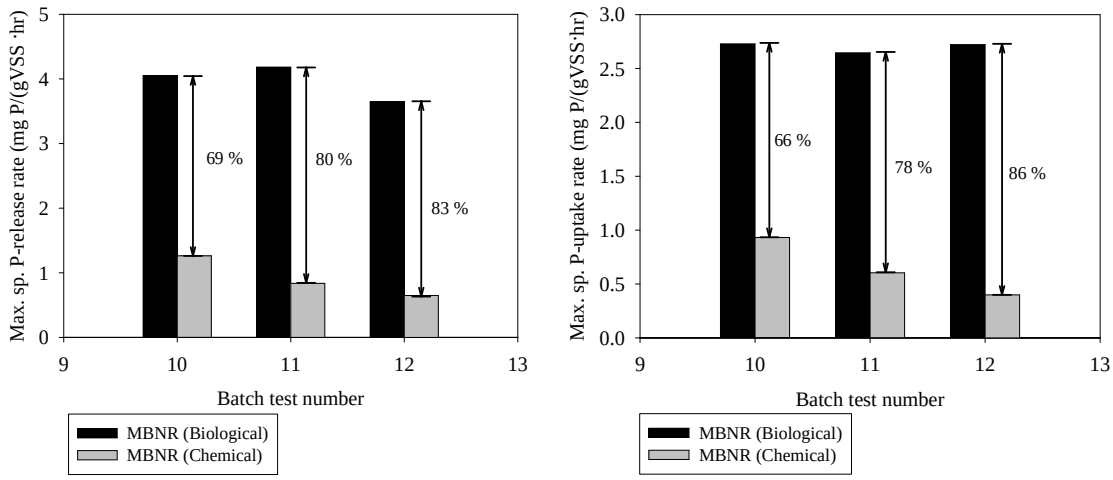


Figure 6.28 Batch test maximum specific phosphorus release and uptake profile (Phase V)

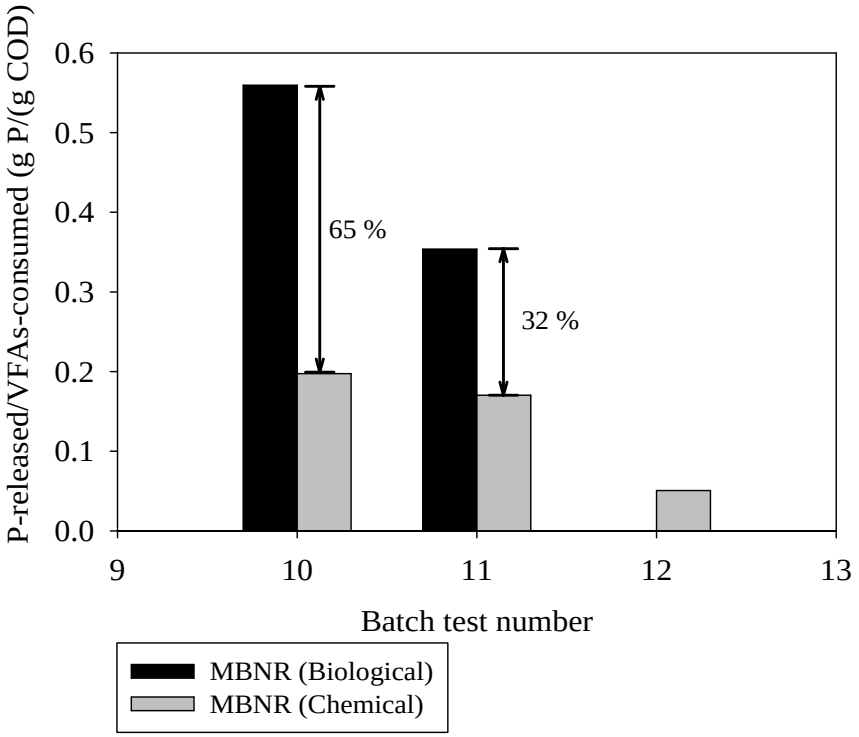


Figure 6.29 Batch test P-released/VFAs-consumed profile (Phase V)

Figures 6.30 to Figure 6.35 summarize molar K^{+1} and Mg^{+2} vs. molar P plots for Phase V batch tests. The broad agreement from the six figures is that a linear relationship existed between the cations and phosphorus in the anaerobic and aerobic periods of the batch tests. R^2 values were usually higher than 0.90 and provided evidence for the above hypothesis. Molar K^{+1} and Mg^{+2} vs. molar P ratios were in the range of 0.20 to 0.35 for the MBNR (Biological) system, whereas higher ratios were observed for the MBNR (Chemical) system. It can be proposed that although linear relationships existed, alum adversely influenced the equilibrium between the cations and phosphorus.

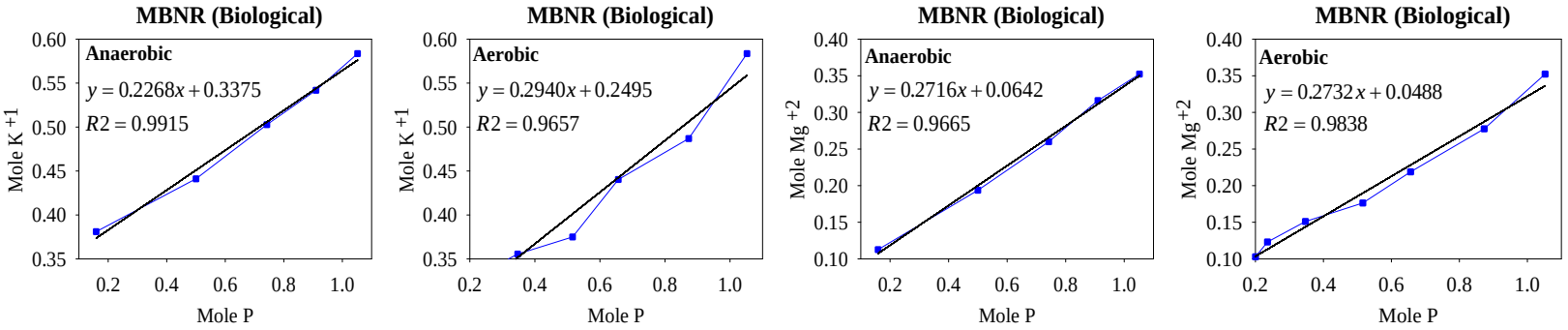


Figure 6.30 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 10 of MBNR (Biological) system (Phase V)

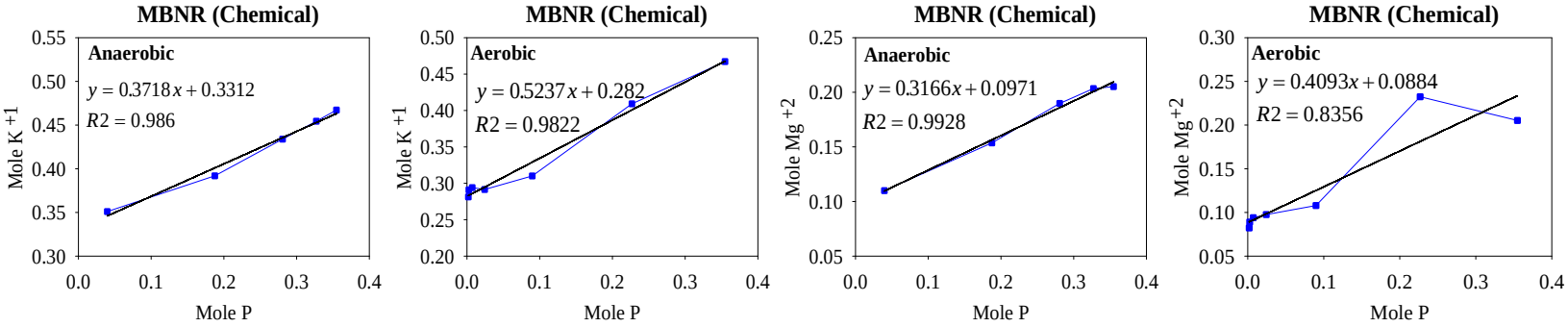


Figure 6.31 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 10 of MBNR (Chemical) system (Phase V)

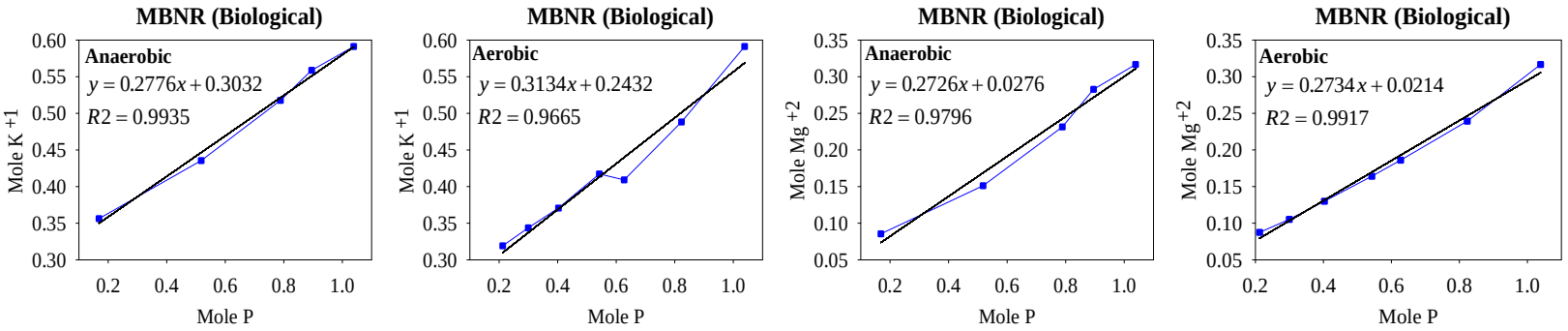


Figure 6.32 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 11 of MBNR (Biological) system (Phase V)

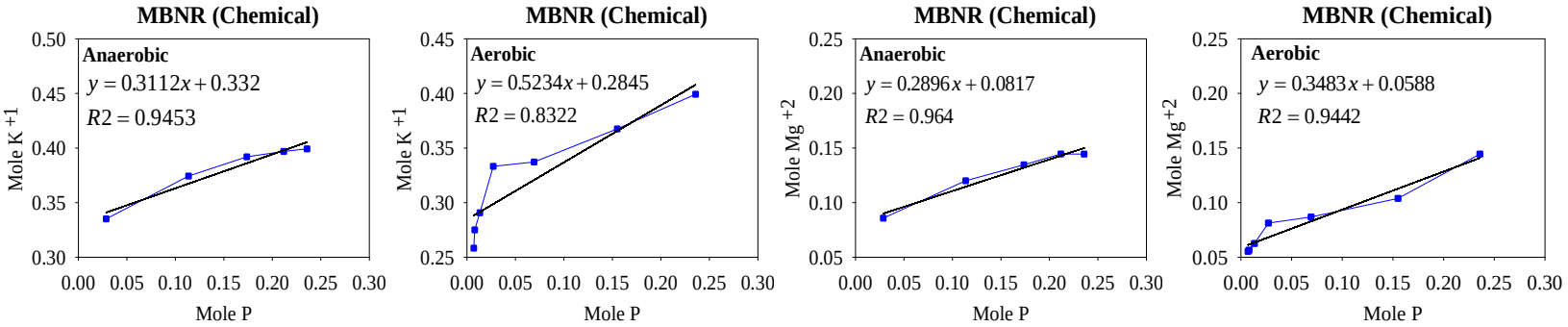


Figure 6.33 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 11 of MBNR (Chemical) system (Phase V)

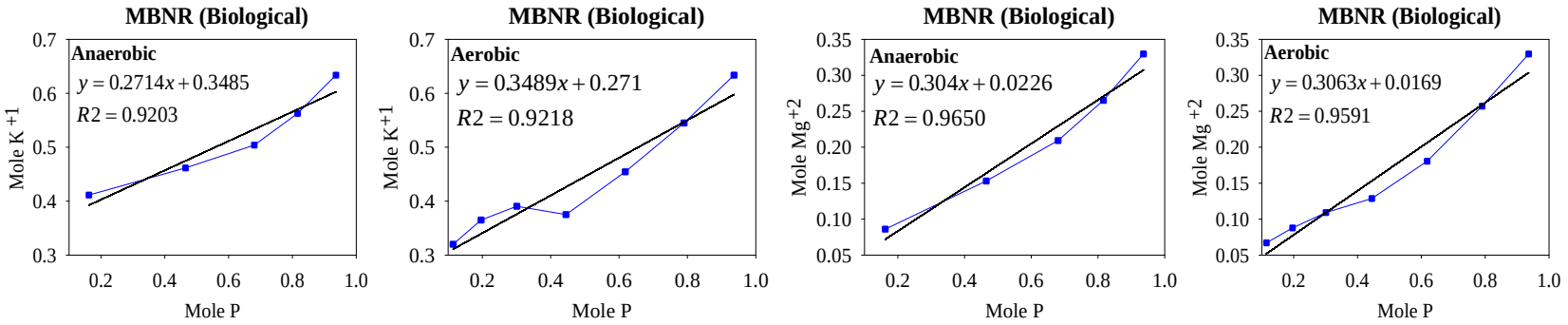


Figure 6.34 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 12 of MBNR (Biological) system (Phase V)

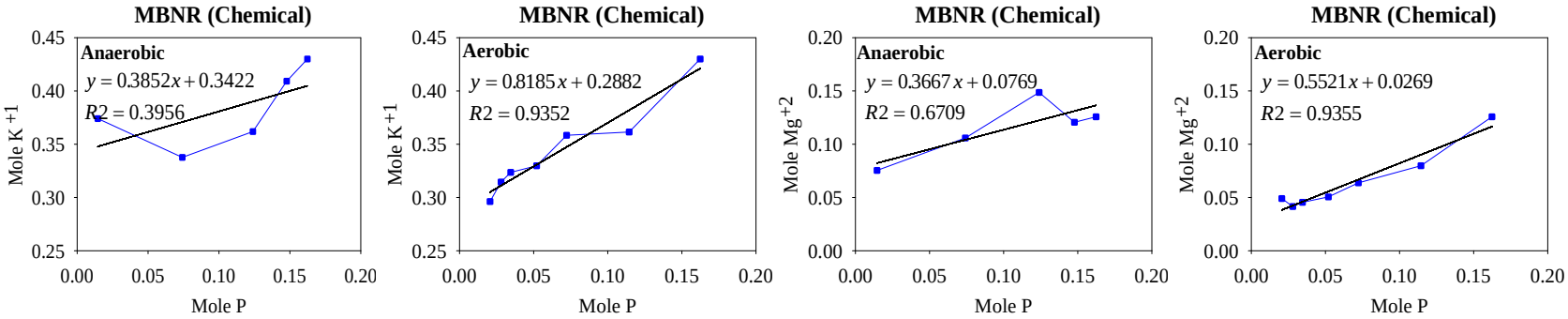


Figure 6.35 Mole K^{+1} and mole Mg^{+2} vs. mole P in Batch Test 12 of MBNR (Chemical) system (Phase V)

The phosphorus removal performances of the parallel MBNR systems were evaluated for the period of the Phase V batch studies and these are illustrated in Table 6.4. The difference in average anaerobic PO_4-P concentration suggests inhibition of phosphorus release in the MBNR (Chemical) system. On the other hand, very low aerobic reactor and permeate PO_4-P concentrations could only be achieved due to the 80 mg/L of alum supplementation. In conclusion, continuous flow system data support the findings of the batch tests conducted in Phase V of the operation.

Table 6.4 Parallel MBNR system P-profiling during the period of batch studies (Phase V)

	MBNR (Biological) system PO_4-P (mg/L) (between day 374-404)	**MBNR (Chemical) system PO_4-P (mg/L) (between day 374-404)**
Anaerobic Reactor	7. 9 (±0.9)	4.3 (±0.6)
Aerobic Reactor	2.2 (±0.5)	0.2 (±0.1)
Permeate	1.8 (±0.5)	0.06 (±0.03)

6.6 Kinetic and Stoichiometric Parameter Evaluation

The parallel MBNR system EBPR kinetic and stoichiometric parameters, estimated from the batch studies, were compared with data from other studies in the literature and the results are summarized in Table 6.5. For the present study, data included values from all 12 batch studies. The maximum anaerobic specific phosphorus release rate and maximum aerobic specific phosphorus uptake rate for the two MBNR systems were below the ranges reported in other studies. However, it has to be mentioned that the parallel MBNR systems were operated with an SRT of 25 days, which is longer than what is typically practised. Longer SRT means higher VSS in the system. Since the kinetic parameter values were normalized against VSS, comparatively lower values have been presented in Table 6.5 for the parallel MBNR systems. Nonetheless, stoichiometric parameter (P/VFA) values from the present study were in the range of values reported by others.

Table 6.5 Kinetics and stoichiometry of EBPR sludge acclimatized to municipal wastewater (Modified from Monti, 2006)

Study	Max. Ana P Re. Rate	P/VFA	Max Aer P Upt. Rate
	mg P/(g VSS·h)	g P/g COD	mg P/(g VSS·h)
Kuba *et al.* (1997b)	7 - 19	0.22 - 0.40	5.7 – 13.0
Brdjanovic *et al.* (2000)	6	0.29	2.2
Kerrn-Jespersen and Henze (1993)			0.6 – 1.3*
Petersen *et al.* (1998)	15	0.58	14
Mamais *et al.* (1992)	7 - 20	0.5	
Rabinowitz and Oldham (1986)		0.85	
Carlsson *et al.* (1996)		0.35 - 0.4	
Tykesson *et al.* (2002)	7		
Monti (2006)	5 - 30	0.5 - 0.6	2.0 – 10.0
MBNR (Biological) System	3.9 (Average) 2.1-6.1(Min.-Max.) ±1.1 (Std. Dev.)	0.5 (Average) 0.2-1.0 (Min.-Max.) ±0.2 (Std. Dev.)	2.7 (Average) 1.2-3.9 (Min.-Max.) ±0.7 (Std. Dev.)
MBNR (Chemical) System	2.6 (Average) 0.7-4.2 (Min.-Max.) ±1.3 (Std. Dev.)	0.4 (Average) 0.1-1.0 (Min.-Max.) ±0.3 (Std. Dev.)	2.0 (Average) 0.4-3.7 (Min.-Max.) ±1.0 (Std. Dev.)

*measured as mg P/(g SS·h)

Similarly, molar K^{+1} and Mg^{+2} vs. molar P values observed in the parallel MBNR batch studies were compared with data from the literature and the results are shown in Table 6.6. For the present study, average, minimum-maximum and standard deviation values of 8 batch tests are reported. It is evident from Table 6.6 that the values estimated from the present study are comparable to the literature data.

Table 6.6 Literature data for $\Delta Mg^{+2}/\Delta P$ and $\Delta K^{+}/\Delta P$ (Modified from Barat *et al.*, 2005)

Reference	$\Delta Mg^{+2}/\Delta P$ (mol Mg^{2+} mol P^{-1})	$\Delta K^{+1}/\Delta P$ (mol K^{+1} mol P^{-1})
Choi *et al.* (2011)	0.21 (Anaerobic) 0.10 (Aerobic and Anoxic)	0.20 (Anaerobic) 0.16 (Aerobic and Anoxic)
Rickard and McClintock (1992)	0.30	0.21
Jardin and Pöpel (1996)	0.34	0.26
Wild *et al.* (1997)	0.32	
Brdjanovic *et al.* (1996)	0.33	0.33
Comeau *et al.* (1987)		0.33
Miyamoto-Mills *et al.* (1983)	0.26	0.27
Arvin and Kristensen (1985)	0.32	0.23
Pattarkine and Randall (1999)	0.36	
MBNR (Biological) System	0.27 (Average) 0.18-0.34 (Min.-Max.) ±0.05 (Std. Dev.)	0.30 (Average) 0.20-0.44 (Min.-Max.) ±0.07 (Std. Dev.)
MBNR (Chemical) System	0.31 (Average) 0.15- 0.55 (Min.-Max.) ±0.09 (Std. Dev.)	0.35 (Average) 0.11- 0.82 (Min.-Max.) ±0.17 (Std. Dev.)

6.7 Conclusions

The important conclusions from the batch studies are the following.

- The sequential anaerobic-aerobic batch tests demonstrated that while the parallel MBNR systems were operating in bio-P only mode, EBPR capability appeared to be greater in the MBNR (Biological) system.
- The dosage of alum up to 40 mg/L did not impact MBNR (Chemical) system EBPR potential adversely. This observation was based on batch test phosphorus release/uptake profile as well as kinetic parameter values, which were reasonably similar to those of the MBNR (Biological) system.
- The batch tests conducted with MBNR (Chemical) mixed liquor during the period of 80 mg/L of alum dosing illustrated considerable reduction in anaerobic P release and aerobic P uptake potential. Moreover, much lower EBPR kinetic parameter values

were calculated when compared with the MBNR (Biological) mixed liquor. High dosage of alum and competition for same source of phosphorus was thought to inhibit PAO activity in the MBNR (Chemical) system.

- The batch studies confirmed that the relationship between chemical P removal and EBPR was dynamic and was driven by the alum dosing concentrations. This was consistent with the trends observed in the two continuous flow MBNR system operations.
- Acetate utilization was variable, even though similar levels of phosphorus release were occurring in some batch tests. The presence of non-PAOs utilizing acetate anaerobically were the most probable explanation for the observation.
- Cation profiling, i.e. molar K^{+} and Mg^{+2} vs. molar P, was linear in both the anaerobic and aerobic periods of the batch tests. The observation was true for both MBNR systems. Moreover, the estimated ratios were within the range of values reported in the literature.
- The maximum specific phosphorus release and maximum specific phosphorus uptake rates, i.e. mg P/ (g VSS·h), calculated from the batch tests of the parallel MBNR systems, were at the lower end of the ranges of literature data. Greater VSS concentration in the present study due to long SRT of 25 days contributed to the low values. On the other hand, the stoichiometric parameter (i.e. P/VFA) values were comparable to those of other studies.

7 Steady State and Dynamic Modeling of the Parallel MBNR Systems

7.1 Introduction

Modeling was an important component of the present research as its suitability in predicting performance was evaluated in parallel MBNR systems targeting LoT effluent nutrient goals. This task was undertaken by incorporating relevant information about the treatment system and the wastewater influent into BioWin^{TM} and running simulations. Simulated data were compared with measured data for suspended solids concentration, nitrification, denitrification, EBPR and combined chemical-biological phosphorus removal. Initially, steady state modeling was conducted for the MBNR (Biological) system using the *BioWin^{TM} General Model* default parameters. This was followed by a parameter sensitivity analysis and calibration of the model. Dynamic modeling (with calibrated parameters) was conducted to validate prediction accuracy under moderately variable influent conditions. Consecutive operation periods, i.e. days 236 to 356 for steady state modeling and days 359 to 449 for dynamic modeling were chosen for the MBNR (Biological) system. The rationale for selecting this particular data period was the maintenance of steady operating conditions of the system as far as internal recycling flow rates, external methanol dosing, influent COD concentrations and nutrient removal was concerned. The calibrated MBNR (Biological) model was then applied in dynamic simulation of the MBNR (Chemical) system for the period during which it was supplemented with different dosages of alum (operating day 226 to day 470). The idea was to assess model prediction capability of the influence of alum on TSS, nitrification, denitrification and biological phosphorus removal. In BioWin^{TM}, this objective was accomplished by adding the *Chemical Phosphorus Precipitation Model* to the *General Model.*

7.2 Steady State Modeling of MBNR (Biological) System with Default Parameters

Predicted and measured average steady state TSS and VSS data for each reactor are shown in Table 7.1 and Table 7.2 respectively. Suspended solids was chosen as the preliminary indicator of modeling data fit because TSS and VSS concentrations in a biological wastewater system depend on important modeling parameters such as fraction of non-biodegradable particulate COD (F_{up}), heterotrophic yield (Y_H) and heterotrophic decay (b_H). In addition, the estimation of the correct ratio of VSS/TSS depends on the concentration of inorganic suspended solids (ISS) in the influent wastewater.

Data from both tables illustrate that there were considerable differences between the predicted and the measured data. Special focus was given to TSS and VSS percent errors for the aerobic reactor, which exceeded 20 percent for both parameters. The aerobic reactor was critical to this analysis as it contained the largest inventory of suspended solids in the MBNR (Biological) system. The comparative suspended solids analysis put forward the case for further tuning of the model parameters. However, to evaluate the relative importance of individual model parameters, a sensitivity analysis was chosen as the next step.

Table 7.1 Measured and predicted steady state (with default BioWin^{TM} parameters) TSS data for the MBNR (Biological) system

Reactor	**Measured TSS* (mg/L)**	**Model TSS (mg/L)**	**% Error**
Anaerobic	1120 (±181)	1376	19.6
Pre-anoxic	2329 (±527)	2606	10.6
Aerobic	**3115 (±217)**	**4242**	**26.6**
Post-anoxic	3078 (±206)	4192	26.6
Membrane	4992 (±532)	6293	20.7

*: data period for operating days 236 to 356

Table 7.2 Measured and predicted steady state (with default BioWin^{TM} parameters) VSS data for the MBNR (Biological) system

Reactor	Measured VSS* (mg/L)	Model VSS (mg/L)	% Error
Anaerobic	1078 (±180)	1183	8.9
Pre-anoxic	2186 (±447)	2231	2.0
Aerobic	**2776 (±208)**	**3613**	**23.2**
Post-anoxic	2760 (±212)	3577	22.8
Membrane	4452 (±504)	5366	17.0

*: data period for operating days 236 to 356

7.3 Sensitivity Analysis

A sensitivity analysis was conducted with four selected parameters for the MBNR (Biological) system, i.e. F_{up}, Y_H, Y_{PO4} (yield of PAOs) and b_H. The approach used in the sensitivity analysis was to change the chosen parameter value from the default value in steps of 10 percent. At each step, a simulation was run to quantify the percent change in suspended solids concentration. The parameter variation range was from -50 percent to + 50 percent of the default value. The resulting simulated suspended solids concentration change was quantified for the aerobic reactor. Sensitivity analysis was carried out with one parameter at a time, while keeping all other parameters at their default values during the exercise. In addition to the above four parameters, the impact of ISS (influent inert suspended solids) variability was analysed with respect to TSS and VSS/TSS ratio.

Figure 7.1 shows modeling results for several influent ISS values (from 0 to 45 mg/L) on the abscissa and the resulting simulated aerobic reactor suspended solids data on the ordinate. As expected, the TSS concentration in the aerobic reactor of the MBNR (Biological) system increased with increasing influent ISS concentration. Moreover, the VSS/TSS ratio declined from 92 percent to 58 percent when the ISS was increased from 0 mg/L to 45 mg/L. This ratio was of particular interest as the experimental average VSS/TSS was 88 percent for the aerobic reactor. Therefore, it was inferred that proper model calibration would require a smaller influent ISS value. The chosen value for the calibrated model was 5 mg/L (Table 7.3).

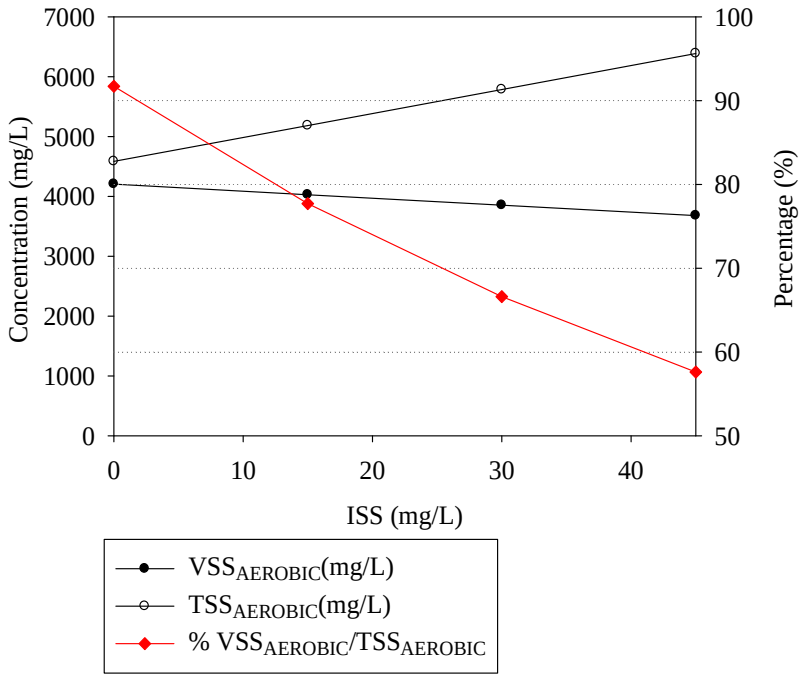


Figure 7.1 Sensitivity analysis of ISS for the MBNR (Biological) system

The sensitivity analysis summary for non-biodegradable particulate COD (F_{up}) is illustrated in Figure 7.2. This parameter had a strong impact on TSS and VSS as the values of these varied approximately -10 percent to +10 percent from their default values. Also, a decrease or an increase in F_{up} resulted in lower and higher suspended solids concentrations respectively. Since the predicted suspended solids concentration was higher than the measured values in the MBNR (Biological) system, the calibrated F_{up} value was reduced from that of the default value in BioWin^{TM} for achieving a reasonable fit. For that reason, the F_{up} value was modified to 0.065 from the default value of 0.130 (Table 7.3). A smaller F_{up} value was justified as significant amount of non-biodegradable particulate COD was known to be removed by settling in influent wastewater holding tanks (refer to **Section 5.4** for details).

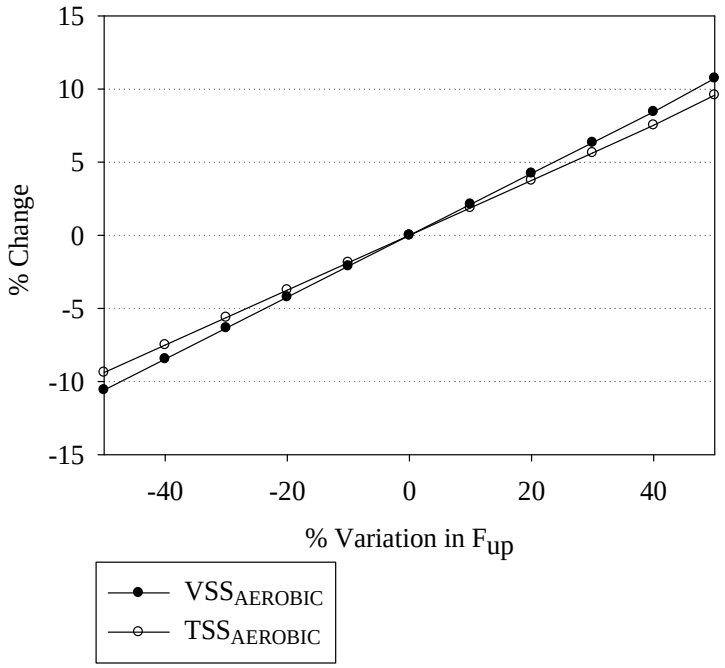


Figure 7.2 Sensitivity analysis of F_{up} for the MBNR (Biological) system

Figure 7.3 and Figure 7.4 present the sensitivity analysis results for Y_H (aerobic and anoxic) and Y_{PO4} (aerobic and anoxic) respectively. All four parameters had a significant impact on suspended solids concentration, although aerobic heterotrophic yield and aerobic PAO yield exerted the largest influence. Predicted TSS and VSS concentrations in the aerobic reactor varied from approximately -13 percent to +36 percent for the defined range of aerobic Y_H. Similarly, TSS and VSS concentrations of the aerobic reactor varied approximately -6 percent to +5 percent for the defined range of aerobic Y_{PO4}. Another observation from the sensitivity analysis was that lower values of the four yield parameters produced comparatively lower suspended solids concentrations in the MBNR (Biological) system. Hence, the lesson from sensitivity analysis of Y_H and Y_{PO4} was that the recommended default value of BioWin^{TM} would need to be reduced to achieve a better fit between the measured and the predicted data. As shown in Table 7.3, aerobic yield (heterotrophic, PAO and propionic) and anoxic yield (heterotrophic and PAO) values were reduced to 0.60 mg COD/mg COD and 0.46 mg COD/mg COD, respectively from the BioWin^{TM} default values of 0.639 mg COD/mg COD and 0.52 mg COD/mg COD.

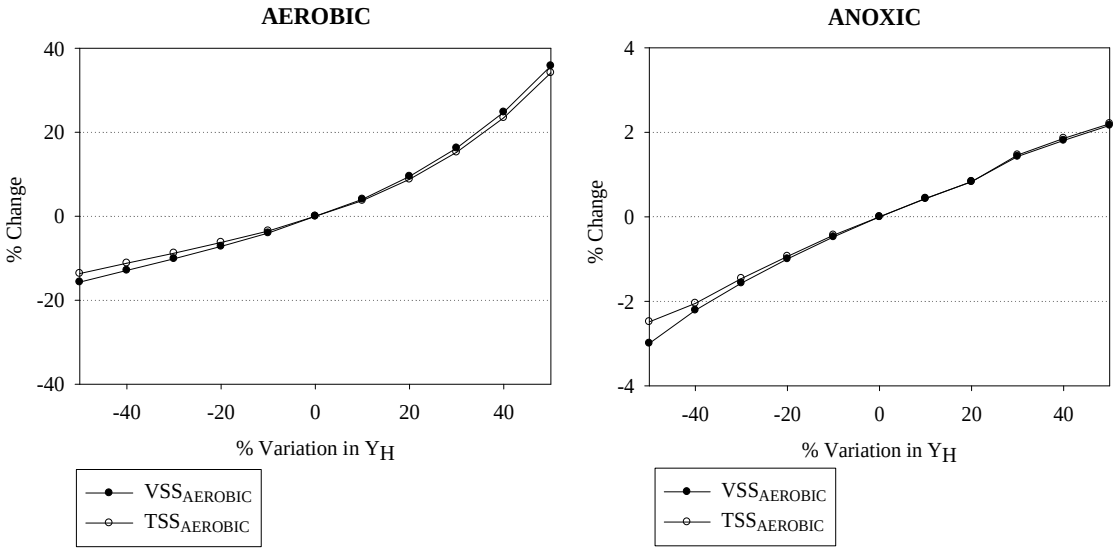


Figure 7.3 Sensitivity analysis of Y_H for the MBNR (Biological) system

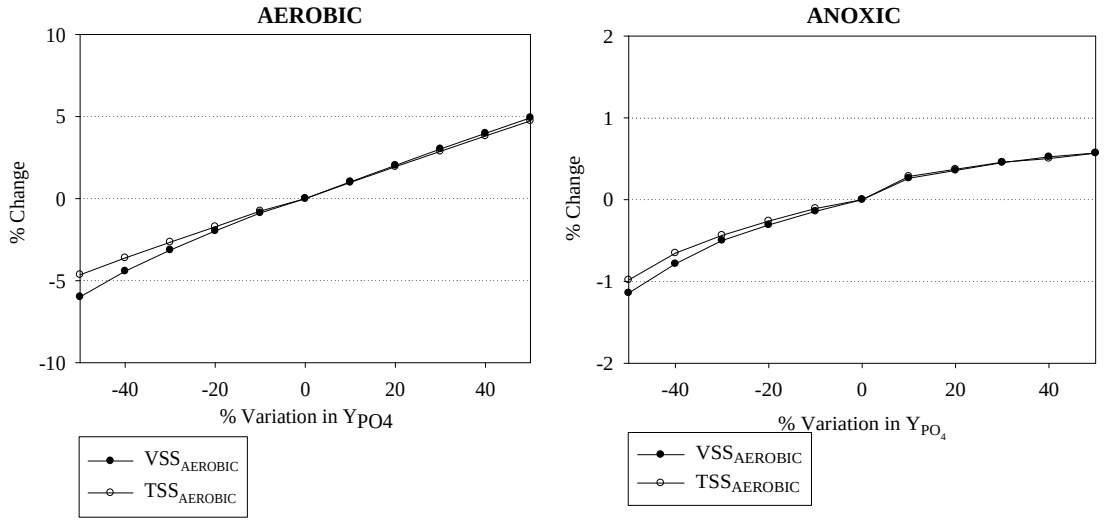


Figure 7.4 Sensitivity analysis of Y_{PO4} for the MBNR (Biological) system

The sensitivity analysis results for heterotrophic decay (b_H) are shown in Figure 7.5. The TSS and VSS concentration changes were approximately -7 percent to +3 percent for the defined range of b_H in x-axis. An increase in b_H value resulted in a decrease in suspended solids concentration in the aerobic reactor. For that reason, higher value of 0.70 d^{-1} (default value was 0.62 d^{-1}) was chosen for heterotrophic decay parameter in calibration of the MBNR (Biological) model (Table 7.3).

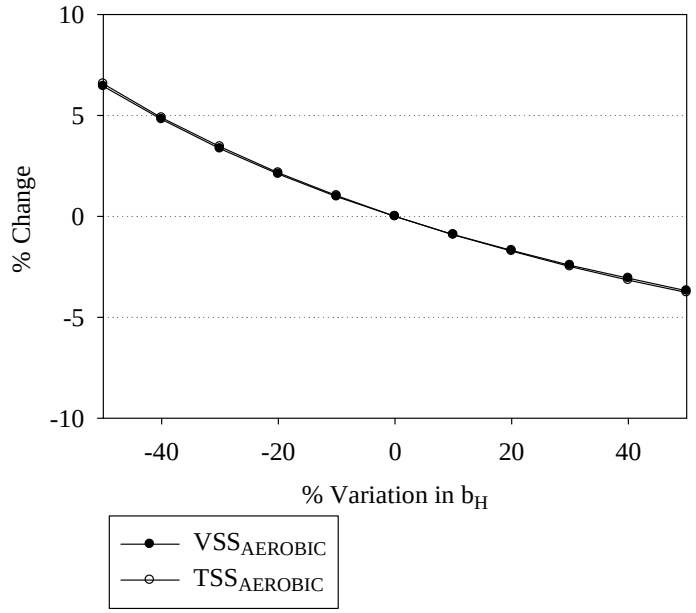


Figure 7.5 Sensitivity analysis of b_H for MBNR (Biological) system

7.4 Calibration of Model

7.4.1 Parameter modification

Table 7.3 summarizes the modified parameter values for calibration of the MBNR (Biological) system model. As mentioned above, the selected parameters were identified from the literature and were evaluated for their impact on MBNR (Biological) aerobic zone suspended solids concentrations through a sensitivity analysis. The calibrated values shown in Table 7.3 were determined through a trial and error approach, in which simulations were run with different parameter values, until predicted data and measured data were reasonably close for the MBNR (Biological) system. Nonetheless, it is important to explain the rationale for choosing the parameter values. ISS and F_{up} are absolutely critical modeling parameters as far as system sludge production and VSS/TSS ratio are concerned. A high measured VSS/TSS ratio in the MBNR (Biological) system and multi-step settling of the wastewater influent were the major factors for choosing values for both parameters that were lower than the default values. For heterotrophic

yield, different values have been proposed in the literature for MBR systems (refer to **Table 2.4**). Nonetheless, the calibrated value of 0.60 mg COD/mg COD was comparable to the values reported in Table 2.4. For the modified anoxic heterotrophic yield parameters in Table 7.3, their ratio to aerobic heterotrophic yield, i.e. Y_H (Anoxic)/ Y_H (Aerobic), was 0.77. The calculated ratio was very close to the recommended range of 0.78-0.85, validated both experimentally and theoretically by various researchers (summarized in Muller *et al.*, 2003). The heterotrophic decay (b_H) value in BioWin^{TM} (and ASM1) was originally determined for conventional activated sludge system (CAS) systems that typically operate with SRTs in the range of 3-15 days and HRTs of 3-5 hours (Tchobanoglous *et al.*, 2003). In contrast, the MBNR (Biological) system was operated with an SRT of 25 days and an HRT of 13.83 hours. It was theorized that these operating conditions would cause higher biomass decay rates and therefore, justified a b_H value of 0.70 d^{-1} for modeling.

Table 7.3 Calibration summary

Parameter	**BioWin^{TM} Default Value**	**Modified Value**
F_{up} (Unbiodegradable particulate COD fraction)	0.130	0.065
ISS (mg/L)	15-45	5
Y_H (Heterotrophic yield) (Aerobic)	0.66	0.60
Y_H (Heterotrophic yield) (Anoxic)	0.54	0.46
Y_{PAO} (PAO yield) (Aerobic)	0.639	0.60
Y_{PAO} (PAO yield) (Anoxic)	0.52	0.46
Y_H (Propionic) (Aerobic)	0.64	0.60
b_H (Aerobic Decay) (d^{-1})	0.62	0.70

7.4.2 Measured and calibrated model suspended solids data

Parameter sensitivity analysis and calibration was followed by steady state simulation of the MBNR (Biological) system. Tables 7.4 and 7.5 compare model predicted TSS and VSS data respectively, with measured data. It could be concluded that a reasonably good fit was obtained when compared to modeling with the default parameter values. Even so,

the error for aerobic reactor suspended solids was 12.5 percent and could have potentially been reduced with further calibration of parameters. A further calibration step was not taken as it had been noticed that a significant quantity of biomass was attached to the walls of the five reactors throughout the study period that otherwise would have contributed to higher measured suspended solids concentrations. In full scale and pilot scale plants, wall growth is less significant due to the smaller surface to volume ratios of large scale reactors. For the lab scale MBNR (Biological) system, it was hypothesized that the presence of significant wall growth resulted in lower measured suspended growth and therefore, no further calibration was warranted.

Table 7.4 Measured and predicted steady state (with calibrated BioWin^{TM} parameters) TSS data for the MBNR (Biological) system

Reactor	**Measured TSS* (mg/L)**	**Predicted TSS (mg/L)**	**% Error**
Anaerobic	1120 (±181)	1156	3.1
Pre-anoxic	2329 (±527)	2192	-6.3
Aerobic	**3115 (±217)**	**3561**	**12.5**
Post-anoxic	3078 (±206)	3523	12.6
Membrane	4992 (±532)	5289	5.6

*: data period for operating days 236 to 356

Table 7.5 Measured and steady predicted steady state (with calibrated BioWin^{TM} parameters) VSS data for the MBNR (Biological) system

Reactor	**Measured VSS* (mg/L)**	**Predicted VSS (mg/L)**	**% Error**
Anaerobic	1078 (±180)	1064	-1.3
Pre-anoxic	2186 (±447)	1987	-10.0
Aerobic	**2776 (±208)**	**3208**	**13.5**
Post-anoxic	2760 (±212)	3175	13.1
Membrane	4452 (±504)	4765	6. 6

*: data period for operating days 236 to 356

7.4.3 Measured and calibrated model nitrogen data

Modeling of biological nitrogen removal was useful to assess the potential to achieve LoT effluent objectives and to estimate the external methanol requirement for enhanced denitrification. Table 7.6 summarizes measured and predicted steady state NH_4-N and

NO_3-N data for the MBNR (Biological) system. Modeling activity was conducted for the period during which the post-anoxic reactor was supplemented with 72 mg/L of methanol. Comparison of the measured and predicted data in Table 7.6 demonstrates a satisfactory fit of the *BioWin^{TM} General Model* for both nitrification and denitrification (Table 7.6). The model also provided confirmation that the methanol supplementation was adequate in removing nitrate to extremely low levels in the permeate. The default kinetic and stoichiometric parameter values used in BioWin^{TM} were able to predict MBNR (Biological) system steady state nitrogen removal performance accurately and therefore, no further calibration was required.

Table 7.6 Measured and predicted steady state (with calibrated BioWin^{TM} parameters) NH_4-N and NO_3-N data for the MBNR (Biological) system

Reactor	**Measured NH_4-N* (mg/L)**	**Predicted NH_4-N (mg/L)**	**Measured NO_3-N* (mg/L)**	**Predicted NO_3-N (mg/L)**
Anaerobic	22 (±3.7)	23.3	0.1 (±0.1)	0
Pre-anoxic	13 (±2.2)	14.2	0.3 (±0. 5)	0.0
Aerobic	0.4 (±0.2)	0.7	7.0 (±1.6)	7.2
Post-anoxic	0.6 (±0.5)	1.0	0.9 (±1.0)	0.0
Membrane	0.2 (±0.2)	0. 3	1.4 (±1.0)	0.5
Permeate	0.0 (±0.0)	0.3	1.3 (±1.1)	0.5

***:** data period for operating days 236 to 356

7.4.4 Measured and calibrated model phosphorus data

Steady state EBPR predicted and measured data for the MBNR (Biological) system are summarized in Table 7.7. The key observation is that even though permeate PO_4-P concentrations were comparable in the predicted vs measured cases; the EBPR mechanism was completely different. The two different mechanisms are explained below.

According to the measured EBPR data, major PO_4-P release occurred in the anaerobic reactor. This was followed by uptake in the pre-anoxic reactor and the aerobic reactor. The elevated average PO_4-P concentration in the aerobic reactor was attributed to an incomplete uptake mechanism (Table 7.7). Furthermore, neither phosphorus release nor

uptake was specifically observed in the post-anoxic reactor or the membrane tank. For detailed information on experimental EBPR performance, please refer to **Section 5.3.4.1**.

According to the predicted EBPR data in Table 7.7, very little PO_4-P release occurred in the anaerobic reactor, while it was prominent in the pre-anoxic reactor and the post-anoxic reactor. In addition, excellent phosphorus uptake took place in the aerobic reactor.

The two completely diverging mechanisms illustrated that the default EBPR kinetic and stoichiometric parameters proposed by the *BioWin^{TM} General Model* were inadequate for MBNR (Biological) system modeling. Hence, parameter calibration was the next step in realizing a good fit between the measured and predicted PO_4-P data.

Table 7.7 Measured and predicted steady state (with calibrated BioWin^{TM} parameters) PO_4-P data for the MBNR (Biological) system

Reactor	**Measured PO_4-P* (mg/L)**	**Predicted PO_4-P (mg/L)**
Anaerobic	6.3 (±0.1)	3.9
Pre-anoxic	4.2 (±0.5)	4.4
Aerobic	2.5 (±1.6)	0.2
Post-anoxic	3.0 (±1.0)	2.9
Membrane	2.2 (±1.0)	2.1
Permeate	2.0 (±1.2)	2.1

*: data period for operating days 236 to 356

Table 7.8 shows EBPR stoichiometric and kinetic parameters that were modified from their default values. The calibrated parameter values were selected by adopting a trial and error approach, although they were selected based on continuous flow system performance observation. The first parameter, mg of phosphorus uptake per mg of PHA, was reduced to 0.795 from its default value of 0.95. A modification of this parameter value was indicated from the consistent, incomplete phosphorus uptake observed in the aerobic reactor of the MBNR (Biological) system. The second parameter in Table 7.8, defined as the anoxic zone rate constant for VFA sequestration to form PHA (stored substrate), was changed from the default value of 6 d^{-1} to 0 d^{-1}. In the MBNR (Biological) system, phosphorus uptake occurred concurrently with PHA utilization in the pre-anoxic reactor, while no release or uptake took place in the post-anoxic reactor between

operating days 236 to 356. Therefore, modifying the sequestration rate parameter value to zero was thought to be a valid assumption for the present study.

Table 7.8 2nd level calibration of BioWin™ (EBPR parameters)

Parameter	BioWin™ Default Value	Modified Value
Aerobic P/PHA Uptake (mg P/mg PHA)	0.95	0.795
Local Kinetic Parameter -Pre-anoxic sequestration rate (1/d) -Post-anoxic sequestration rate (1/d)	 6 6	 0 0

Modification of EBPR parameters was followed by further simulation of the MBNR (Biological) system. The results are shown in Table 7.9. The predicted phosphorus profile followed the same trend as the measured data with P release in the anaerobic reactor, P uptake in the pre-anoxic reactor and the aerobic reactor and negligible activity in the post-anoxic reactor and the membrane tank. Also, predicted PO_4-P concentrations for the individual reactors were in satisfactory agreement with the measured values, as demonstrated in Table 7.9. In conclusion, calibration enabled successful modeling of the steady state MBNR (Biological) system EBPR performance.

Table 7.9 Measured and predicted steady state (with 2nd level calibration of BioWin™) PO_4-P data for the MBNR (Biological) system

Reactor	Measured PO_4-P* (mg/L)	Predicted PO_4-P (mg/L)
Anaerobic	6.3 (±0.1)	8.2
Pre-anoxic	4.2 (±0.5)	5.7
Aerobic	2.5 (±1.6)	2.1
Post-anoxic	3.0 (±1.0)	2.4
Membrane	2.2 (±1.0)	2.2
Permeate	2.0 (±1.1)	2.2

*: data period for operating days 236 to 356

7.5 Dynamic Modeling of the MBNR (Biological) System

Dynamic modeling was conducted to evaluate the response of the calibrated steady state model to varying influent conditions. Since the influent flow rate was constant for the MBNR (Biological) system, dynamic conditions were expected only in terms of wastewater constituent concentrations. Data for dynamic modeling were taken from the operating period from day 359 to 449. Figures 7.6 to 7.9 summarize model predicted and measured suspended solids data, NH_4-N data, NO_3-N data and PO_4-P data, respectively. The comparability of both sets of data is discussed below.

Model predicted total suspended solids and % volatile suspended solids data demonstrated satisfactory agreement with the measured data. This observation was consistent for all five reactors (Figure 7.6).

The NH_4-N data, both measured and predicted, were in excellent agreement for all five reactors (Figure 7.7). Both sets of data also demonstrated complete nitrification in the MBNR (Biological) system. Nevertheless, the model did not predict the failure of nitrification that occurred on day 363 of operation. As described in **Section 5.3.2**, the failure was attributed to low alkalinity in the influent. Although alkalinity was measured during that period, it was measured only twice (Monday and Friday) per week. In the dynamic modeling, an average influent alkalinity value determined from measurements made between operating day 359 and 449 was used for the simulations. For that reason, it is believed that the model assumed a higher alkalinity concentration than was present in the wastewater near day 363 and, thus, was unsuccessful in predicting nitrification failure.

Dynamic modeling and measured NO_3-N data in general agreed well, as illustrated in Figure 7.8. For the anaerobic and pre-anoxic reactor, the predicted values were close to 0 mg/L consistently, whereas some variability was observed in the measured data, which were predominately between 0 mg/L and 0.5 mg/L. Both sets of data compared very well as far as NO_3-N production in the aerobic reactor was concerned. The major discrepancy

was observed in the post-anoxic reactor. With 72 mg/L of methanol supplementation, the model predicted 100 percent removal of NO_3-N (exception on day 429) in the post-anoxic reactor. The model assumed complete mixing of methanol, whereas, as described earlier, floating foam was thought to have hindered the mixing in the post-anoxic reactor of the MBNR (Biological) system (refer to **Section 5.3.3**). For the subsequent membrane tank and permeate, the predicted and measured NO_3-N concentrations mimicked the profile of the post-anoxic reactor.

Measured PO_4-P data were matched quite well by the predictions of the dynamic model. This was true for the reactors, as well as the permeate (Figure 7.9). For example, the model was able to predict the initial downward and subsequent upward trend of phosphorus release in the anaerobic reactor. Similarly, the predicted permeate PO_4-P concentration profile matched that of the experimental data between day 415 to 443, when the average PO_4-P concentration was unexpectedly lower than 0.5 mg/L.

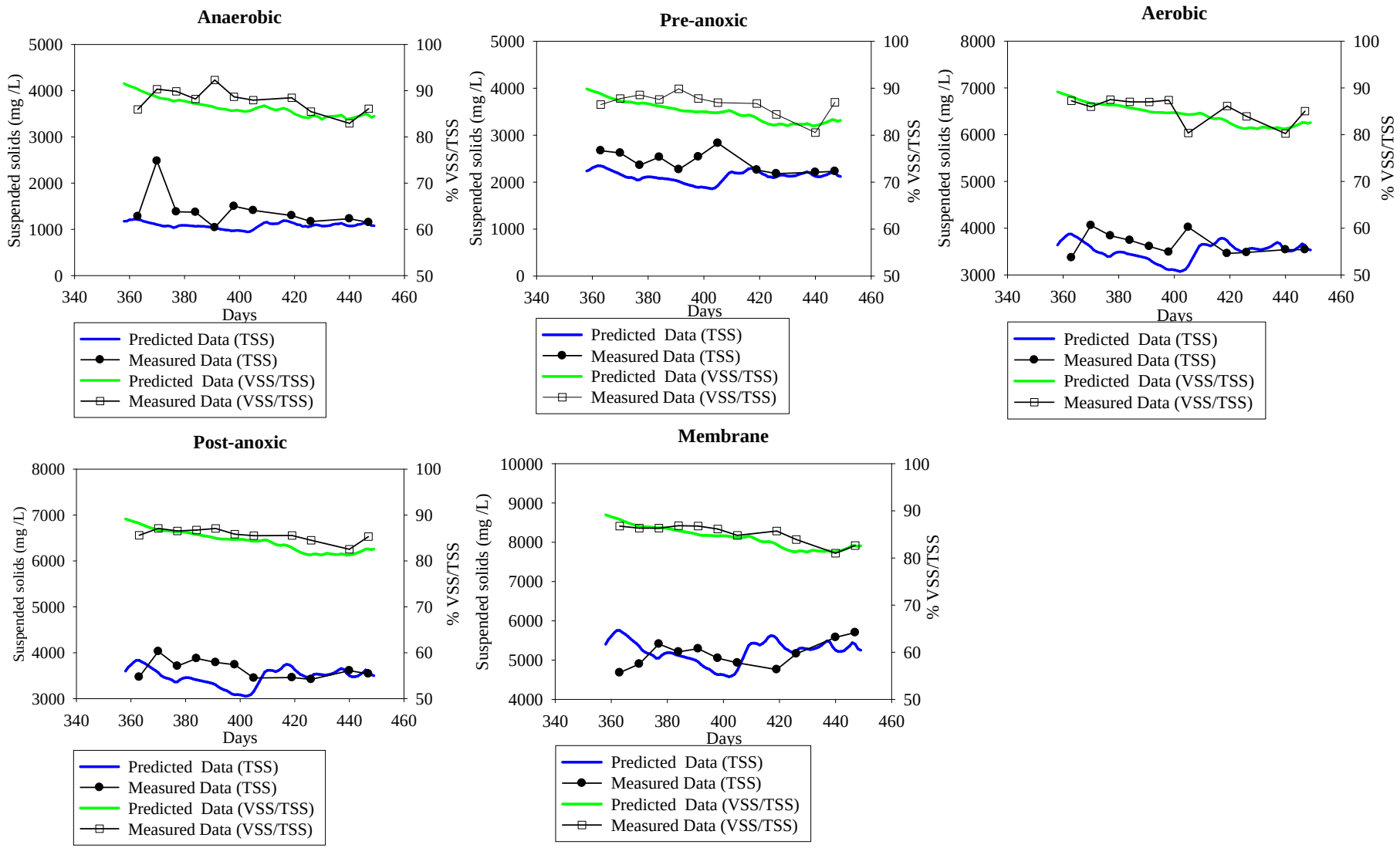


Figure 7.6 MBNR (Biological) system measured and dynamic modeling (with calibrated BioWin^{TM} parameters) suspended solids data

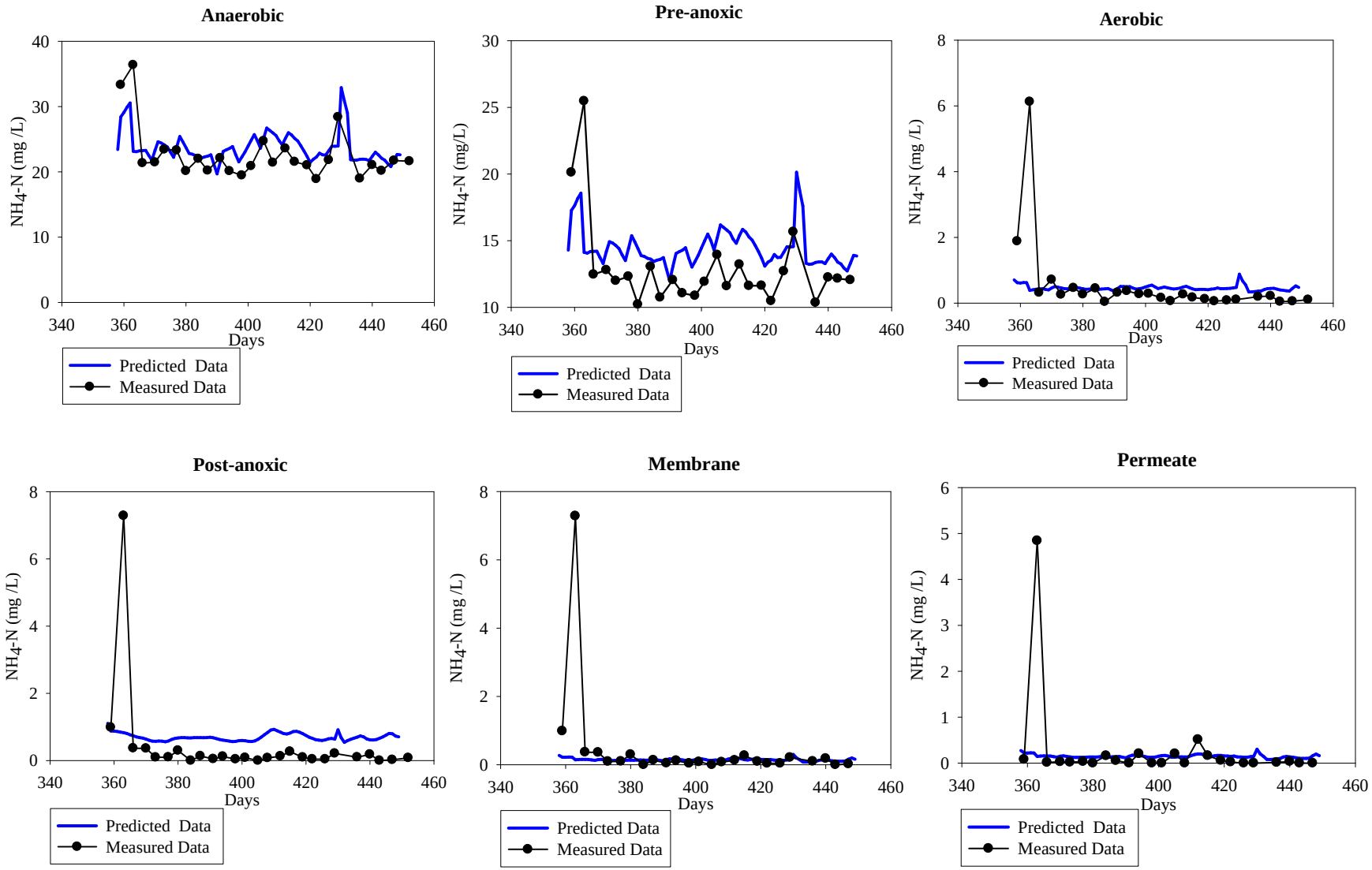


Figure 7.7 MBNR (Biological) system measured and dynamic modeling (with calibrated BioWin^{TM} parameters) NH_4-N data

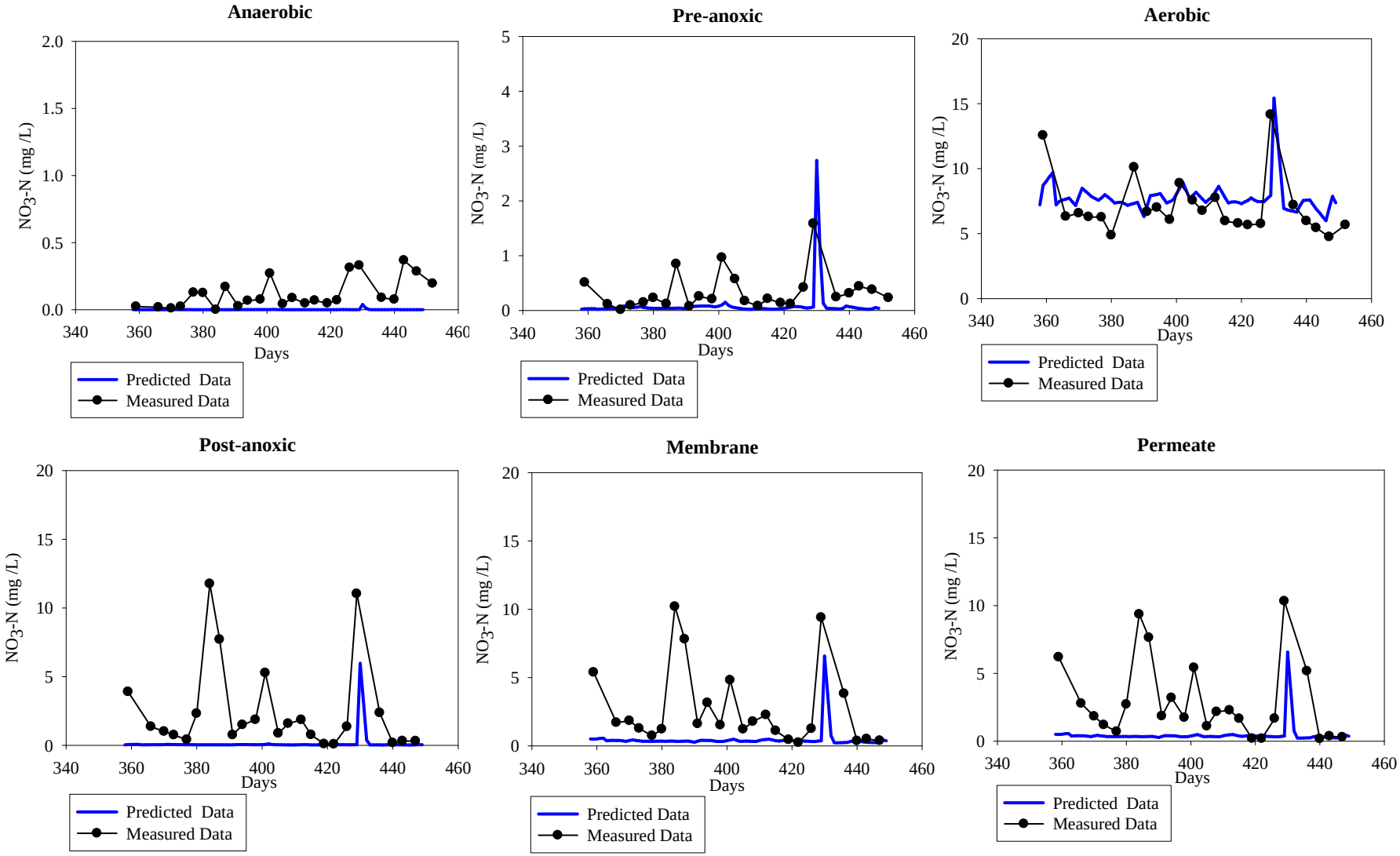


Figure 7.8 MBNR (Biological) system measured and dynamic modeling (with calibrated BioWin^{TM} parameters) NO_3-N data

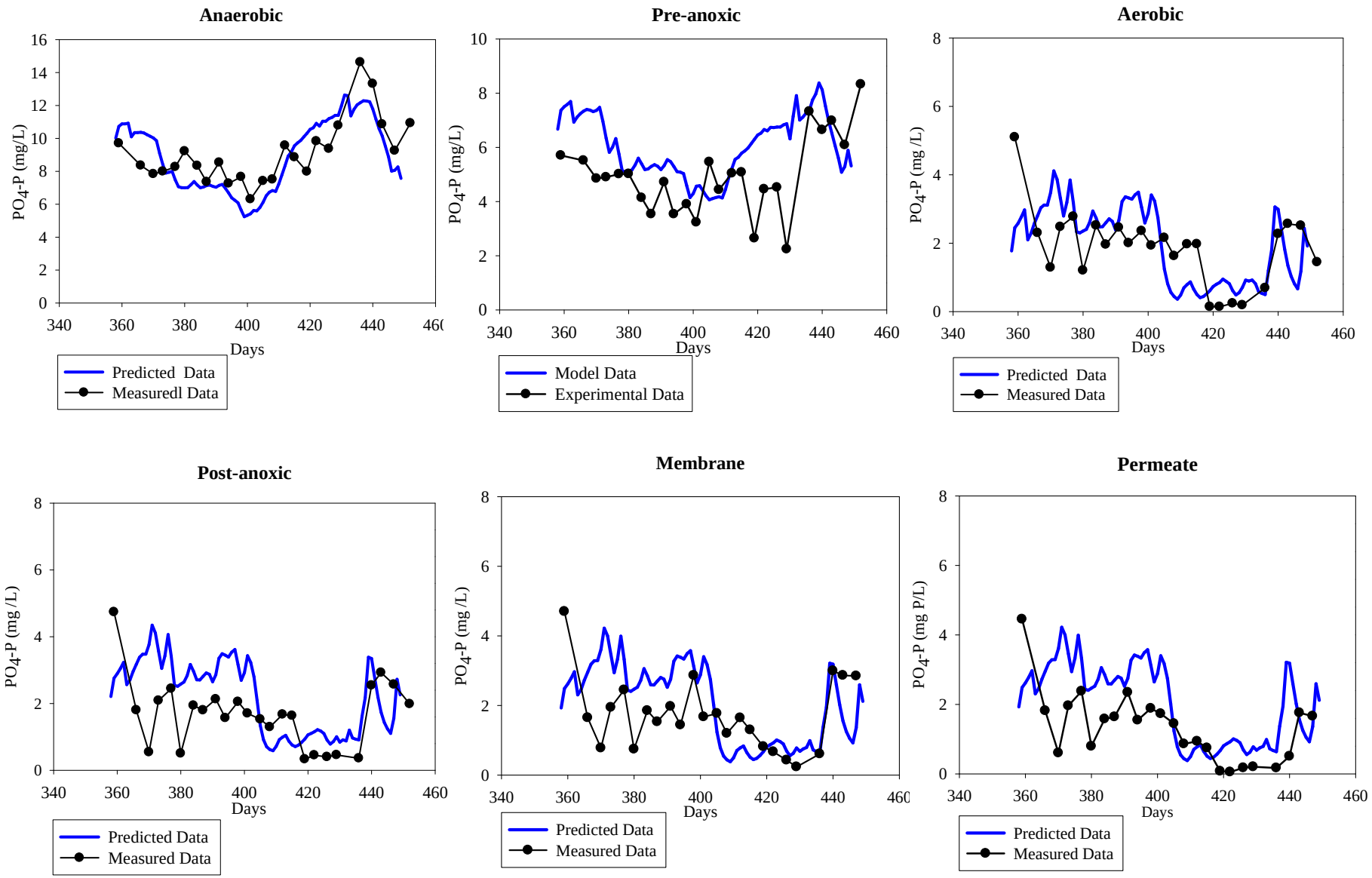


Figure 7.9 MBNR (Biological) system measured and dynamic modeling (with calibrated BioWin^{TM} parameters) PO_4-P data

7.6 Application of the Model to the MBNR (Chemical) System

The calibrated MBNR (Biological) model was applied to the MBNR (Chemical) system to assess prediction capability when alum was added to enhance P removal to LoT levels. A more specific objective was to compare predicted solids concentration, nitrification, denitrification and EBPR performance with measured data at different alum dosing rates. As mentioned earlier, the MBNR (Chemical) system was operating as an EBPR-only process for the first 225 days. The nutrient removal performance of the parallel MBNR systems was comparable for that period. Consequently, it was assumed that the simulation model parameter calibration completed for the MBNR (Biological) system also would be applicable to the MBNR (Chemical) system. Dynamic simulation was conducted for only the period of alum dosing, beginning on operating day 226 (first day of 20 mg/L of alum dosing) and ending on operating day 470. Figure 7.10, Figure 7.11, Figure 7.12 and Figure 7.13 illustrate predicted and measured suspended solids data, NH_4-N data, NO_3-N data and PO_4-P data respectively. The comparability of both set of results is discussed below.

The predicted total suspended solids and % volatile suspended solids data exhibited agreement with the measured data for all five reactors (Figure 7.10). However, the impact of the predicted accumulation of alum precipitates on % VSS/TSS was greater in the model predictions than was observed in the measured data.

Measured and predicted NH_4-N data were in excellent agreement for all five reactors and permeate, the only exception being the period between day 354 to day 380 (Figure 7.11). As mentioned in **Section 5.3.2**, nitrification inhibition was observed in the MBNR (Chemical) system during that period and this was attributed to combined low influent alkalinity and the initiation of 80 mg/L of alum supplementation. The model however, demonstrated uninterrupted complete NH_4-N removal. The two different results may be explained by the denitrification performance in Figure 7.12. The model predicted complete denitrification with sufficient alkalinity generation in the system to counter low influent alkalinity conditions as well as alum-related alkalinity consumption.

Dynamic model and measured NO_3-N data mimicked the observations noted previously for the MBNR (Biological) system. In general, a very good fit was achieved for all five reactors and the permeate (Figure 7.12). For the anaerobic and pre-anoxic reactor, the model predicted values that were close to 0 mg/L consistently, whereas some variability was observed in the measured data. It should be mentioned that the measured NO_3-N concentrations fell within a narrow range, predominately between 0 mg/L and 0.5 mg/L. The data sets compared very well as far as NO_3-N production in the aerobic reactor was concerned. However, discrepancy was observed in the post-anoxic reactor. With 72 mg/L of methanol supplementation, the model predicted complete removal of NO_3-N (although a few exceptions could be noticed in Figure 7.12) in the post-anoxic reactor. On the other hand, the measured data were much more dynamic, with periods of high and low NO_3-N concentrations. The reasons for variable measured data vs. consistent predicted data could not be identified in the present study. Finally, for the membrane tank and the permeate, predicted and measured NO_3-N concentration mimicked the profile of the post-anoxic reactor.

The phosphorus removal performance, measured in the MBNR (Chemical) system was completely different than that was predicted in the BioWin^{TM} model. Both sets of data are summarized in Figure 7.13. The model predicted failure of EBPR during 20 mg/L of alum dosing, whereas in reality, the progress of EBPR decline was very slow and started only after reaching 80 mg/L of alum dosing. Moreover, predicted individual reactor PO_4-P concentrations became close to zero after only 24 days of alum dosing. This was in total contrast to the measured data (Figure 7.13) and this provides evidence of PAOs being much more resilient than predicted by the BioWin^{TM} model. As far as permeate PO_4-P concentration is concerned, predicted data demonstrated that 20 mg/L of alum was sufficient to realize excellent permeate PO_4-P concentrations. On the other hand, measured data indicated that 80 mg/L of alum was required to achieve similarly low levels.

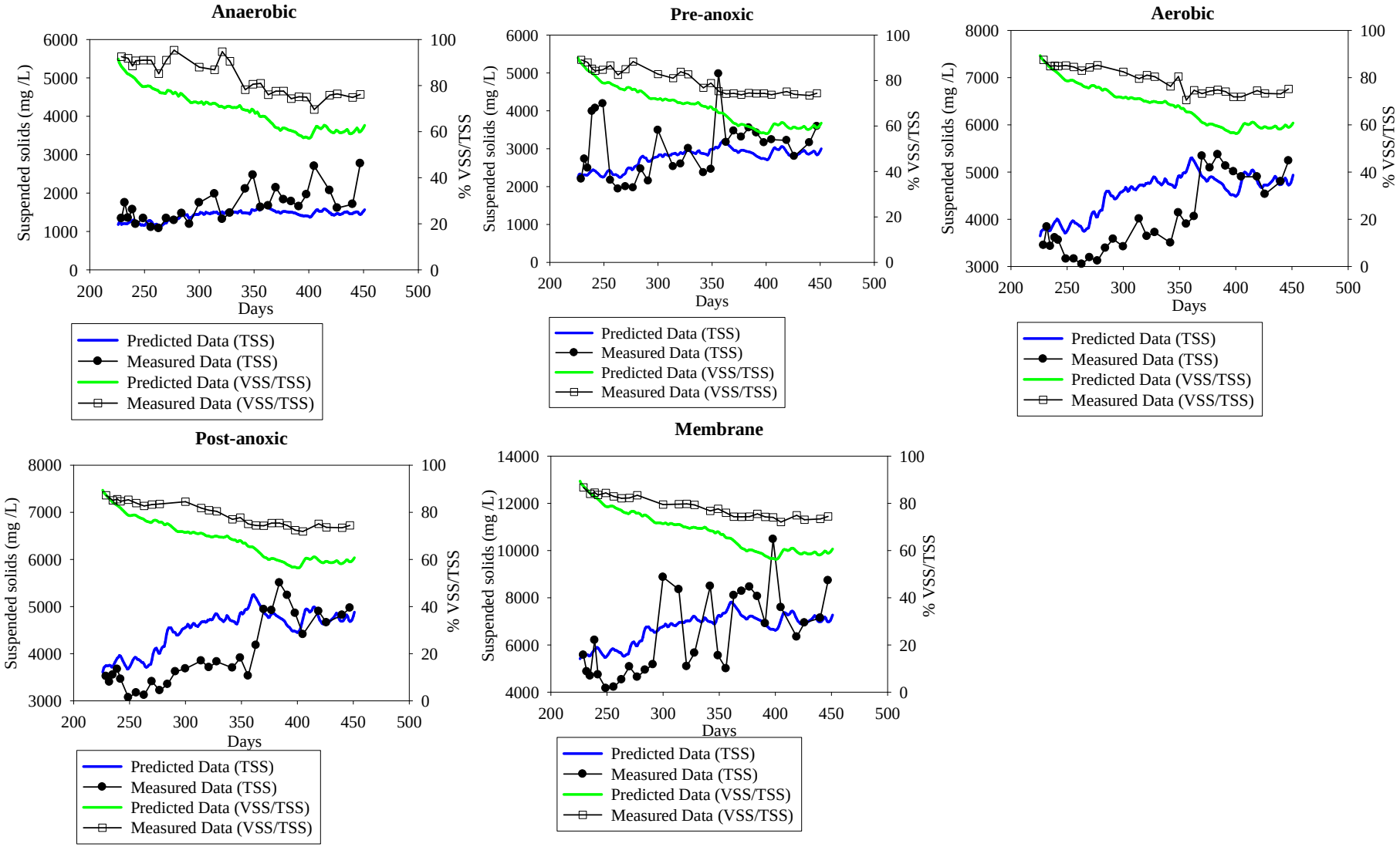


Figure 7.10 MBNR (Chemical) system measured and dynamic modeling (with calibrated BioWin^{TM} parameters) suspended solids data

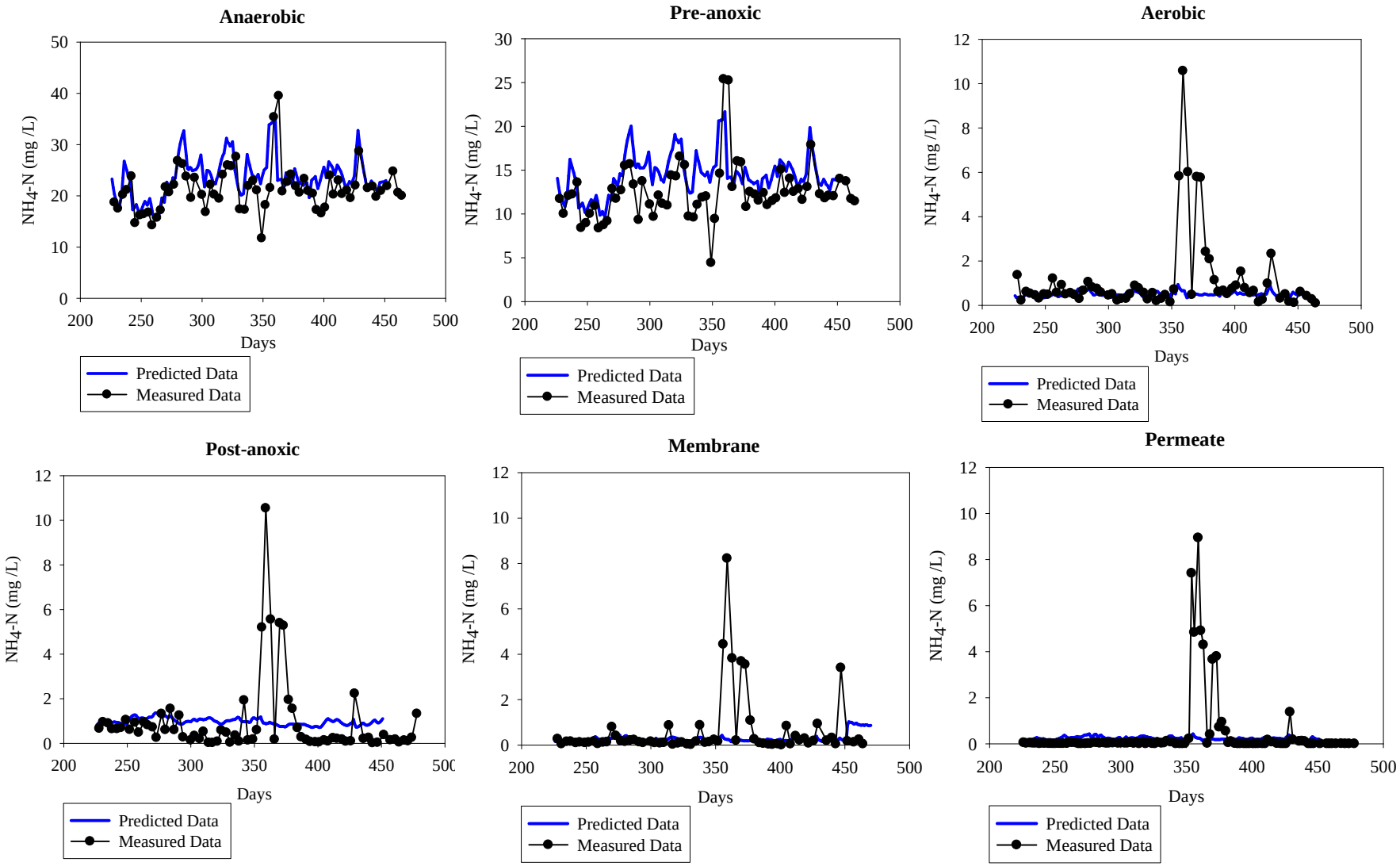


Figure 7.11 MBNR (Chemical) system measured and dynamic modeling (with calibrated BioWin^{TM} parameters) NH_4-N data

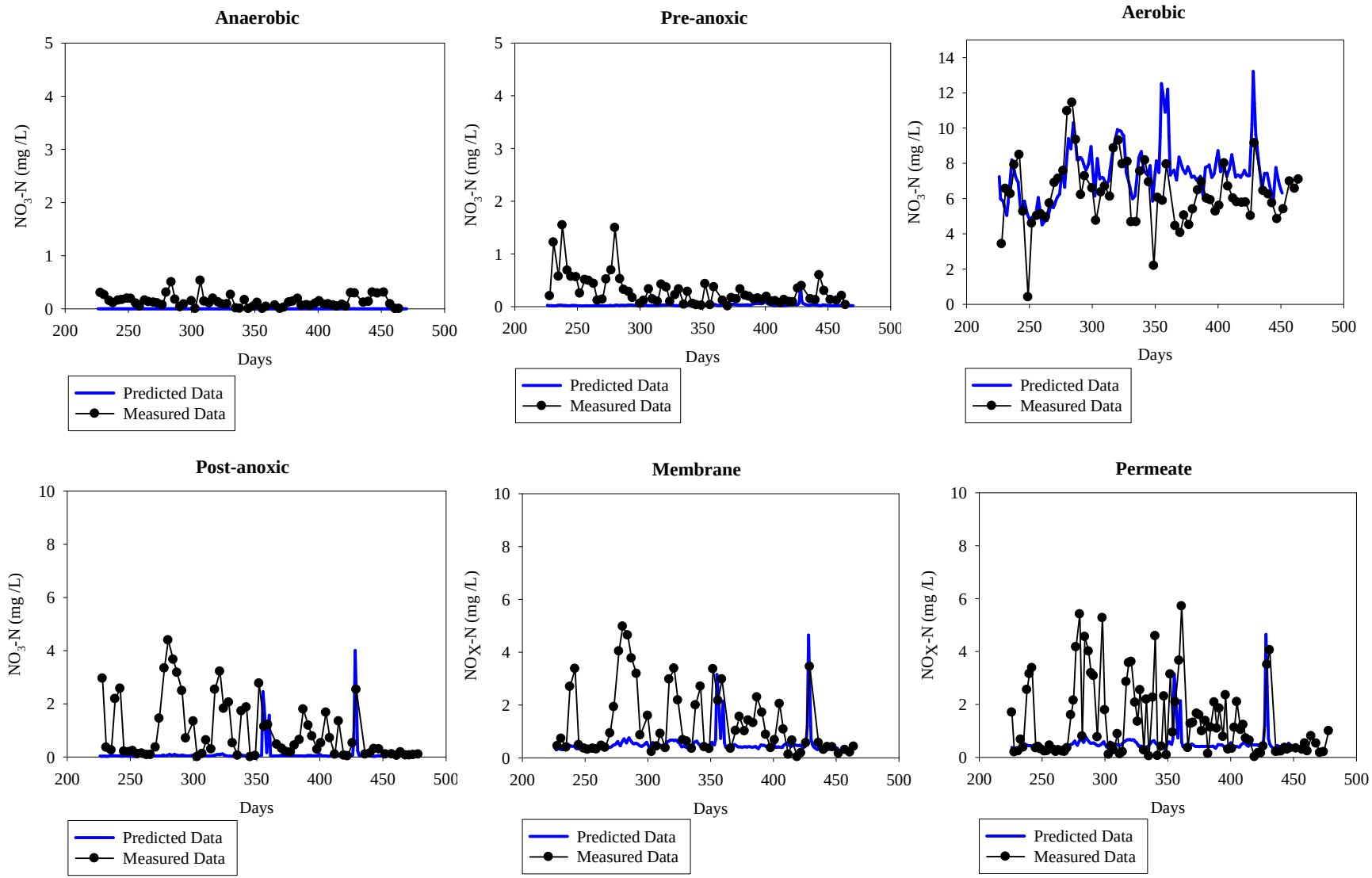


Figure 7.12 MBNR (Chemical) system measured and dynamic modeling (with calibrated BioWin^{TM} parameters) NO_3-N data

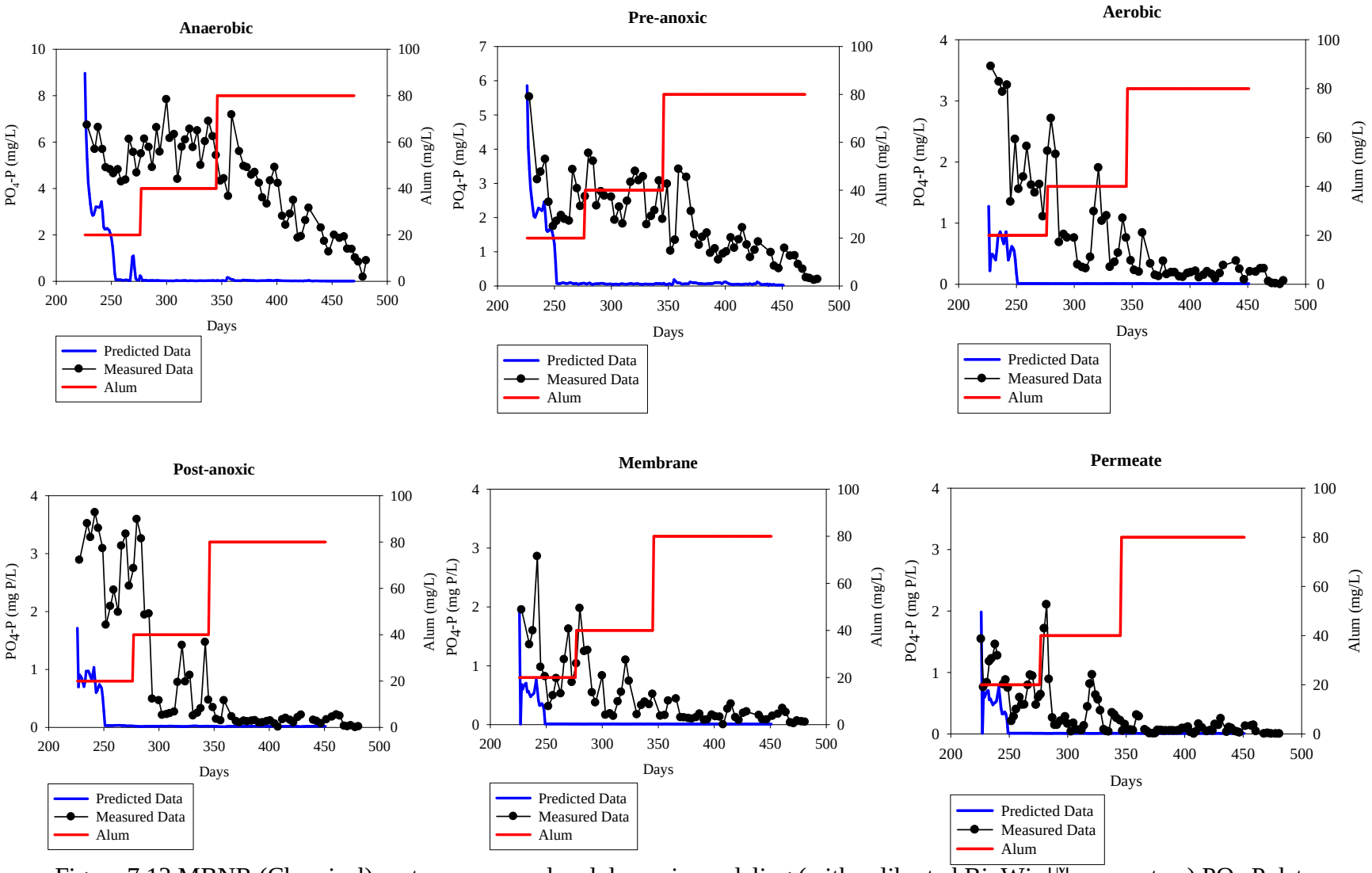


Figure 7.13 MBNR (Chemical) system measured and dynamic modeling (with calibrated BioWin™ parameters) PO_4-P data

7.7 Assessment of the ASM-Based Model

The application of BioWin^{TM} provided important insights into the suitability of ASM in the modeling of the parallel MBNR systems. The model overpredicted solids production in the MBNR (Biological) system. The issue was addressed by calibrating values of F_{up}, Y_H, Y_{PO4}, b_H and ISS from their default settings. As far as nitrogen removal modeling was concerned, an excellent fit was achieved with default nitrification and denitrification parameter values. Prediction was successful in both steady state and dynamic modeling modes. A key objective of the modeling task was to understand whether the design of the MBNR system along with external methanol addition could result in LoT nitrogen removal capability. The model results were affirmative as approximately 100 percent NH_4-N and NO_3-N removal was indicated. The phosphorus removal mechanism, as predicted by the steady state model with default EBPR parameters, was different than that suggested by the MBNR (Biological) system measured data. Conversely, predicted and measured permeate PO_4-P concentrations were greater than 2 mg/L, signifying incomplete EBPR activity. It could therefore be said that the EBPR-only strategy in the case of the MBNR (Biological) system would not realize the LoT phosphorus goal. Nonetheless, to achieve a fit between the predicted and measured EBPR performance, aerobic P/PHA Uptake (mg P/mg PHA) and sequestration rate was reduced to 0.795 and switched off respectively. Subsequent steady state and dynamic modeling efforts resulted in very good fit with the measured data.

Alum dosing in the MBNR (Chemical) system was modeled in the present study by combining the *General Model* with the *Chemical Phosphorus Precipitation Model*. As expected, alum contributed to an increase of TSS in the bioreactors. Alum addition was also predicted to cause a decrease in the % VSS/TSS ratio to a slightly greater extent than suggested by the measured data. As far as nitrogen removal was concerned, the model did not show any impact of alum on either the nitrification or denitrification mechanisms. Approximately 100 percent NH_4-N and NO_3-N removal was the outcome of modeling of the MBNR (Chemical) system. On the whole, this was in agreement with the measured data and provided strong evidence of the potential for accomplishing LoT nitrogen

removal concurrently with 80 mg/L of alum addition. The impact of alum on EBPR was not mapped well by the calibrated model when compared to the measured data, although both sets of data indicated LoT phosphorus removal capability. The model predicted a rapid biological P removal failure, while the measured data indicated that EBPR capability declined slowly in the continuous flow system. Moreover, the model predicted that > 97 percent PO_4-P removal would result with 20 mg/L of alum dosing, while, in reality, 80 mg/L was required to achieve this removal efficiency. The difference between the alum requirements could be attributed to the way the model is set up. The model assumed that ideal mixing conditions enabled alum-phosphorus complexation and hence, low dosing was required. On the other hand, aeration-induced vigorous mixing in the membrane tank of the MBNR (Chemical) system might not have provided optimum conditions, or even disrupted floc formation. Therefore, a greater amount of alum was required to achieve very low permeate PO_4-P concentrations. Nevertheless, it could be concluded that the MBNR (Chemical) model realized LoT nitrogen and phosphorus removal objective with 72 mg/L of methanol and 80 mg/L of alum.

Interpretation by the combined *General Model* and *Chemical Phosphorus Precipitation Model* on the relationship between simultaneous biological and chemical P removal is an area of research where little information is currently available. As mentioned previously, Liu *et al.* (2011) used the two models to predict P removal performance in a pilot UCT-MBR system. They concluded that while the model predicted inhibition of alum on EBPR, the measured data did not support that finding. The authors however did not elaborate on how to calibrate the model so that predicted data could adequately describe the relationship between the two P removal mechanisms. Ingildsen *et al.* (2006) are probably the only researchers who calibrated model parameters with the sole objective of fitting measured data from a combined chemical P removal and EBPR plant. The authors applied the ASM2d model to simulate phosphorus removal dynamics in AveØre WWTP (Denmark), resulting in poor model fit. To address the gap between the predicted and measured data, they initially calibrated two model parameters, i.e. decrease in k_{PRE} (rate constant for P precipitation) value to 0.1 d^{-1} from the default value of 1 d^{-1} and k_{RED} (rate constant for P dissolution) value to 0.06 d^{-1} from the default value

of 0.6 d^{-1}. The two parameters were modified as the authors hypothesized that the simulated precipitation process was too fast when compared to reality. Subsequent simulations provided only partially improved results. Applying a trial and error approach, Ingildsen *et al.* (2006) further found that an increased K_{PHA} (Monod saturation constant for PHA) value of 0.1 (default value of 0.01) yielded the most consistent model fit. However, the authors did not have any microbiological evidence to support this particular calibration strategy. In conclusion, the task of identifying the right parameters with a strong microbiological rationale will require very good understanding of the fundamentals of the two models.

7.8 Conclusions

The key conclusions from the modeling activity are the following.

- A steady state version of the *BioWin^{TM} General Model* applied on the MBNR (Biological) system resulted in overprediction of suspended solids concentrations in the bioreactors. Four model parameters, i.e. F_{up}, Y_H, Y_{PO4}, b_H and ISS were modified from their default values to realize a satisfactory fit to the measured suspended solids data.
- Nitrification and denitrification steady state predicted data exhibited excellent agreement with the measured data of the MBNR (Biological) system. Hence, no further parameter calibration was required in the *BioWin^{TM} General Model*.
- The steady state *BioWin^{TM} General Model* misinterpreted the prevailing EBPR mechanism in comparison to that suggested by the measured data from the MBNR (Biological) system. A calibration of one kinetic parameter, i.e. anoxic sequestration rate and one stoichiometric parameter, i.e. aerobic P/PHA Uptake (mg P/mg PHA) enabled a good fit with the measured data.
- Dynamic simulation of the MBNR (Biological) system validated the suitability of the modified *BioWin^{TM} General Model*. This was accomplished by successful fitting of the predicted solids production, nitrification, denitrification and EBPR data with the measured data.

- The *BioWin^{TM} General Model* calibrated for the MBNR (Biological) system along with the *Chemical Phosphorus Precipitation Model* was applied on the MBNR (Chemical) system to simulate behaviour during the alum dosing period. The predicted suspended solids production, ammonium and nitrate concentrations in all the reactors as well as permeate, were in satisfactory agreement with the measured data.
- Predicted ammonium and nitrate removal was approximately 100 percent at high alum dosing concentrations (80 mg/L), indicating no negative impact of alum addition on nitrification or denitrification. This was mostly in agreement with the measured data, although measured data were more variable in the permeate.
- The combined *BioWin^{TM} General Model* and *Chemical Phosphorus Precipitation Model* predicted failure of EBPR at a relatively low alum dosing concentration (20 mg/L). This was not supported by the measured data, which indicated that EBPR failure started only at 80 mg/L of alum dosing in the MBNR (Chemical) system. Also, the model predicted that greater than 97 percent PO_4-P removal could be accomplished with 20 mg/L of alum dosing, while in reality, 80 mg/L of alum was required.
- The contrasting observation regarding the alum requirement was attributed to the model set up. The model assumed that ideal mixing conditions enabled stoichiometric removal rates and predicted very low effluent PO_4-P concentrations. In reality, vigorous aeration in the membrane tank may have created non ideal mixing conditions which resulted in decreased metal salt-phosphorus complexation. Therefore, greater requirement of alum for similar level of removal.
- Dynamic modeling of the MBNR (Chemical) system indicated that the selected modified Bardenpho configuration along with external methanol and alum dosing has the capability to accomplish LoT nutrient removal goals. This was supported by the measured data generated by laboratory scale continuous flow system.

8 Novel Batch Method for Direct Measurement of Dissolved Organic Nitrogen in the Parallel MBNR Systems

8.1 Introduction

Dissolved organic nitrogen (DON) was measured in the present study to assess its contribution in the permeate of the parallel MBNR systems targeting TN ≤ 3 mg/L. The task was expanded to individual reactors with the goal of finding specific DON production and/or utilization trends. Indirect measurement methods were not preferred due to their limitations (Refer to **Section 2.3.4.2**). Thus, a direct batch DON measurement method was developed in the present study with the twin objectives of obtaining reliable results and processing bulk samples relatively quickly. The method involved two steps, i.e. adsorption on anion exchange resin followed by persulfate digestion. The first step was expected to remove nitrate from samples to background levels and the second step was to convert DON (and ammonium if present) to nitrate so that direct measurement could be made. Validation of both steps was accomplished by comprehensive process of quality control (QC). After successful completion of QC, DON was measured in permeate of the parallel MBNR systems between operating days 403 and 464 and the individual reactors between operating days 405 and 464.

8.2 Quality Control for Batch Anion Exchange Resin Method

The main purpose of the batch anion exchange resin method was to adsorb nitrate from samples while not impacting other forms of nitrogen (i.e. ammonium and dissolved organic nitrogen). The success of the method was dependent on the determination of the contact time between the sample and resin and the quantity of resin required for removing sample nitrate to background levels. By adopting a trial and error approach, contact time and resin requirement was determined in the present study for nitrate levels that were typical in the permeate and reactors of the parallel MBNR systems. Moreover, their impact on samples containing ammonium and dissolved organic nitrogen (urea) was also

evaluated. The step by step execution of the batch anion exchange resin experiments is detailed in **Section 4.5.1**.

8.2.1 Nitrate removal

The nitrate adsorption efficiency of the batch anion exchange resin method was evaluated and the results are summarized in Table 8.1. The quantity of anion exchange resin used was constant at 0.75 g while two contact times (1 hour and 24 hour) were assessed in the experiments. Samples containing initial NO_3-N concentrations of 2, 3, 5 and 7 mg/L were contacted with the anion exchange resin followed by measurement of residual concentrations. An initial NO_3-N range of 2-7 mg/L was selected because similar concentrations were generally observed in the parallel MBNR system bioreactors and permeates. The data in Table 8.1 show greater than 93 percent removal efficiency in all cases and thus, demonstrate the suitability of the batch adsorption method. Another key observation was that 1 hour contact time was sufficient to realize maximum removal potential. As a result, it was determined that a 1 hour contact time would be utilized in all future batch tests.

Table 8.1 NO_3-N removal efficiency of batch anion exchange resin method (samples with different initial NO_3-N concentrations)

Sample	Initial Concentration* (mg NO_3-N/L)	Final Concentration (mg NO_3-N/L)	% Removal	Description
Tap water	2	0.12 (±0.004)	94 (±0.0)	0.75 g dry resin -1 hour contact time
	2	0.12 (±0.002)	94 (±0.0)	0.75 g dry resin -24 hour contact time
	3	0.14 (±0.109)	95 (±3.5)	0.75 g dry resin -1 hour contact time
	3	0.21 (±0.003)	93 (±0.0)	0.75 g dry resin -24 hour contact time
	5	0.35 (±0.006)	93 (±0.0)	0.75 g dry resin -1 hour contact time
	5	0.35 (±0.005)	93 (±0.0)	0.75 g dry resin -24 hour contact time
	7	0.38 (±0.014)	95 (±0.6)	0.75 g dry resin -1 hour contact time
	7	0.42 (±0.003)	94 (±0.0)	0.75 g dry resin -24 hour contact time

*: Samples in triplicate
±: Standard deviation

The capability of the batch anion exchange resin adsorption method was further verified with samples of MBNR system mixed liquor and permeate. The mixed liquor sample was collected from the MBNR (Chemical) system aerobic reactor and was filtered (0.45 µm) before adsorption with the anion exchange resin. The aerobic reactor was selected as it had the highest concentration of nitrate among all the reactors. Three different quantities of resin, i.e., 0.75 g, 1.00 g and 1.25 g were used, while the contact time was fixed at 1 hour. The results in Table 8.2 illustrate that 1.25 g of dry resin resulted in the best adsorption efficiencies. It was therefore decided that 1.25 g of anion exchange resin would be used for samples from the reactors. As far as permeate samples were concerned, 0.75 g dry resin and 1 hour contact time was sufficient in removing NO_3-N to background levels (Table 8.2).

Table 8.2 NO_3-N removal efficiency of batch anion exchange resin method (MBNR system mixed liquor and permeate samples)

Sample	Initial Concentration* (mg NO_3-N/L)	Final Concentration (mg NO_3-N/L)	% Removal	Description
MBNR (Chemical) aerobic mixed liquor	7.0	0.42 (±0.04)	95 (±0.6)	0.75 g dry resin -1 hour contact time
	7.0	0.28 (±0.01)	96 (±0.0)	1.00 g dry resin -1 hour contact time
	7.0	0.25 (±0.02)	97 (±0.6)	1.25 g dry resin -1 hour contact time
MBNR (Biological) permeate	2.0	0.04 (±0.04)	98 (±1.8)	0.75 g dry resin -1 hour contact time
MBNR (Biological) permeate	0.2	0.00 (±0)	100 (±0.0)	0.75 g dry resin -1 hour contact time

8.2.2 Ammonium recovery

The anion exchange resin adsorption of NH_4-N was investigated and the results are shown in Table 8.3. For initial NH_4-N concentrations in range of 0.5 to 3 mg/L, recovery was consistently close to 100 percent. The tests were done with 0.75 g of dry resin and 1 hour contact time. In conclusion, % NH_4-N recovery data confirmed that the anion exchange resin did not adsorb NH_4-N.

Table 8.3 NH_4-N recovery efficiency of batch anion exchange resin method

Sample	Initial Concentration* (mg NH_4-N /L)	Final Concentration (mg NH_4-N/L)	% Recovery	Description
Tap water	0.5	0.50 (±0.003)	101 (±0.6)	0.75 g dry resin -1 hour contact time
	1	1.00 (±0.020)	103 (±2.1)	
	2	1.99 (±0.005)	99.6 (±0.3)	
	3	3.01 (±0.040)	100.4 (±1.4)	

*: Samples in triplicate
±: Standard deviation

8.2.3 DON recovery

Evaluation of the impact of anion exchange resin on DON was a key step in the development of the procedure because no removal was expected during the resin adsorption step. However, if statistically significant removal of DON occurred along with nitrate, underestimation of final DON concentration would occur in samples. Urea was chosen as a model for DON at initial concentrations varying from 0.90 to 2.90 mg/L. The selected DON range is typical of values observed in secondary wastewater treatment plant effluents (Bratby *et al.*, 2008). Nonetheless, test results in Table 8.4 demonstrate excellent urea recovery rates in all cases, with 0.75 g of dry resin and 1 hour contact time. For that reason, it could be presumed that the batch anion exchange resin method did not have any impact on DON concentrations in samples, for the quantity of dry resin and contact time used.

Table 8.4 Urea recovery efficiency of batch anion exchange resin method

Sample	Initial Concentration* (mg Urea-N/L)	Final Concentration (mg Urea-N/L)	% Recovery	Description
Tap water	0.90	0.93 (±0.015)	102 (±1.5)	0.75 g dry resin -1 hour contact time
	2.00	1.93 (±0.036)	93.7 (±1.5)	
	2.90	2.84 (±0.050)	99.7 (±1.5)	

*: Samples in triplicates
±: Standard deviation

8.3 Quality Control for Persulfate Digestion Method

The main objective of the persulfate digestion method was to convert DON (also ammonium if present) to nitrate. In the present study, this was accomplished by 4500-N C. Persulfate Method (APHA *et al.*, 2005). This step is more effective for samples with very little ammonium present in them. In such situations, the sample would contain only nitrate and organic nitrogen. As nitrate is adsorbed on the anion exchange resin in the first step, only organic nitrogen is left for persulfate digestion conversion and subsequent

direct measurement. Nevertheless, in the present study, quality control tests were conducted to assess the conversion consistency of standards with known concentrations of ammonium, urea and glutamic acid to nitrate. In addition, recovery tests were conducted on standards containing known concentrations of nitrate. The results are discussed below.

8.3.1 Ammonium conversion

The persulfate digestion tests were done with a range of initial NH_4-N concentrations, i.e. 0.5, 1.0, 2.0 and 3.0 mg/L. Table 8.5 illustrates that NH_4-N conversion results were excellent with ≥ 100 percent in three cases. So, it can be said that the method is capable of accomplishing complete conversion of ammonia to nitrate in samples.

Table 8.5 NH_4-N conversion efficiency of persulfate digestion method

Sample	Initial Concentration* (mg NH_4-N/L)	Final Concentration (mg NO_3-N/L)	% Conversion
Tap water	0.5	0.45 (±0.008)	90 (±1.6)
	1	1.02 (±0.048)	102 (±4.9)
	2	2.10 (±0.053)	105 (±2. 7)
	3	3.15 (±0.072)	105 (±2.4)

*: Samples in triplicate
±: Standard deviation

8.3.2 DON conversion

Urea and glutamic acid were the chosen model DON compounds for assessing the efficacy of the persulfate digestion method for DON oxidation. Four different initial concentrations were selected, i.e. 0.5, 1.0, 2.0 and 3.0 mg/L. The results in Table 8.6 and Table 8.7 show approximately 100 percent conversion of DON compounds to nitrate in all cases. Thus it was clear that persulfate digestion could successfully convert both forms of DON to nitrate, as assessed by direct NO_3-N measurement.

Table 8.6 Urea conversion efficiency of persulfate digestion method

Sample	Initial Concentration* (mg Urea-N/L)	Final Concentration (mg NO_3-N/L)	% Conversion
Tap water	0.5	0.53 (±0.055)	106 (±11.1)
	1	1.00 (±0.018)	100 (±1.9)
	2	2.02 (±0.052)	101 (±2.6)
	3	3.06 (±0.059)	102 (±2.0)

*: Samples in triplicate
±: Standard deviation

Table 8.7 Glutamic acid conversion efficiency of persulfate digestion method

Sample	Initial Concentration* (mg Glutamic Acid-N/L)	Final Concentration (mg NO_3-N/L)	% Conversion
Tap water	0.5	0.46 (±0.019)	92 (±3.9)
	1	1.16 (±0.130)	116 (±13.6)
	2	2.0 (±0.044)	100 (±2.2)
	3	2.97 (±0.059)	99 (±1.6)

*: Samples in triplicate
±: Standard deviation

8.3.3 Nitrate recovery

The influence of persulfate digestion on a sample containing nitrate was also evaluated. A sample containing an initial NO_3-N concentration of 5 mg/L was digested and then measured for NO_3-N. A final concentration of 5 mg/L and recovery of 100 percent illustrated that no reaction took place as far as NO_3-N was concerned (Table 8.8).

Table 8.8 NO_3-N recovery efficiency of persulfate digestion method

Sample	Initial Concentration* (mg NO_3-N/L)	Final Concentration (mg NO_3-N/L)	% Recovery
Tap water	5	5 (±0.105)	100 (±2.1)

*: Samples in triplicate
±: Standard deviation

8.4 Parallel MBNR System Permeate TN Speciation

Following validation of the batch anion exchange resin method and persulfate digestion method, direct measurement of DON in permeates of the parallel MBNR systems was undertaken. The resulting profiles are summarized in Figure 8.1. The range of DON was 0.16-2.60 mg/L and 0.11-2.08 mg/L for the MBNR (Biological) system and the MBNR (Chemical) system, respectively. Nevertheless, no clear indication could be established regarding which system generated lower DON concentrations in the permeate. Thus it could be inferred that 80 mg/L of alum dosing in case of the MBNR (Chemical) system was unable to achieve additional DON removal when compared to the MBNR (Biological) system. The most probable reason would be that low molecular weight DON compounds were predominately present in the permeate of the parallel MBNR systems and coagulants like alum are typically not effective for their removal (refer to Chapter Two for detailed description).

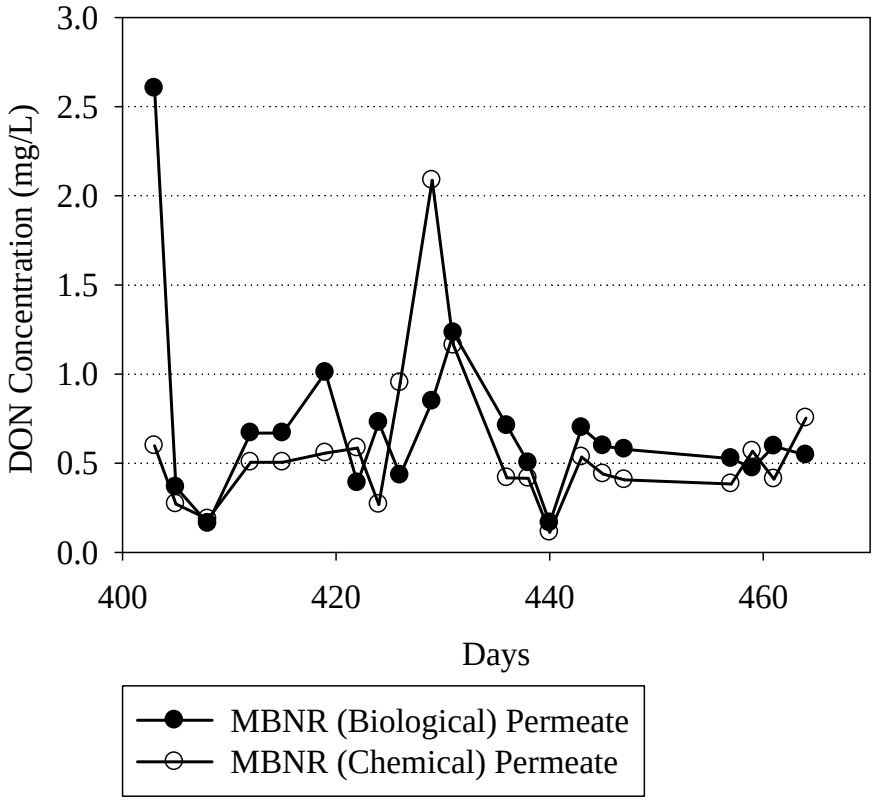


Figure 8.1 Permeate DON concentration of the parallel MBNR systems

Figure 8.2 illustrates permeate TN (total nitrogen) speciation for the parallel MBNR systems for the period between day 403 and 464. TN was calculated based on direct measurement of NH_4-N, NO_X-N and DON. From the figure, it can be observed that all of the samples contained less than 3 mg/L of total nitrogen. This is because the contribution of each component needed to be quantified at times at which the LoT nitrogen effluent goals were being realized in the parallel MBNR systems. From the figure, it is evident that either DON or NO_X-N was the dominant fraction when permeate TN $\leq$ 3 mg/L. NH_4-N contribution was negligible, as nitrification was essentially complete in both MBNR systems during the sampling period. DON contribution, on other hand, varied from 7 percent to 82 percent in the MBNR (Biological) system and 12 percent to 96 percent in the MBNR (Chemical) system.

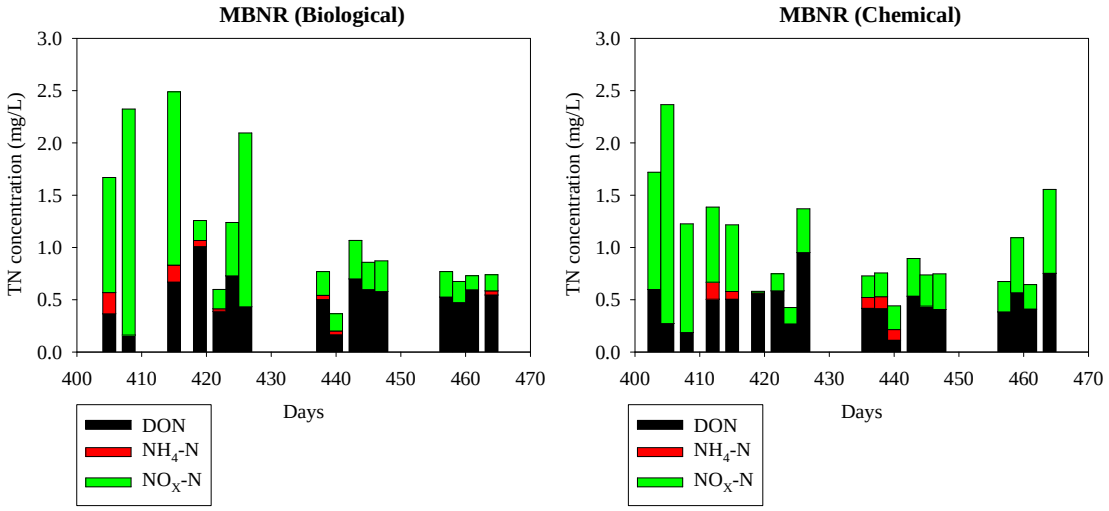


Figure 8.2 TN speciation of the parallel MBNR systems

The permeate DON and TN speciation data indicate challenges in accomplishing the LoT TN goal. DON from longer SRT systems, such as the parallel MBNRs, has typically a greater refractory fraction than a biodegradable fraction (Parkin and McCarty, 1981a). For that reason, excellent nitrification and denitrification performance becomes an absolute necessity. In the context of the parallel MBNR systems, nitrification performance was consistently good, with very little ammonium in the permeate, the LoT goal success was always dependent on denitrification performance.

8.5 Parallel MBNR System Reactor DON Profiling

DON production/utilization was profiled in the individual reactors of the parallel MBNR systems using the combined batch anion exchange resin method and persulfate digestion method. During the experiments, mixed liquor samples were taken from the reactors and were passed through 0.45 µm filters. Subsequently, the batch anion exchange resin method was used to remove nitrate from the samples. In the final step, persulfate digestion was conducted to convert ammonium and organic nitrogen to nitrate for measurement. Reactor DON was estimated by subtracting the ammonium concentration (measured independently) from the nitrate concentrations measured in the persulfate digestates. Figures 8.3 aand 8.4 illustrate DON concentrations of the MBNR (Biological) system and the MBNR (Chemical) system, respectively.

DON concentrations were predominantly negative in the anaerobic reactor and pre-anoxic reactor while being positive in the last three reactors of the parallel MBNR systems. This suggested an inadequacy of the persulfate digestion method. Ammonium was present in high concentrations in the first two reactors and all ammonium was expected to be converted to nitrate during persulfate digestion. However, it seemed that this may not have been accomplished, resulting in negative DON values. It is true that the testing described in **Section 8.3.1** demonstrated successful ammonium conversion, however, the highest concentration tested was 3 mg/L. This problem did not occur with samples from the downstream reactors as ammonium was present in these zones only at very low concentrations. DON concentration varied in those reactors and was typically in the range of 0-2 mg/L. The observation was true for both MBNR systems. Moreover, the absence of any specific trend made it very difficult to profile production or utilization of DON in individual reactors.

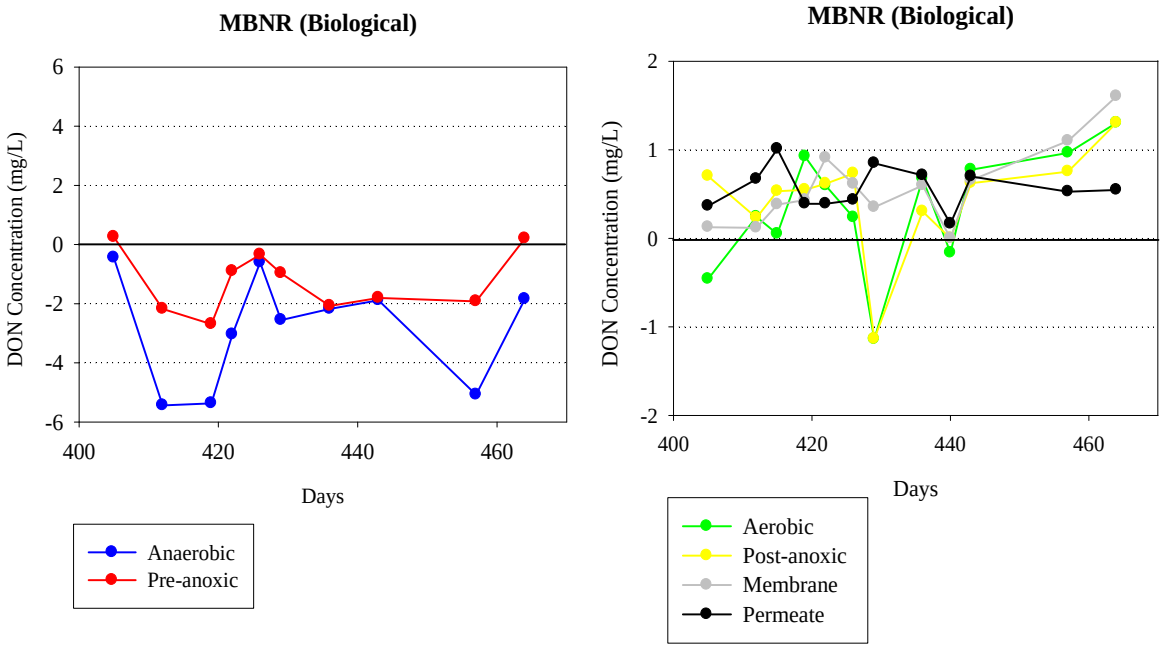


Figure 8.3 DON concentration in individual reactors of the MBNR (Biological) system

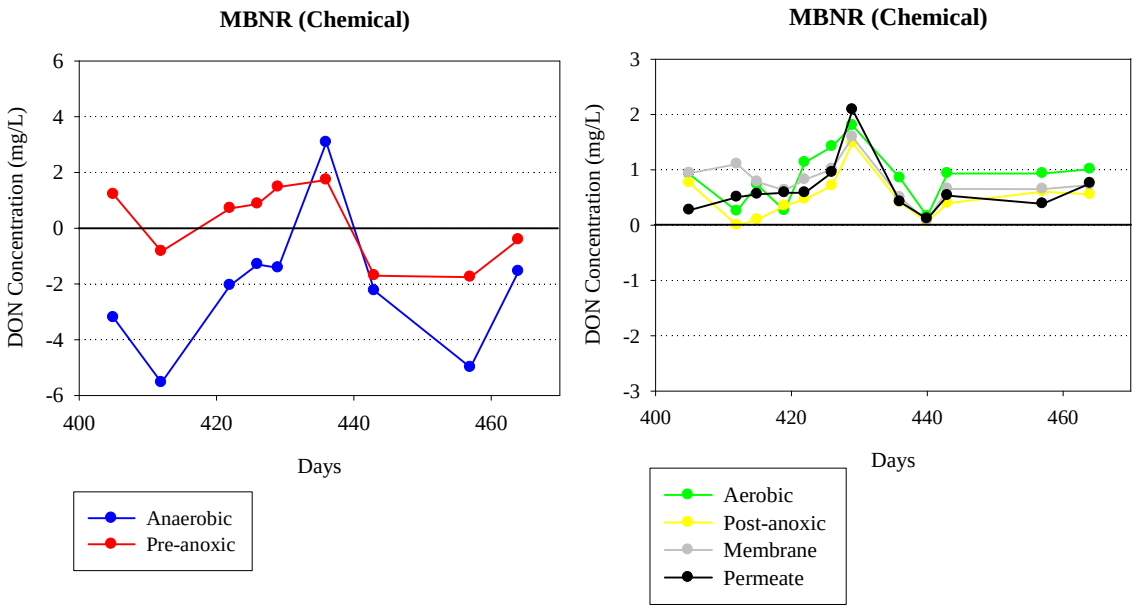


Figure 8.4 DON concentration in individual reactors of the MBNR (Chemical) system

8.6 Conclusions

The key conclusions of the novel batch method for DON determination are the following.

- The two-step direct DON measurement method was validated successfully by quality control procedures in the present study.
- The first step, utilizing an anion exchange resin method, was effective in removing nitrate to background levels without interfering with ammonium or DON concentrations. The optimum resin contact time was determined to be 1 hour while 0.75 g and 1.25 g resin was required for parallel MBNR permeate and bioreactor samples respectively.
- The second step, persulfate digestion, completed approximately 100 percent conversion of ammonium and DON to nitrate, whereas, no affect was observed on initial nitrate concentrations.
- The measured permeate DON concentration range was comparable in the parallel systems with 0.16-2.60 mg/L for the MBNR (Biological) system and 0.11-2.08 mg/L for the MBNR (Chemical) system between operating days 403 and 464. Contrary to

some studies in the literature, a high alum dosage did not enable greater DON removal in the MBNR (Chemical) system.

- The DON contribution to total nitrogen varied from 7 percent to 82 percent in the MBNR (Biological) system and from 12 percent to 96 percent in the MBNR (Chemical) system when permeate TN concentrations were less than 3 mg/L. It signified a requirement for consistently high level nitrification and denitrification performance in the parallel MBNR systems.
- Parallel MBNR system bioreactor DON measurement was partially successful in the current study. It could not be profiled in the anaerobic reactors and pre-anoxic reactors as persulfate digestion method was inadequate in converting high ammonium concentrations to nitrate. In the last three bioreactors of the parallel MBNR systems, DON concentration was typically in 0-2 mg/L range.
- There was no specific DON production and/or utilization trend in bioreactors of the parallel MBNR systems.

9 Conclusions and Recommendations

9.1 Research Conclusions

The key conclusions of the research project are the following.

1.

i. The parallel MBNR system performance data signified the importance of external methanol and alum dosing in accomplishing the LoT nutrient removal goal. The average permeate TP concentration was 0.19 mg/L (including periods when TP concentration was < 0.1 mg/L) with 80 mg/L of alum dosing and average permeate TN concentration was 2.4 mg/L with 72 mg/L of methanol dosing in the MBNR (Chemical) system.

ii. The stoichiometric methanol ratio, i.e. mg methanol required / mg NO_3-N removed, was calculated to be 6.1 in reducing average permeate NO_3-N concentration to 1.4 mg/L.

iii.

a. An average molar Al/TP ratio of 1.9 was required to reduce PO_4-P concentration to 0.07 mg/L in the permeate of the MBNR (Chemical) system.

b. The relationship between chemical P removal and EBPR was dynamic and was dependent on alum dosage concentration in the MBNR (Chemical) system. Alum dosing up to a concentration of 40 mg/L complemented EBPR in improving permeate P removal. On the other hand, 80 mg/L of alum supplementation competed with and finally, inhibited EBPR until the MBNR (Chemical) system was converted to chemical P removal system.

c. Alum addition did not have any influence on COD removal and denitrification in the MBNR (Chemical) system when compared with the MBNR (Biological) system. A similar conclusion can be made for

nitrification except when permeate ammonium concentration was observed to be elevated at the beginning of the period of 80 mg/L of alum dosing. It is thought that low influent alkalinity in combination with alum-induced alkalinity consumption impeded nitrification temporarily.

2. Sequential anaerobic-aerobic batch tests demonstrated that the mixed liquor maximum specific phosphorus release (mg P/(g VSS· hr) and maximum specific phosphorus uptake (mg P/(g VSS· hr) values were similar in the parallel MBNRs although alum dosage was up to 40 mg/L in the MBNR (Chemical) system. Conversely, values of the two kinetic parameters were reduced considerably when the batch tests were conducted with MBNR (Chemical) mixed liquor during 80 mg/L of alum dosing period. This was consistent with the trends observed in the continuous flow MBNR (Chemical) system.

3.

   i. A steady state version of the *BioWin^{TM} General Model* applied on the MBNR (Biological) system required modification of F_{up}, Y_H, Y_{PO4}, b_H and ISS from their default values to realize a good fit against the measured suspended solids data. Nitrification and denitrification steady state predicted data, on the other hand, were in excellent agreement with the measured data of the MBNR (Biological) system.

   ii. The steady state *BioWin^{TM} General Model* misinterpreted the prevailing EBPR mechanism in comparison to that suggested by the measured data from the MBNR (Biological) system. A calibration of anoxic sequestration rate and aerobic P/PHA Uptake (mg P/mg PHA) enabled a good fit of the measured data.

   iii. Dynamic simulation of the MBNR (Biological) system validated the suitability of the modified *BioWin^{TM} General Model*. This was accomplished by successful fitting of the predicted solids production, nitrification, denitrification and EBPR data with the measured data.

   iv. The *BioWin^{TM} General Model* calibrated for the MBNR (Biological) system along with the *Chemical Phosphorus Precipitation Model* was applied on the

MBNR (Chemical) system to simulate behaviour with alum dosing. The predicted suspended solids production, ammonium and nitrate concentrations in all the reactors as well as permeate, were in good agreement with measured data.

v. The combined *BioWin*^{TM} *General Model* and *Chemical Phosphorus Precipitation Model* predicted failure of EBPR at a relatively low alum dosing concentration (20 mg/L). This was not supported by the measured data, which indicated that EBPR failure started only at 80 mg/L of alum dosing in the MBNR (Chemical) system. Also, the model predicted that greater than 97 percent PO_4-P removal could be accomplished with 20 mg/L of alum dosing, while in reality, 80 mg/L of alum was required.

vi. The contrasting observations regarding alum requirement was attributed to the model set up. The model assumed that ideal mixing conditions enabled stoichiometric removal rates and predicted very low effluent PO_4-P concentrations. In reality, vigorous aeration in the membrane tank may have created non-ideal mixing conditions, resulting in decreased metal salt-phosphorus complexation. Therefore, a greater requirement of alum was identified to achieve a similar level of removal.

vii. Dynamic modeling of the MBNR (Chemical) system indicated that the selected modified Bardenpho configuration, along with external methanol and alum dosing has the capability to accomplish LoT nutrient removal goals. This is in agreement with indications from the measured data of the continuous flow system.

4.

i. The direct DON measurement method, batch anion exchange resin adsorption followed by persulfate digestion, was validated successfully by a quality control procedure in the present study.

ii. The measured permeate DON concentration range was comparable in the parallel systems with 0.16-2.60 mg/L for the MBNR (Biological) system and 0.11-2.08 mg/L for the MBNR (Chemical) system between operating days

403 and 464. Contrary to some studies in the literature, a high alum dosage did not enable greater DON removal in the MBNR (Chemical) system.

iii. The DON contribution to permeate total nitrogen varied from 7 percent to 82 percent in the MBNR (Biological) system and from 12 percent to 96 percent in the MBNR (Chemical) system when permeate TN concentrations were less than 3 mg/L. It signified a requirement for consistently high level nitrification and denitrification performance in the parallel MBNR systems.

iv. There was no specific DON production and/or utilization trend in bioreactors of the parallel MBNR systems.

9.2 Engineering Significance

MBR technology has successfully been combined with nutrient removal in recent years. In the current research, the author evaluated its suitability in accomplishing LoT level removal, i.e. $\leq$ 3 mg/L TN and $\leq$ 0.1 mg/L TP in the effluent. An MBR system, combined with modified Bardenpho configuration, demonstrated that the above stated goal could be realized. However, it was also observed that external dosing of methanol and alum had a significant role to play in enhancing nitrogen and phosphorus removal respectively. In the current research project, the author calculated stoichiometric requirements of both compounds, which could be very helpful for design of future WWTPs targeting LoT nutrient removal.

A key objective of the present study was to evaluate the influence of alum on simultaneously occurring EBPR. The author concluded that the relationship between the two P removal mechanisms was dynamic and was alum dose dependent. When free soluble PO_4-P was not available for both mechanisms, due to the fast reaction rates of alum phosphorus complexation, EBPR was inhibited. This is an important conclusion, as alum dosing will definitely be a provision in EBPR systems targeting LoT P removal. Engineers and operators will have to be very careful in alum dosing calculations, so that it only aids as a polishing step to EBPR, rather than competing.

Alum dosing did not inhibit nitrification and denitrification generally in the present study. Nonetheless, for a brief period, elevated ammonium concentrations were observed when alum dosing was increased to 80 mg/L in the membrane tank of the MBNR (Chemical) system. It was speculated that low influent alkalinity, combined with alum-induced low alkalinity conditions in mixed liquor caused temporary failure of nitrification. This is an important lesson for engineers and operators, specifically dealing with WWTPs that have a history of low alkalinity influent wastewater.

Activated sludge modeling was found to be a reliable tool in simulating suspended solids production, nitrification, denitrification and EBPR mechanisms of the parallel MBNR systems. This finding proves that simulation could be handy for design of future WWTPs targeting LoT nutrient levels. The one weakness of the model, however, was a prediction of the relationship between simultaneous chemical P removal and EBPR. A similar observation has been made before by another research group. Hence, the learning point is that WWTP modelers should evaluate results of such a scenario carefully.

The direct DON measurement method developed in the present study will help in analysing greater number of samples while ensuring data reliability. As DON is going to be a major fraction in WWTPs targeting effluent TN $\leq$ 3 mg/L, there is a significant interest in direct and reliable measurement. Indirect methods have been proven to be unreliable. Also, researchers are very interested in understanding DON production and utilization profile in bioreactors. For the above stated tasks, this novel direct DON measurement method could be a very useful tool.

9.3 Recommendations for Future Research

The present study encompassed continuous operation of MBNR process, batch experiments for kinetic and stoichiometry evaluation, modeling and measurement method development. However, due to time constraint, some interesting research ideas could not be addressed. These are outlined below.

Alum dosing will be a definite requirement in LoT P removal. However, more focus should be given on how to optimize dosing requirements. The parameters that could influence dosing are contact time, mixing speed and location of mixing. Optimizing all these parameters in a bioreactor may not be feasible. An area of research exploration could be a dedicated tank for alum and mixed liquor flocculation for enhanced P removal.

The LoT level TN and TP discharge requirements means that DON and DOP are going to be the dominant fractions. However, currently, there is a paucity of information on group of compounds. First, classification of the organic group of compounds that constitute DON and DOP has not been understood completely. Second, their characterization, i.e. whether biodegradable or refractory, in effluent of secondary wastewater treatment plants, has not been defined reliably. Third, the process/operational parameters that impact DON and DOP production/utilization in a wastewater system have to be studied in detail by researchers.

The current study found that the ASM model did not predict simultaneous P removal mechanisms accurately. There is currently a knowledge gap as far as identifying the model parameters that could be modified, to enable a good fit between predicted and measured data. However, it has to be mentioned that selection of the parameters has to be supported by experimental work findings.

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Appendices

Appendix A: Sizing of Reactors of Parallel MBNR Systems

A.1 Design Operating Parameters

Table A.1 Design operating parameters of the bench-scale MBNR systems

Parameter	Value
Net flow (Q)	33.45 L/day
Temperature	20 ^{0}C
Membrane module	ZW-1, submersible
Membrane surface area	0.092903 m^2 (1 ft^2)
Membrane flux	15 L/m^{2}·hr
Net flow (Q)	15 L/m^{2}·hr * 0.092903 m^2 = 1.393545 l/hr = **33.45 L/day**
Total hydraulic retention time (HRT)	13.83 h
Anaerobic HRT	1.5 h
Pre-anoxic HRT	3 h
Aerobic HRT	6 h
Post-anoxic HRT	3 h
Membrane HRT	1/3 h

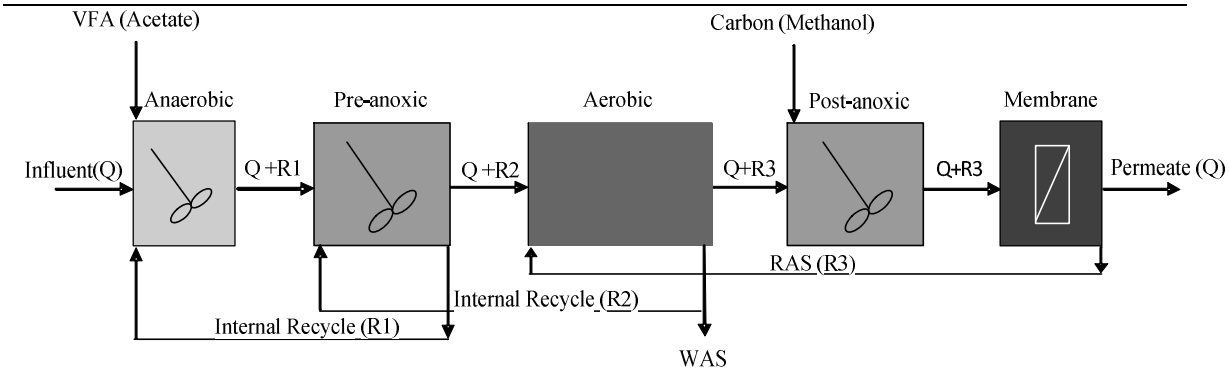

Figure A.1 MBNR (Biological) system

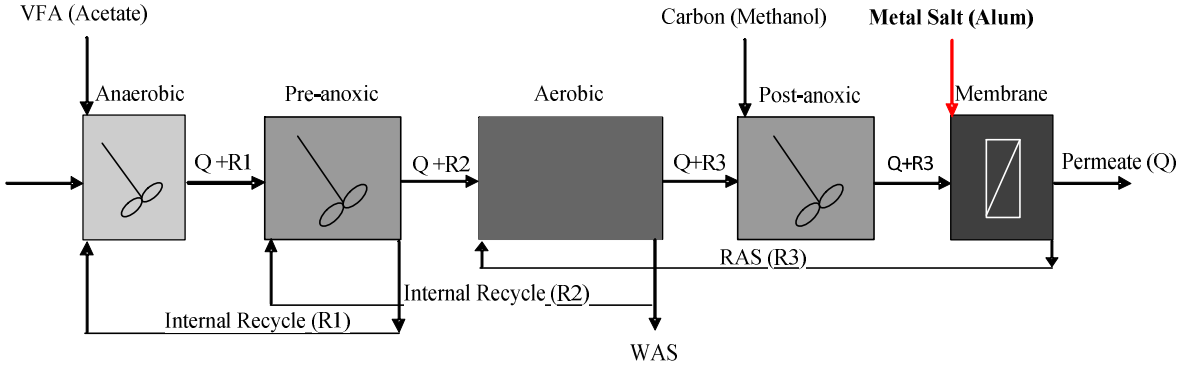

Figure A.2 MBNR (Chemical) system

Table A.2 Design reactor volumes of the bench-scale MBNR systems

	Flow (l/hr)	HRT (hrs)	Q	Volume	Design Reactor Volume
	1.393545	0.5	1	0.696773	
Anaerobic	1.393545	1	1	1.393545	**Final volume = 2.5 L**
	1.393545	**1.5**	**1**	**2.0903**	
	1.393545	1	1	1.393545	
Pre-anoxic	1.393545	2	1	2.78709	**Final volume = 5.6 L**
	1.393545	**3**	**1**	**4.180635**	
	1.393545	4	1	5.57418	
	1.393545	4	1	5.57418	
	1.393545	**6**	**1**	**8.36127**	
Aerobic	1.393545	8	1	11.14836	**Final volume = 14.65 L**
	1.393545	10	1	13.93545	
	1.393545	12	1	16.72254	
	1.393545	2	1	2.78709	
Post-anoxic	1.393545	**3**	**1**	**4.180635**	**Final volume = 5.6 L**
	1.393545	4	1	5.57418	
	1.393545	**1/3**	**1**	**0.466**	
Membrane	1.393545	0.5	1	0.696773	**Final volume = 1.5 L**
	1.393545	1	1	1.393545	

Note:

- Valves at different levels and locations to ensure flexibility of operation
- To maintain real HRT, recycle flow entered at the start of the reactor followed by baffles (to avoid dead zones)

A.2 Sizing of Reactors

A.2.1 Anaerobic Reactor

Quantity = 2

Cylindrical shape for homogeneous mixing

Reactor Design:

Volume = V = 2.5 L

Diameter = D = 0.1016 m = 10. 16 cm = 4 inches

Height = H = 0.308 m = 30.8 cm = 12 inches

For HRT = 0.5 hr, valve was at 0.086 m = 8.6 cm = 3.39 inches

For HRT = 1 hrs, valve was at 0.172m = 17.2 cm = 6.77 inches

For HRT = 1.5 hrs, valve was at 0.258 m = 25.8 cm = 10.16 inches

Overflow valve at = 0.28m = 28.0 cm = 11 inches

Baffle Design:

Total baffle width is 3 inches (1 inch left in all sides)

Distance between baffle and tank bottom was equal or less than 1.5 inches.

A.2.2 Pre-anoxic Reactor

Quantity = 2

Cylindrical shape for homogeneous mixing

Reactor Design:

Volume = V = 5. 6 L

Diameter = D = 0.1524 m = 15. 24 cm = 6 inches

Height = H = 0.3048 m = 30.48 cm= 12 inches

For HRT = 1 hr, valve was at 0.0764 m = 7.64 cm = 3.01 inches

For HRT = 2 hrs, valve was at 0.153m = 15.3 cm = 6.02 inches

For HRT = 3 hrs, valve was at 0.23m = 23 cm = 9.03 inches

Overflow valve at = .268m = 26.8 cm = 10.535 inches

Baffle Design:

Total baffle width is 4 inches (1 inch left in all sides)

Distance between baffle and tank bottom was equal or less than 1.5 inches.

A.2.3 Post-anoxic Reactor

Quantity = 2

Cylindrical shape for homogeneous mixing

Reactor Design:

Volume = V = 5. 6 L

Diameter = D = 0.1524 m = 15. 24 cm = 6 inches

Height = H = 0.3048 m = 30.48 cm = 12 inches

For HRT = 1 hr, valve was at 0.0764 m = 7.64 cm = 3.01 inches

For HRT = 2 hrs, valve was at 0.153m = 15.3 cm = 6.02 inches

For HRT = 3 hrs, valve was at 0.23m = 23 cm = 9.03 inches

Overflow valve at = .268m = 26.8 cm = 10.535 inches

Baffle Design:

Total baffle width is 4 inches (1 inch left in all sides)

Distance between baffle and tank bottom was equal or less than 1.5 inches.

A.2.3 Aerobic Reactor

Quantity = 2

Cylindrical shape for homogeneous mixing

Diameter = D = 0.2032 m = 20. 32 cm = 8 inches

Height = H = 18 inches = 0.4572 m = 45.72 cm

Reactor Design:

Volume = V = 14. 65 L

For HRT = 4 hr, valve was at 0.172 m = 17.2 cm = 6.77 inches

For HRT = 6 hrs, valve was at 0.258m = 25.8 cm = 10.16 inches

For HRT = 8 hrs, valve was at 0.344m = 34.4 cm = 13.54 inches

Overflow valve at = 0.43m = 43 cm = 16.93 inches

Baffle Design:

Total baffle width is 6 inches (1 inch left in each side)

Distance between baffle and tank bottom was equal or less than 1.5 inches.

Distance between two baffles was 6 inches.

A.2.3 Membrane Tank

Quantity = 2

Rectangular shape.

Height = H = 0.30 m = 30 cm = 11.81 inches

Width = W = 0.08 m = 8 cm = 3.15 inches

Length = L = 0.12 m = 12 cm = 4.7 inches.

A.3 Wooden Stairs Dimensions

Forward flow of the wastewater was achieved by gravity. For that reason, reactors were placed in a purposely built wooden stair as shown below. All the units are in inches.

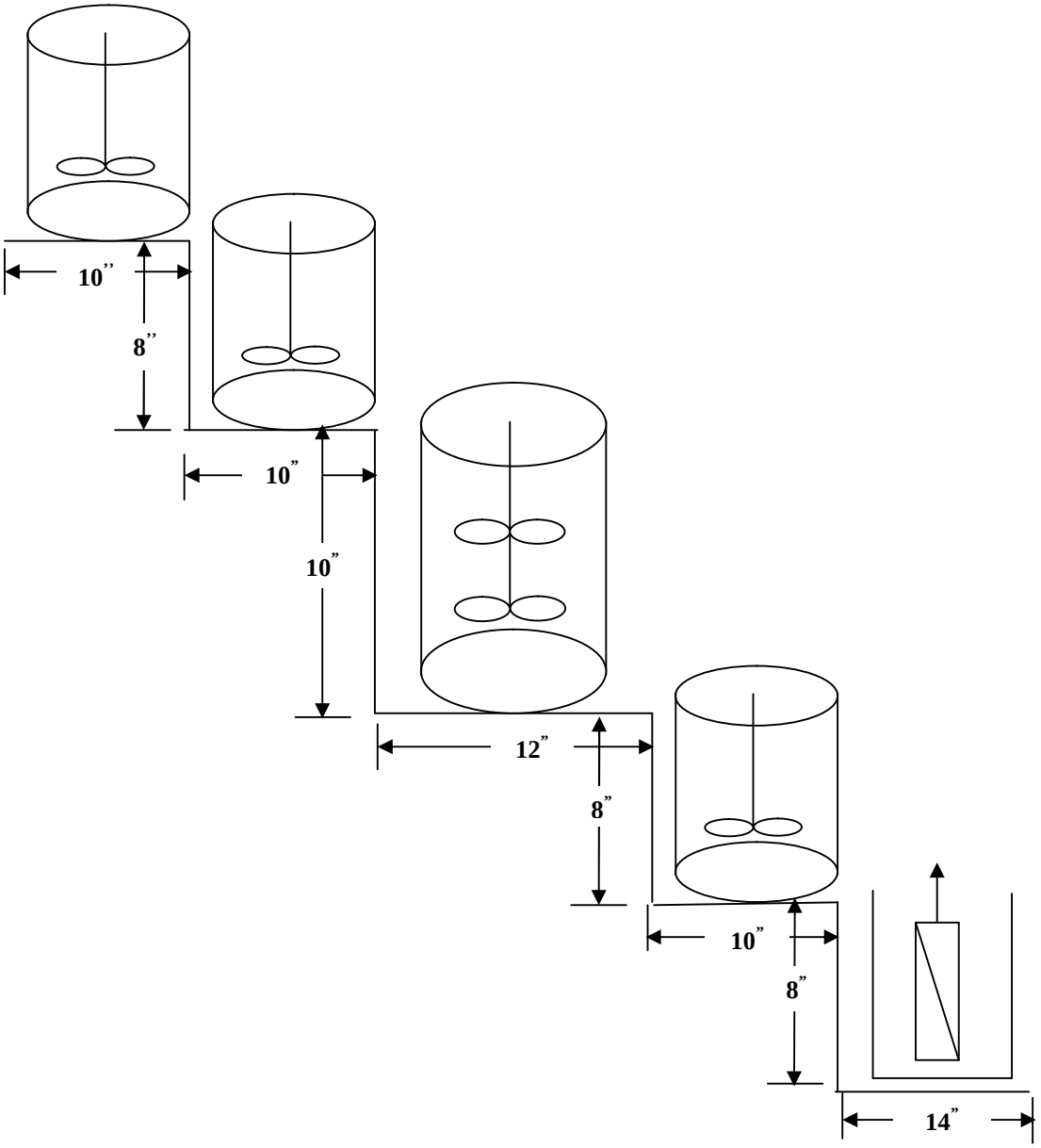

Figure A.3 Wooden stairs dimensions

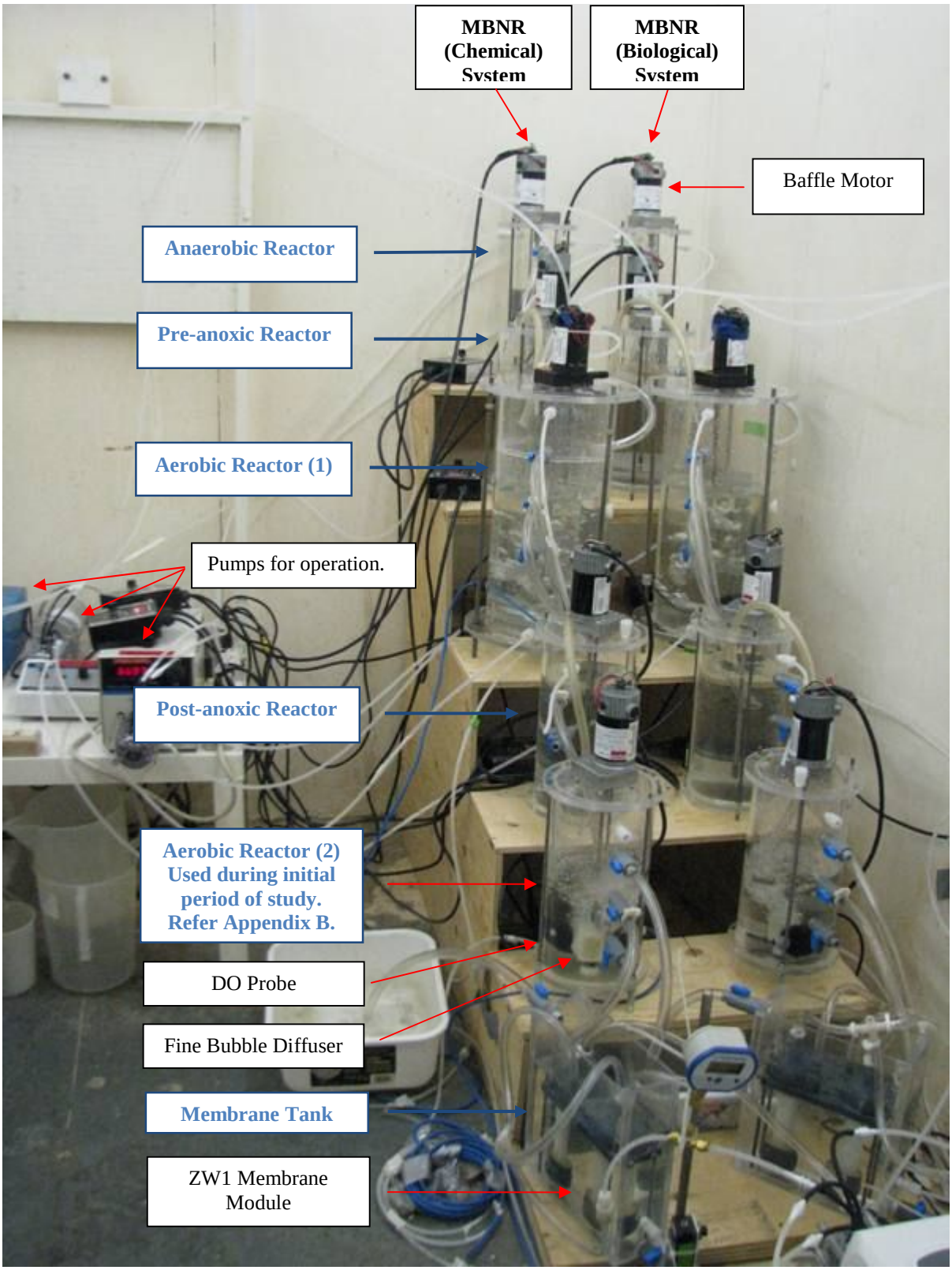

Figure A.4 Lab scale set-up with two parallel MBNR systems

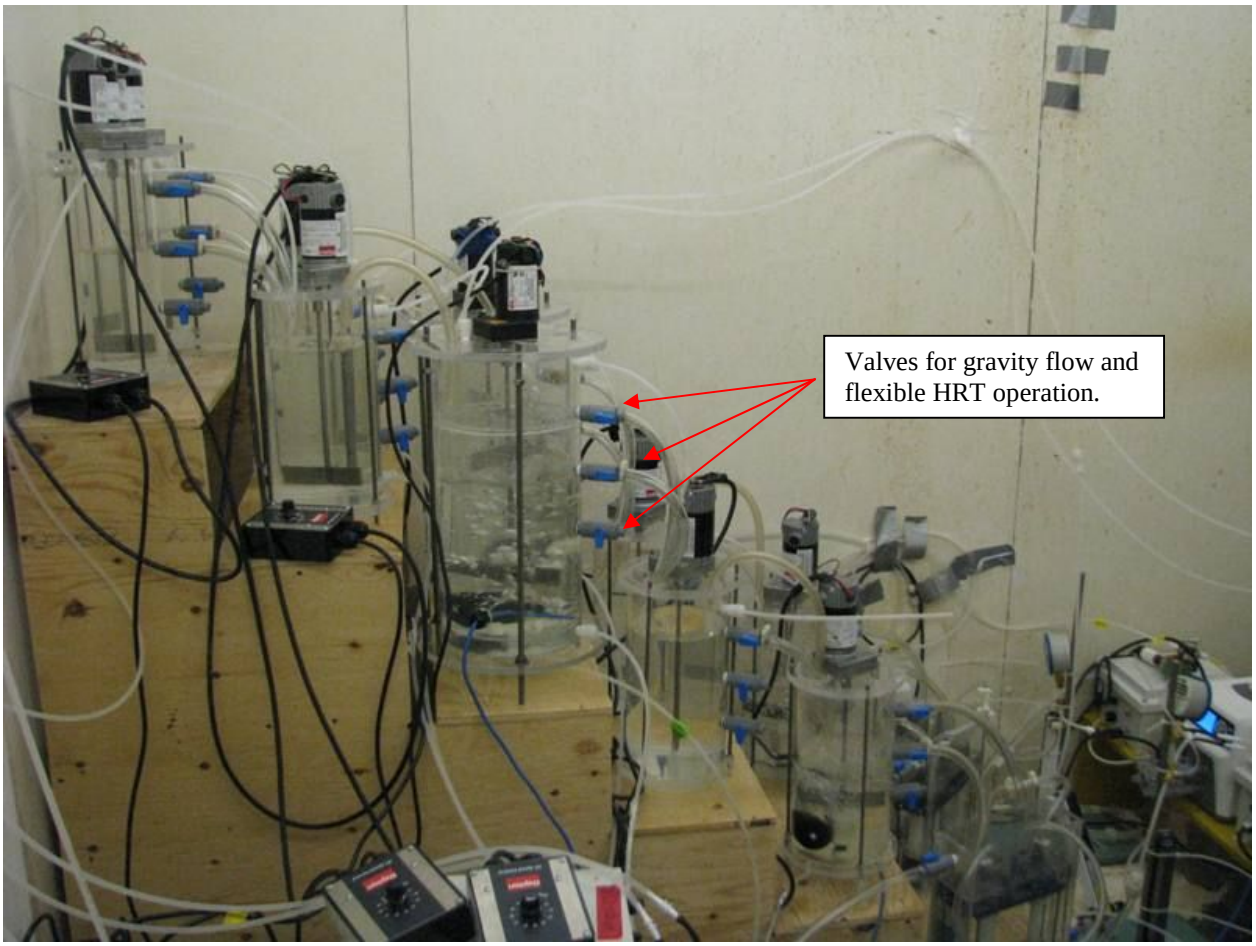

Figure A.5 Valves in lab scale parallel MBNR systems for flexible HRT operation

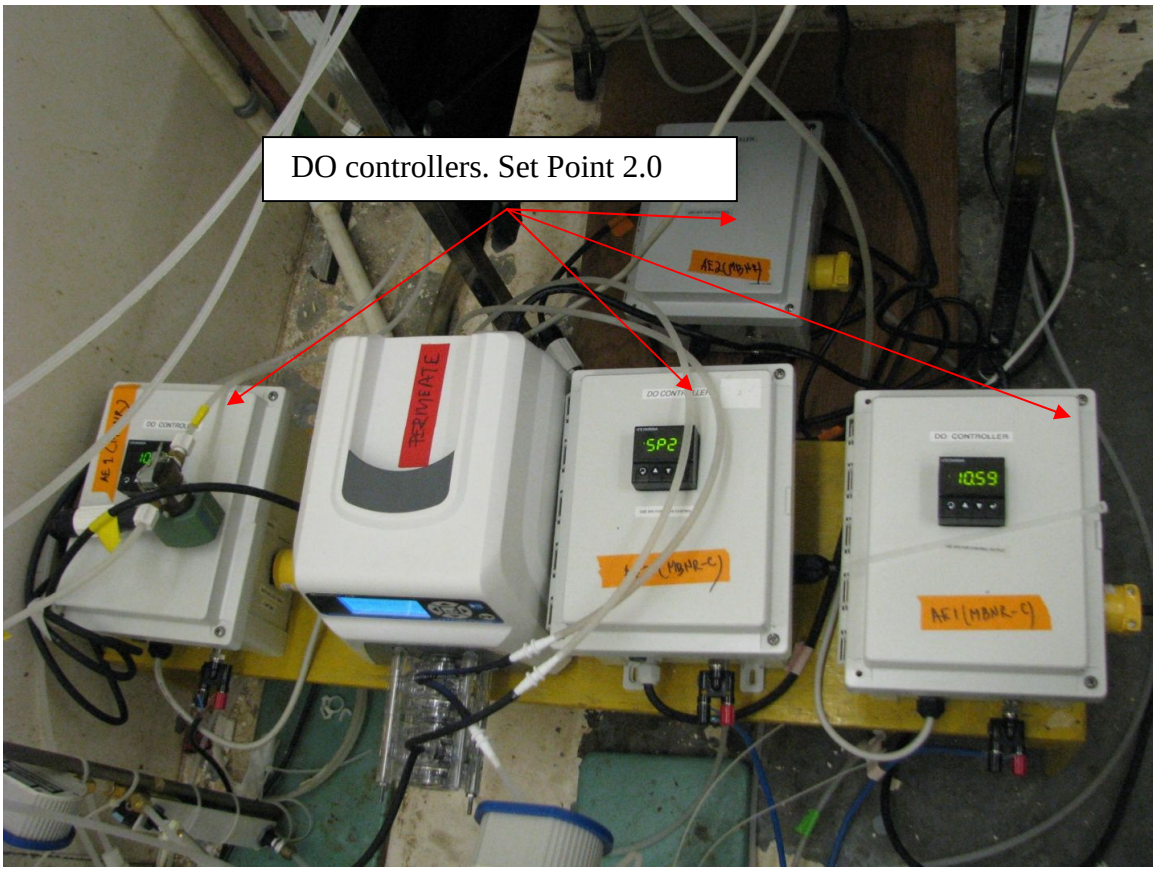

Figure A.6 DO controllers used in the experimental study

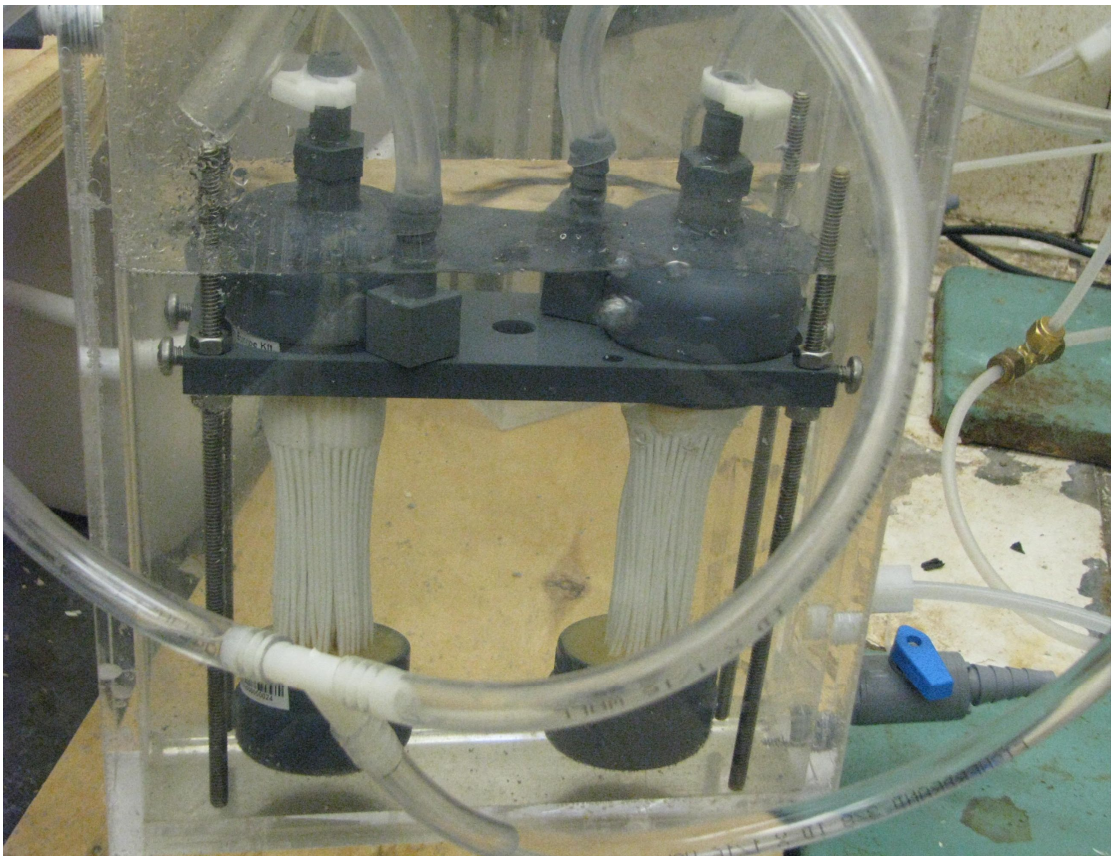

Figure A.7 ZW1 membrane modules used in the present study

Appendix B: Batch Test Data

B.1 Batch Test Number 1

B.1.1 MBNR (Biological) System

Table B.1 Data for batch test number 1 with MBNR (Biological) system mixed liquor

Date : 8/24/2010	Time	Hours	ORP (mV)	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)
2 hrs for denitrification	11:30 AM	0		9.25	2.75	
Target Do = 2-3 mg/L	12:30 PM	1		8.24	2.54	
100 mg/L of acetate before anaerobic phase	1:30 PM	0	78	7.18	3.84	73.1
		0.25	40	5.83	7.13	38.7
		0.5	25	0.503	14	18.6
		0.75	24	0.159	16.9	15.8
		1	19	0.263	22.7	12.6
		1.25	0	0.476	23.3	9.4
		1.5	-36	0.257	25.5	5.8
		1.75	-68	0.259	25.7	7.4
		2	-96	0.246	26.8	0.7
		2.25	-45	0.415	20.3	
		2.5	40	0.529	18.3	7.1
		2.75	50	0.374	16.9	
		3	55	0.396	17.6	0.0
		3.25	59	0.674	16.7	
		3.5	61	0.457	14.2	0.0
		3.75	62	0.549	14.1	
		4	64	0.626	15.8	0.5
		4.25	65	0.519	13.6	
		4.5	66	0.805	13.6	0.6
		4.75	67	0.589	14.9	
	6:30 PM	5	68	0.624	12.2	

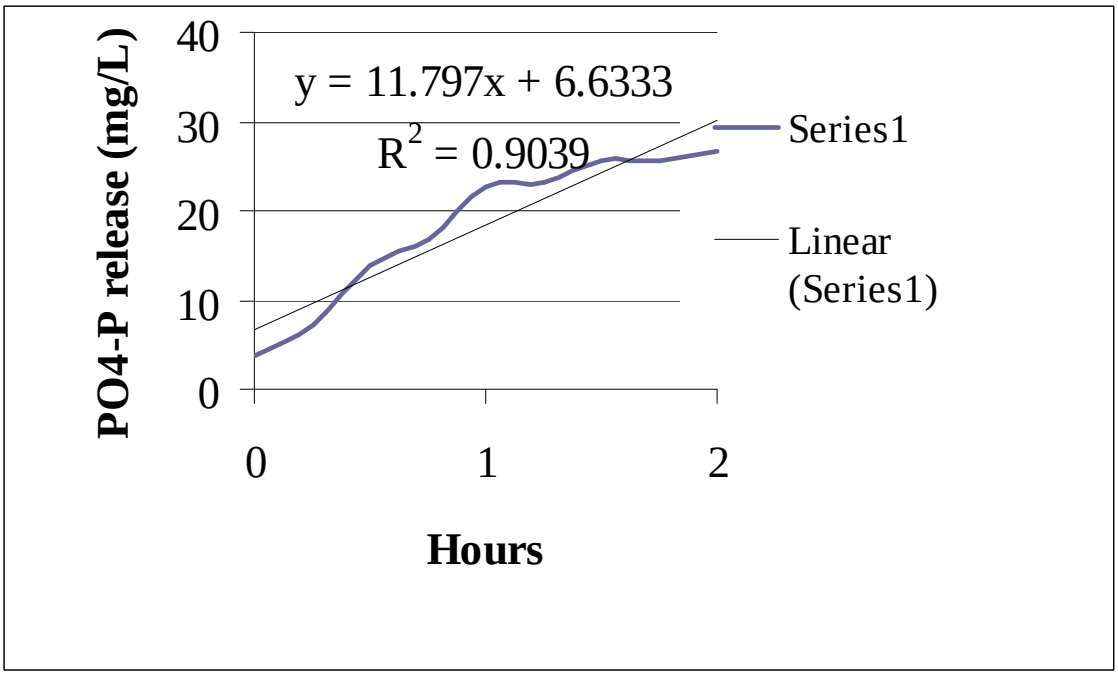

Figure B.1 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 1 (MBNR (Biological) system mixed liquor)

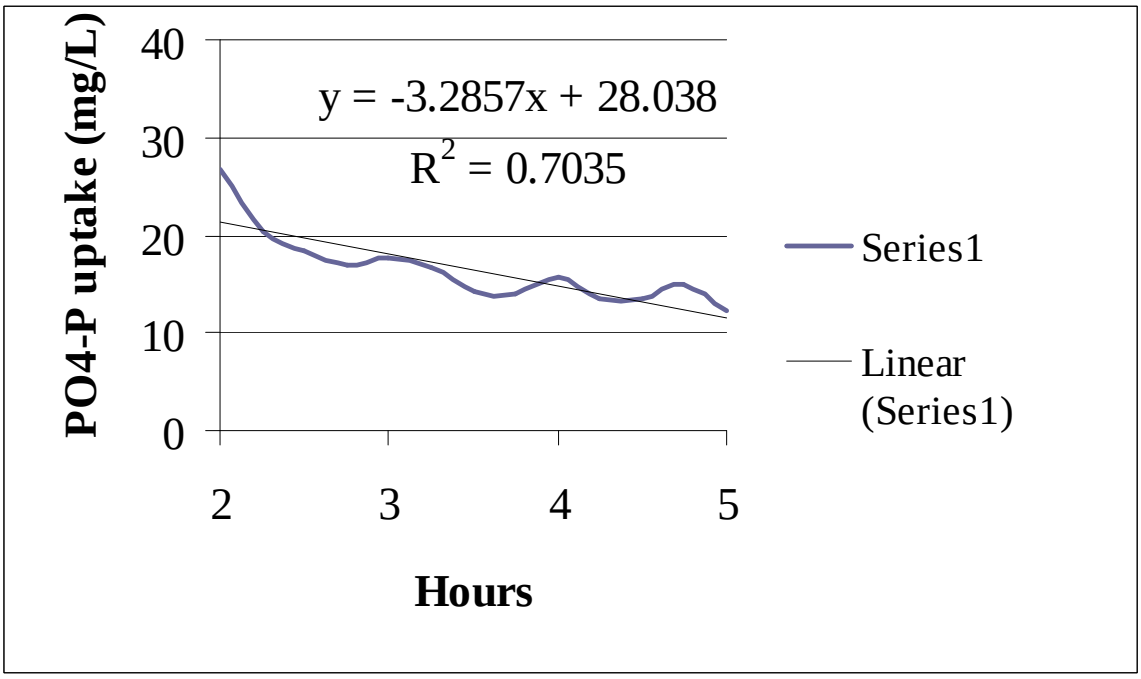

Figure B.2 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 1 (MBNR (Biological) system mixed liquor)

B.1.2 MBNR (Chemical) System

Table B.2 Data for batch test number 1 with MBNR (Chemical) system mixed liquor

Date : 8/26/2010	Time	Hours	ORP (mV)	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)
2 hrs for denitrification	9:30 AM	0		13.9	8.71	
Target DO = 2-3 mg/L	10:30 PM	1		11.8	6.29	
100 mg/L of acetate before anaerobic phase	11:30 AM	0	106	8.81	6.54	100
		0.25	84	5.05	7.23	42.7
		0.5	75	0.0847	9.81	13.8
		0.75	60	0.107	11.7	12.5
		1	11	0.219	11.5	11.6
		1.25	-36	0.00556	11.9	10.7
		1.5	-77	0	13.3	13.5
		1.75	-88	0.136	13.3	11.5
		2	-104	0.223	13.6	15.0
		2.25	31	0.338	11.7	0.8
		2.5	62	0.326	11.7	0.8
		2.75	73	0.322	10.5	0.8
		3	76	0.251	9.87	1.4
		3.25	83	0.306	9.54	1.4
		3.5	87	0.364	8.66	0.8
		3.75	87	0.252	8.56	0.8
		4	87	0.16	7.73	1.2
		4.25	88	0.257	6.87	1.2
		4.5	91	0.159	7.35	0.9
		4.75	90	0.0713	6.94	0.9
	6:30 PM	5	90	0.0912	5.83	0.9

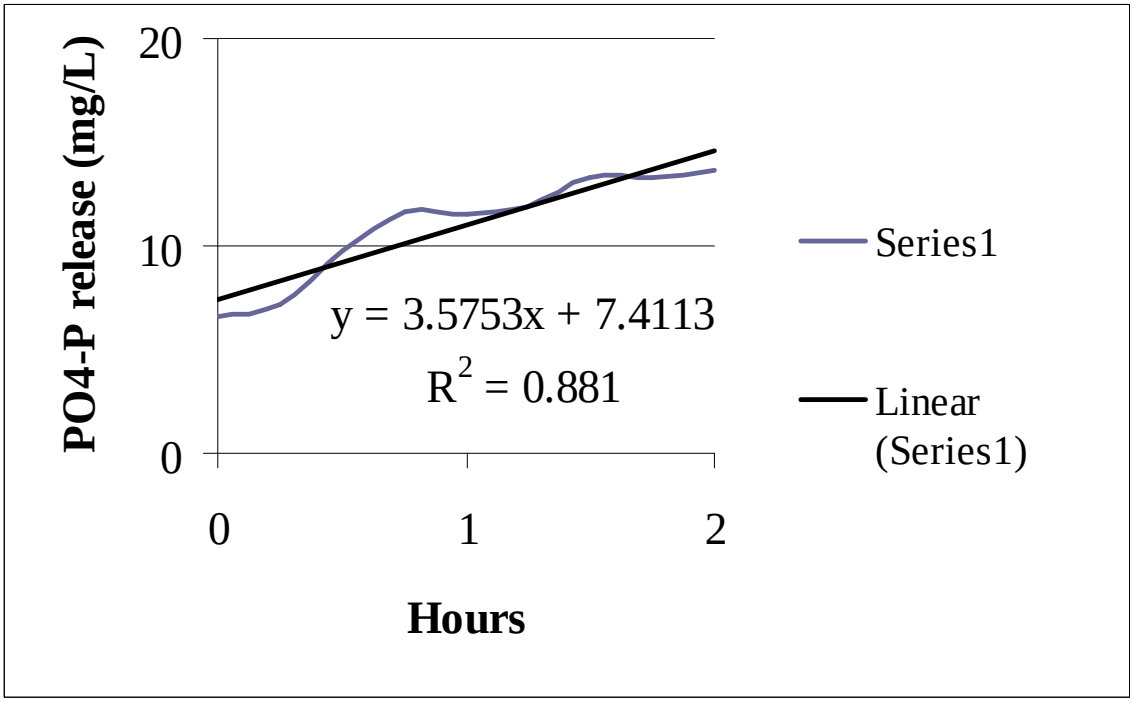

Figure B.3 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 1 (MBNR (Chemical) system mixed liquor)

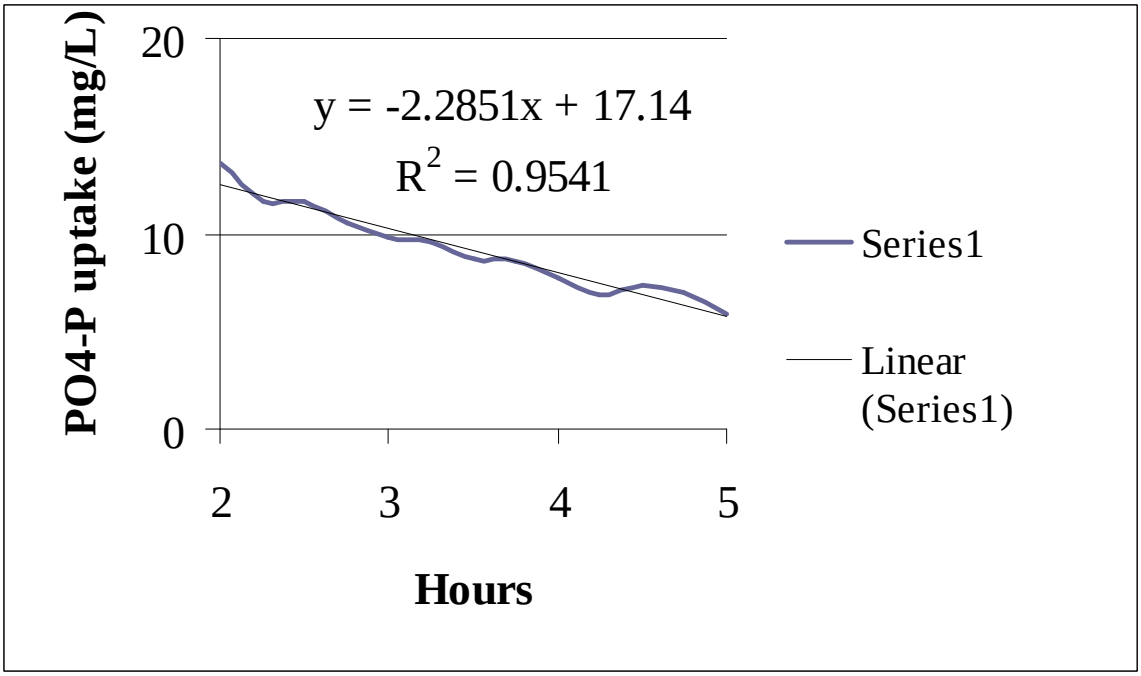

Figure B.4 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 1 (MBNR (Chemical) system mixed liquor)

B.2 Batch Test Number 2

B.2.1 MBNR (Biological) System

Table B.3 Data for batch test number 2 with MBNR (Biological) system mixed liquor

Date : 9/9/2010	Time	Hours	ORP (mV)	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)
2 hrs for denitrification	9:00 AM	0		7.76	1.49	
Target Do = 2-3 mg/L	10:00 AM	1		2.87	0.448	
100 mg/L of acetate before anaerobic phase	11:00 AM	0	120	2.41	4.54	100
		0.25	103	0.333	10.5	42.47
		0.5	77	0.159	27.8	20.53
		0.75	53	0.166	34.8	14.97
		1	27	0.182	39.1	8.29
		1.25	10	0.157	40.6	10.27
		1.5	-2	0.143	41.7	7.36
		1.75	-10	0.32	44.2	6.92
		2	-20	0.386	44.6	2.45
		2.25	46	0.261	38	
		2.5	61	0.265	33.2	3.85
		2.75	69	0.228	29	
		3	77	0.226	22.5	1.08
		3.25	85	0.196	17.7	
		3.5	87	0.118	15.7	4.33
		3.75	86	0.246	15.7	
		4	87	0.171	11.4	2.41
		4.25	93	0.224	11.1	
		4.5	91	0.207	7.33	2.91
		4.75	92	0.304	8.99	
	4:00 PM	5	93	0.23	5.8	0.31

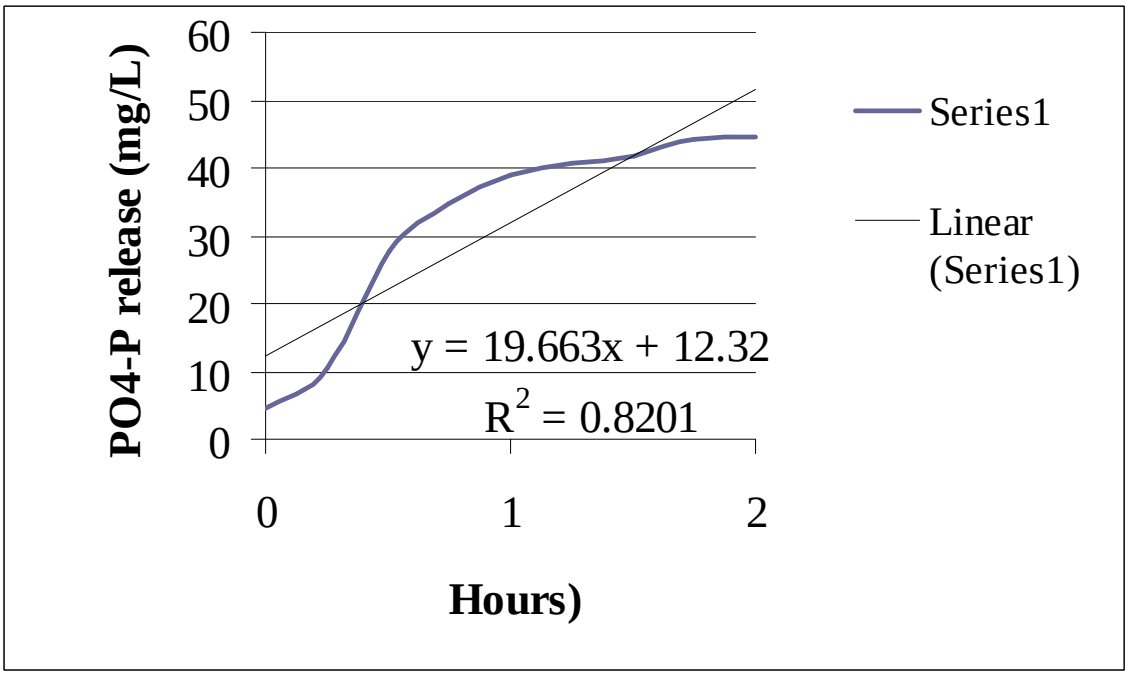

Figure B.5 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 2 (MBNR (Biological) system mixed liquor)

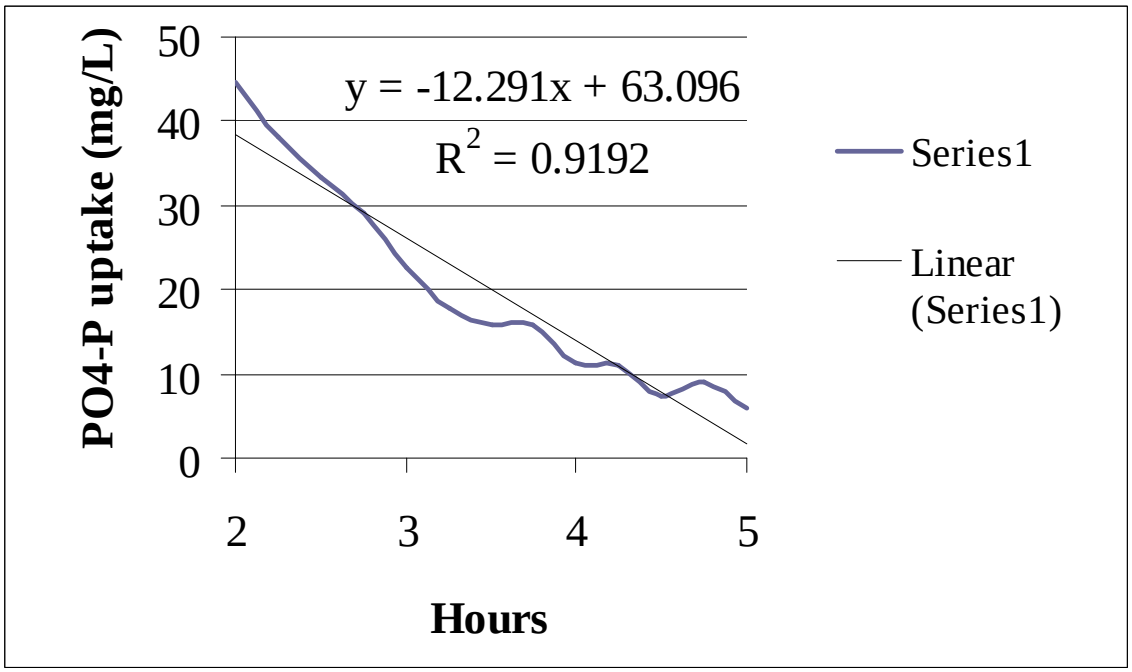

Figure B.6 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 2 (MBNR (Biological) system mixed liquor)

B.2.2 MBNR (Chemical) System

Table B.4 Data for batch test number 2 with MBNR (Chemical) system mixed liquor

Date : 14/9/2010	Time	Hours	ORP (mV)	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)
2 hrs for denitrification	11:00 AM	0		10.2	2.38	
Target Do = 2-3 mg/L	12:00 PM	1		3.9	2.82	
100 mg/L of acetate before anaerobic phase	1:00 PM	0	58	3.84	2.9	100
		0.25	47	0.164	11	33.19
		0.5	-23	0.125	14.6	30.13
		0.75	-88	0.148	19.5	33.76
		1	-127	0.109	23	41.09
		1.25	-149	0.0933	25.1	51.00
		1.5	-160	0.127	25.5	2.39
		1.75	-167	0.107	28	2.86
		2	-167	0.129	28.4	2.86
		2.25	-11	0.507	25.8	26.47
		2.5	13	0.418	20.5	27.58
		2.75	26	0.462	19.3	27.58
		3	38	0.518	14.4	26.75
		3.25	44	0.412	14	26.75
		3.5	48	0.376	11.1	1.60
		3.75	53	0.412	11.8	1.6
		4	59	0.837	8.62	2.60
		4.25	61	0.525	8.43	2.6
		4.5	63	0.491	6.61	0.42
		4.75	67	0.451	8.64	0.42
	6:00 PM	5	68	0.472	5.5	1.64

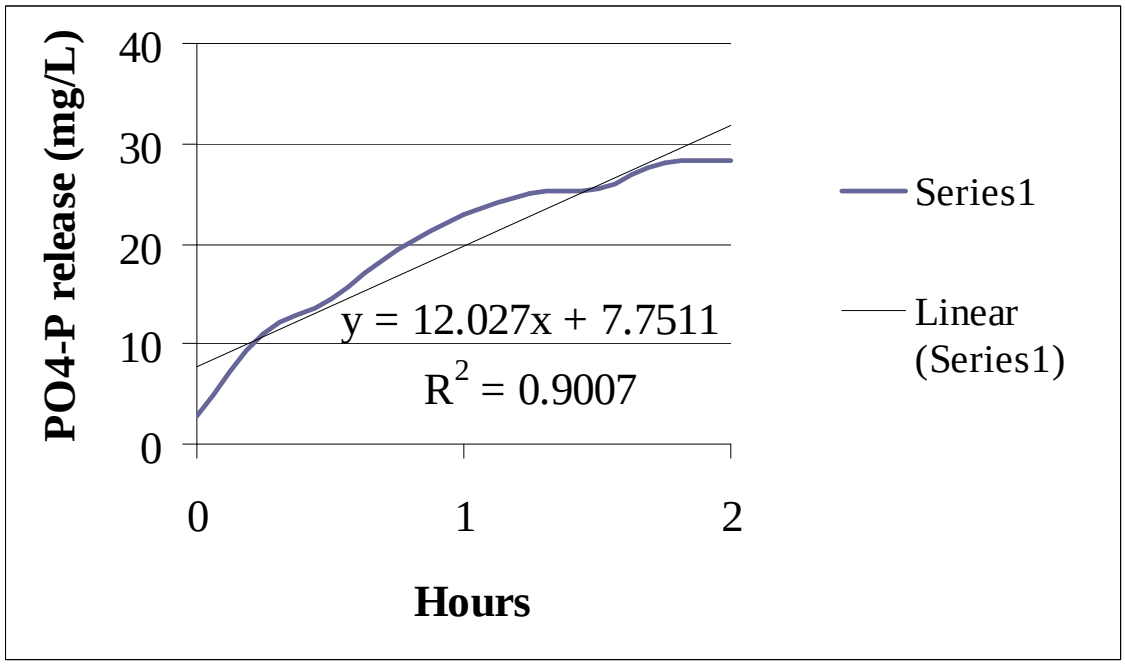

Figure B.7 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 2 (MBNR (Chemical) system mixed liquor)

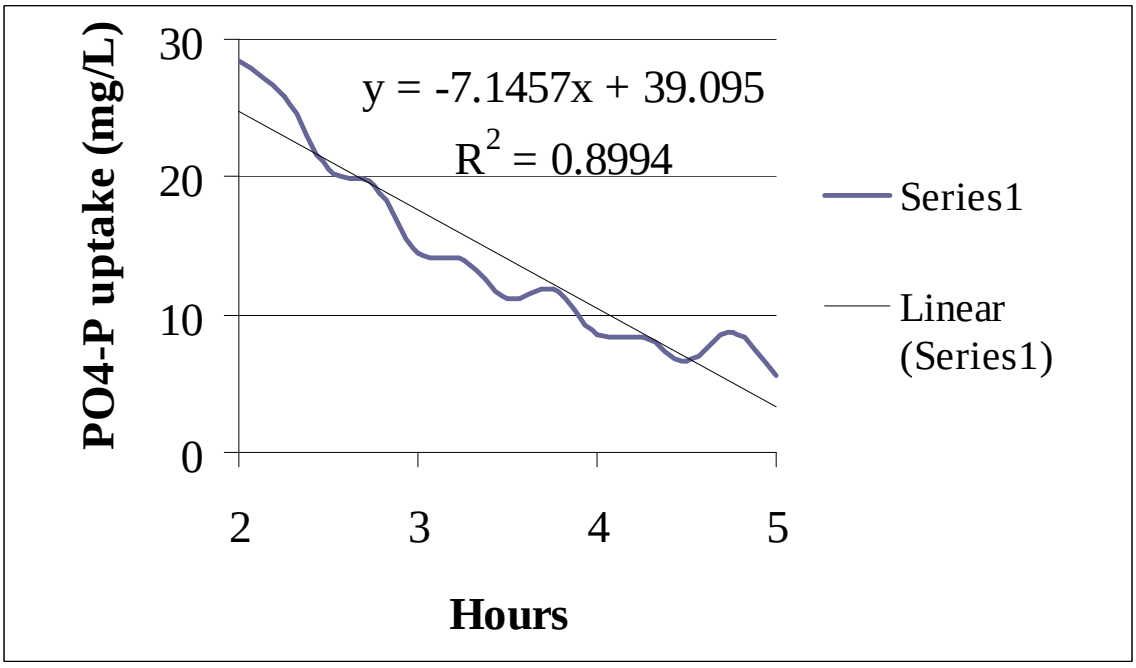

Figure B.8 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 2 (MBNR (Chemical) system mixed liquor)

B.3 Batch Test Number 3

B.3.1 MBNR (Biological) System

Table B.5 Data for batch test number 3 with MBNR (Biological) system mixed liquor

Date : 21/9/2010	Time	Hours	ORP (mV)	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)
2 hrs for denitrification	11:00 AM	0		7.57	1.45	
Target DO = 2-3 mg/L	12:00 PM	1		6.37	1.81	
100 mg/L of acetate before anaerobic phase	1:00 PM	0	75	3.71	7.17	
						100
		0.25	72	0.283	14.5	24.6
		0.5	8	0.0811	21.3	
						12.4
		0.75	-64	0.193	28.2	15.7
		1	-104	0.111	28.7	13.7
		1.25	-126	0.23	21.3	12.6
		1.5	-146	0.177	37	4.7
		1.75	-153	0.198	40.7	2.7
		2	-156	0.162	38.4	3.7
		2.25	-8	0.427	33.2	
		2.5	17	0.24	24.8	1.7
		2.75	29	0.32	19.9	
		3	37	0.263	14	1.9
		3.25	47	0.425	12.3	
		3.5	55	0.215	7.73	3.0
		3.75	58	0.272	7.75	
		4	60	0.287	3.28	2.0
		4.25	64	0.382	5.03	
		4.5	72	0.174	1.27	3.3
		4.75	75	0.272	5.01	
	6:00 PM	5	75	0.199	1.13	3.1

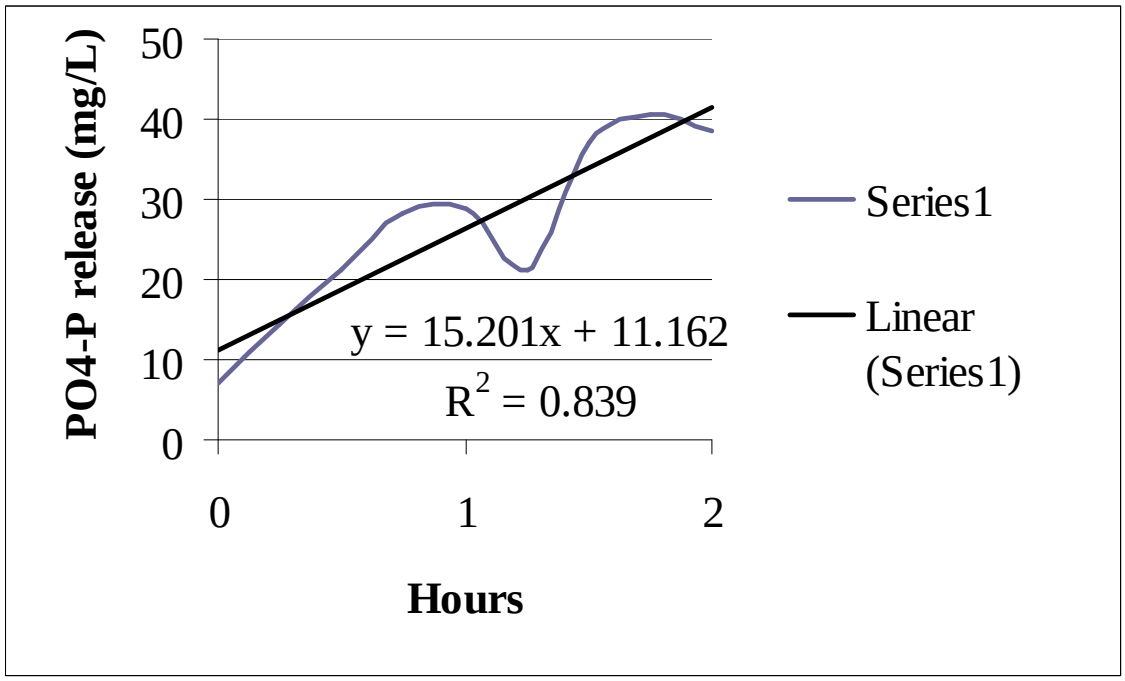

Figure B.9 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 3 (MBNR (Biological) system mixed liquor)

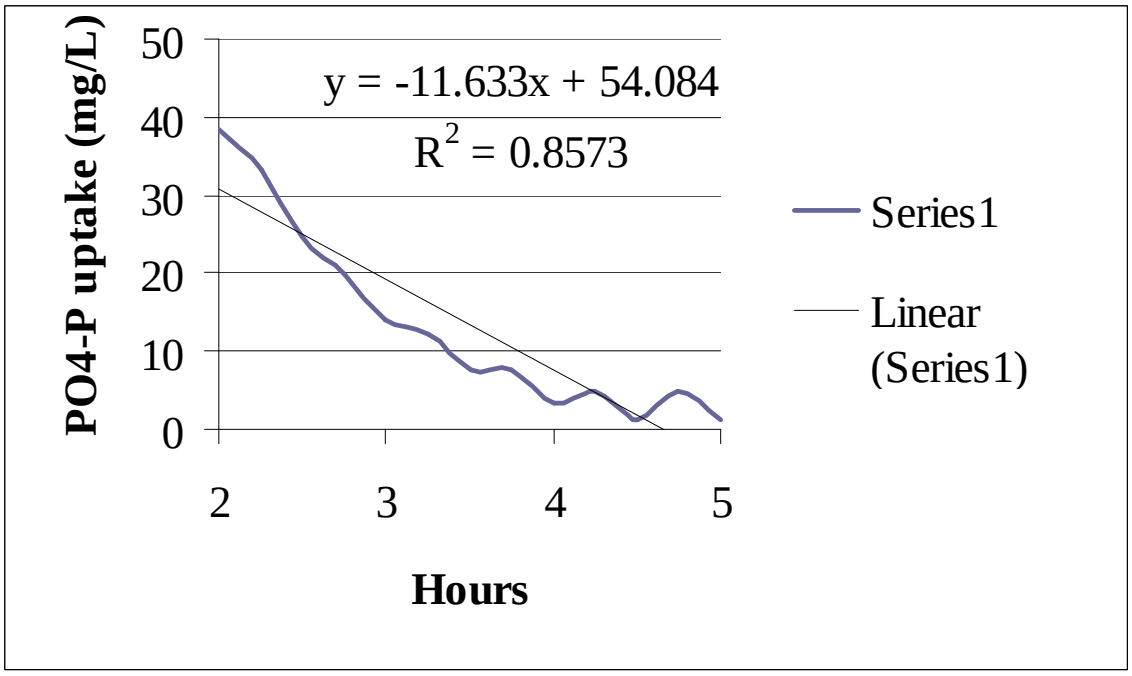

Figure B.10 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 3 (MBNR (Biological) system mixed liquor)

B.3.2 MBNR (Chemical) System

Table B.6 Data for batch test number 3 with MBNR (Chemical) system mixed liquor

Date : 29/9/2010	Time	Hours	ORP (mV)	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)
2 hrs for denitrification	11:45 AM	0		10.2	2.38	
Target DO = 2-3 mg/L	12:45 PM	1		3.9	2.82	
100 mg/L of acetate before anaerobic phase	1:45 PM	0	58	3.84	2.9	100
		0.25	47	0.164	11	33.19
		0.5	-23	0.125	14.6	30.13
		0.75	-88	0.148	19.5	33.76
		1	-127	0.109	23	41.09
		1.25	-149	0.0933	25.1	51.00
		1.5	-160	0.127	25.5	2.39
		1.75	-167	0.107	28	2.86
		2	-167	0.129	28.4	2.86
		2.25	-11	0.507	25.8	26.47
		2.5	13	0.418	20.5	27.58
		2.75	26	0.462	19.3	27.58
		3	38	0.518	14.4	26.75
		3.25	44	0.412	14	26.75
		3.5	48	0.376	11.1	1.60
		3.75	53	0.412	11.8	1.6
		4	59	0.837	8.62	2.60
		4.25	61	0.525	8.43	2.6
		4.5	63	0.491	6.61	0.42
		4.75	67	0.451	8.64	0.42
	6:45 PM	5	68	0.472	5.5	1.64

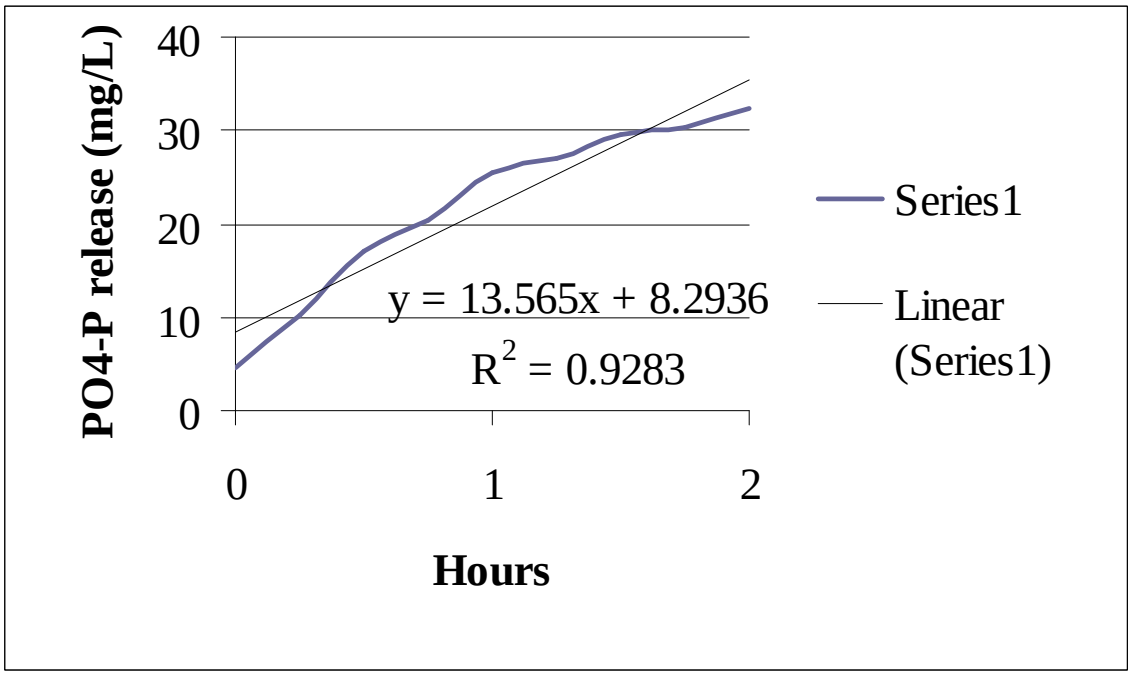

Figure B.11 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 3 (MBNR (Chemical) system mixed liquor)

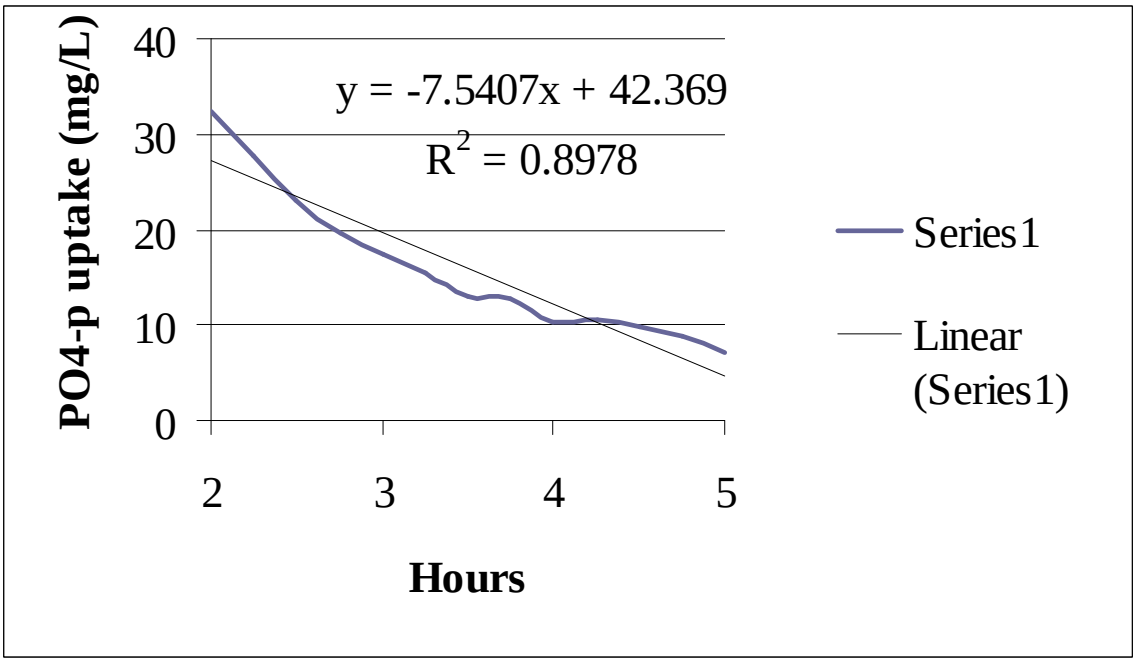

Figure B.12 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 3 (MBNR (Chemical) system mixed liquor)

B.4 Batch Test Number 4

B.4.1 MBNR (Biological) System

Table C.7 Data for batch test number 4 with MBNR (Biological) system mixed liquor

Date: 11/1/2011	Time	Hours	ORP (mV)	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	9:30 AM	0	198	8.603	2.153		12.304	1.94
Target DO = 2-3 mg/L	10:30 AM	1	130	3.047	1.993			
100 mg/L of acetate before anaerobic phase	11:30 AM	2	89	0.097	2.363			
	1:30 PM	0	70	0.117	5.642	100	12.928	1.82
		0.25	33	0.185	9.878	77.5	14.158	1.78
		0.5	-29	0.209	13.311	67.3	13.252	1.806
		0.75	-91	0.289	16.066	74.0	17.412	3.831
		1	-130	0.127	18.237	71.2	18.898	4.029
		1.25	-153	0.118	19.801	70.4	20.54	5.11
		1.5	-164	0.105	21.553	66.6	19.69	5.11
		1.75	-170	0.165	21.956	60.9	22.3	5.54
		2	-178	0.087	22.439	61.7	22.34	6.572
		2.25	14	0.108	22.535		21.74	6.321
		2.5	60	0.148	19.571	0.0	22.94	6.542
		2.75	75	0.162	18.043		22.68	6.647
		3	82	0.186	15.193	0.0	22.68	5.838
		3.25	82	0.184	14.581		21.76	5.529
		3.5	85	0.136	13.05	0.0	19.498	4.46
		3.75	86	0.152	11.946		18.862	4.182
		4	92	0.158	10.995	0	18.256	3.829
		4.25	92	0.15	10.009		19.028	3.726
		4.5	93	0.153	9.24	0	18.476	3.562
		4.75	93	0.134	9.493		17.402	3.346
	6:30 PM	5	93	0.165	8.824	0	17.232	3.513

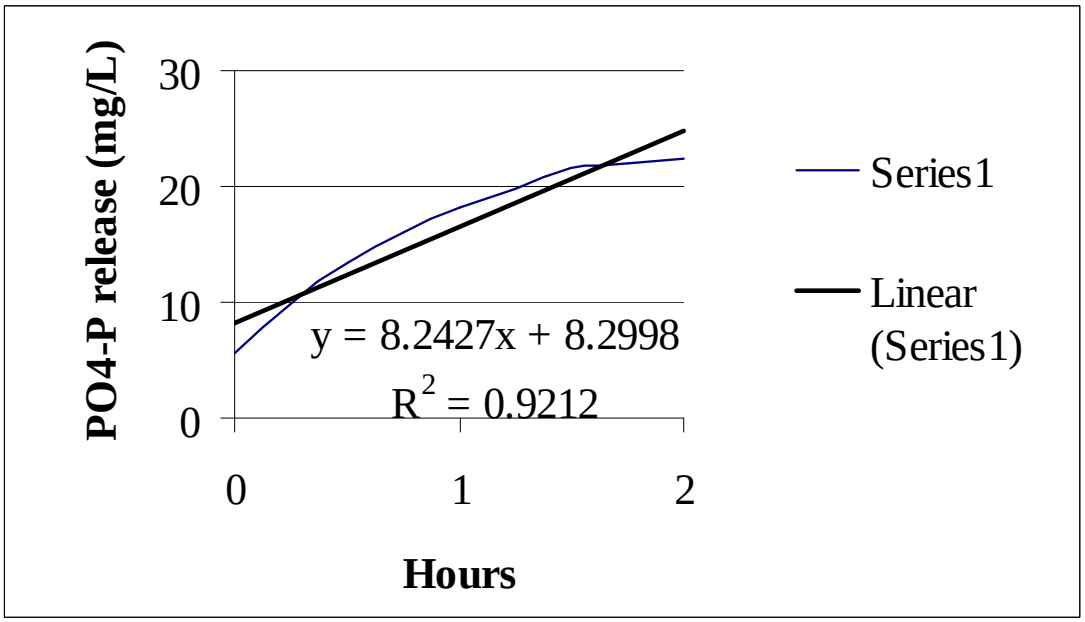

Figure B.13 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 4 (MBNR (Biological) system mixed liquor)

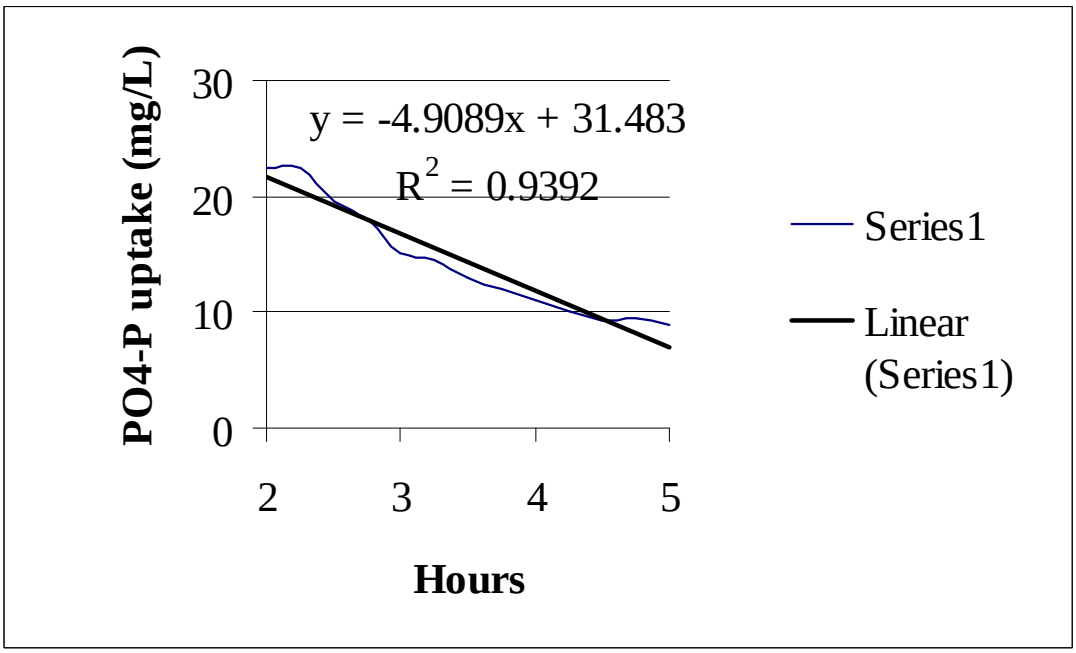

Figure B.14 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 4 (MBNR (Biological) system mixed liquor)

B.4.2 MBNR (Chemical) System

Table B.8 Data for batch test number 4 with MBNR (Chemical) system mixed liquor

Date: 12/1/2011	Time	Hours	ORP (mV)	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	11:00 AM	0	150	5.359	2.52		12.896	2.403
Target DO = 2-3 mg/L	12:00 PM	1	92	0.193	1.422			
100 mg/L of acetate before anaerobic phase	1:00 PM	2	55	0.118	1.573		14.966	2.001
	3:00 PM	0	18	0.113	2.763	100	14.88	2.22
		0.25	-51	0.088	6.745	96.7	15.394	2.973
		0.5	-119	0.179	10.334	81.3	17.492	3.726
		0.75	-149	0.112	13.138	90.9	18.516	4.487
		1	-164	0.213	15.157	94.7	18.874	4.845
		1.25	-173	0.133	17.229	84.4	20.32	5.376
		1.5	-181	0.173	18.916	72.5	20.36	5.787
		1.75	-185	0.086	20.043	78.9	20.6	5.994
		2	-185	0.245	20.802	77.6	19.762	6.25
		2.25	30	0.666	20.429		20.06	6.194
		2.5	50	0.569	20.006	15.7	22.98	6.087
		2.75	65	0.618	19.161		20.02	5.693
		3	68	0.566	17.291	0.0	19.254	5.548
		3.25	71	0.627	15.462		17.43	5.057
		3.5	83	0.594	13.862	0.0	16.784	4.217
		3.75	84	0.516	12.225		16.556	4.089
		4	85	0.486	11.101	0	16.67	3.842
		4.25	85	0.598	9.744		15.866	3.604
		4.5	85	0.443	8.723		16.668	3.188
		4.75	87	0.507	7.608		15.81	3.263
	8:00 PM	5	88	0.393	6.79		17.712	3.026

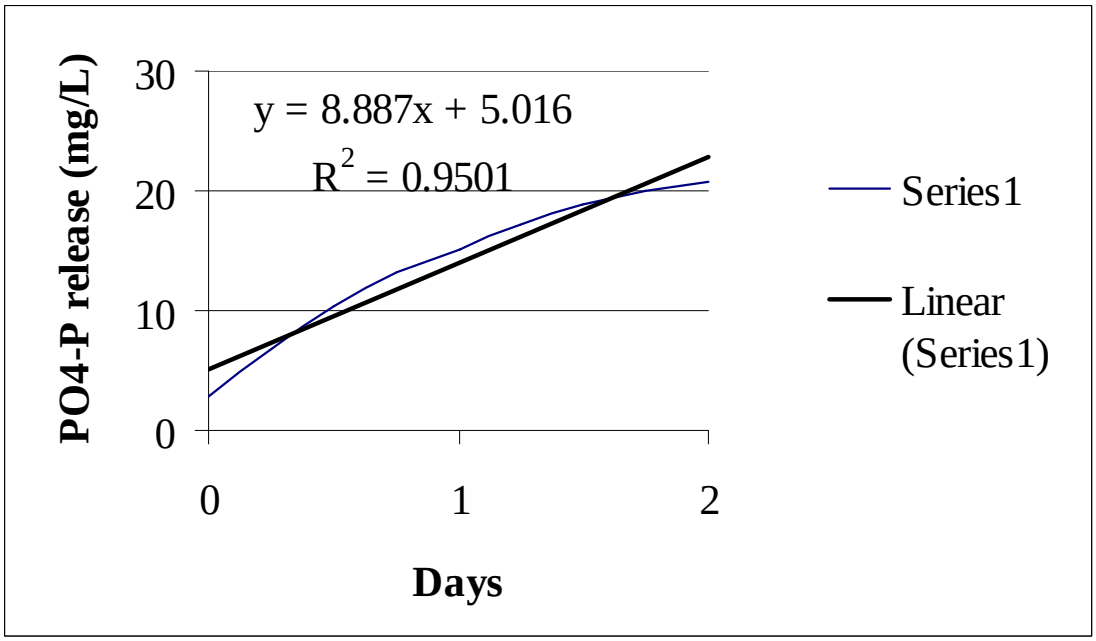

Figure B.15 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 4 (MBNR (Chemical) system mixed liquor)

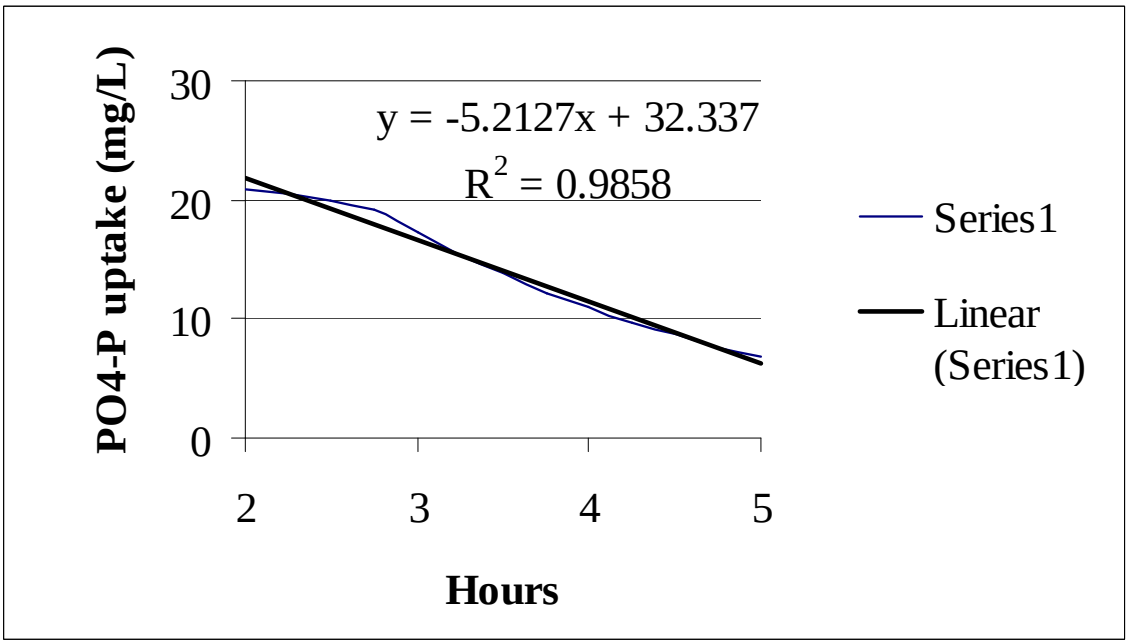

Figure B.16 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 4 (MBNR (Chemical) system mixed liquor)

B.5 Batch Test Number 5

B.5.1 MBNR (Biological) System

Table B.9 Data for batch test number 5 with MBNR (Biological) system mixed liquor

Date: 25/1/2011	Time	Hours	ORP (mV)	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	9:30 AM	0	183	6.91	1.592		13.294	1.317
Target DO = 2-3 mg/L	10:30 AM	1	133	6.92	1.207			
100 mg/L of acetate before anaerobic phase	11:30 AM	2	124	6.77	1.01		13.484	1.139
	12:30 PM	3	129	6.71	0.895			
	1:30 PM	0	124	6.65	1.795	79.05	12.532	1.112
		0.25	101	6.91	5.634	80.36	14.306	0.776
		0.5	62	7.05	10.67	82.79	14.888	0.606
		0.75	0	7.14	14.816	70.02	16.904	1.798
		1	-66	7.23	17.461	60.37	19.56	2.797
		1.25	-104	7.27	19.846	83.39	20.14	3.588
		1.5	-114	7.15	20.446	67.65	20.92	4.221
		1.75	-131	7.26	21.046	1.33	24.46	5.984
		2	-147	7.34	21.597	1.33	23.76	6.582
		2.25	76	7.24	21.315		22.12	5.76
		2.5	91	7.21	19.691	0.41	21.96	5.275
		2.75	95	7.27	17.456		21.48	5.154
		3	101	7.28	15.489	0.57	21.74	4.876
		3.25	110	7.2	13.639		18.998	4.21
		3.5	107	7.22	12.063	0.91	18.168	3.748
		3.75	107	7.29	10.237		19.318	3.575
		4	111	7.21	8.935	0.34	18.644	3.233
		4.25	110	7.25	7.709		17.212	2.838
		4.5	110	7.26	6.582	0.00	17.392	3.361
		4.75	110	7.3	5.604		17.152	3.93
	6:30 PM	5	110	7.3	4.88	0	17.1	3.9

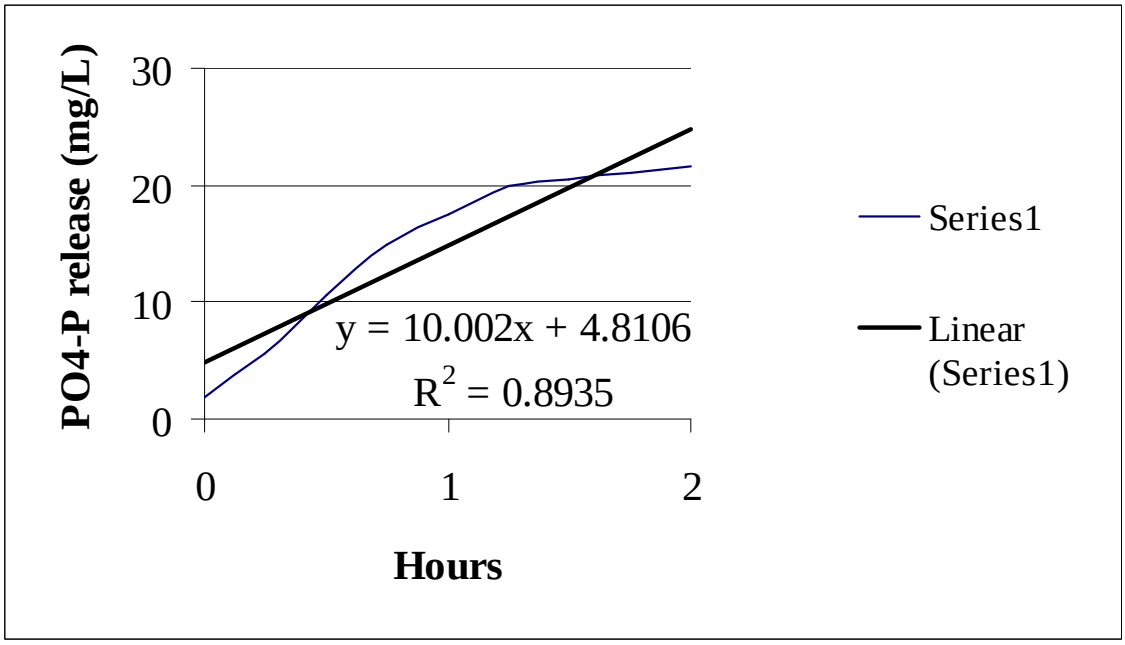

Figure B.17 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 5 (MBNR (Biological) system mixed liquor)

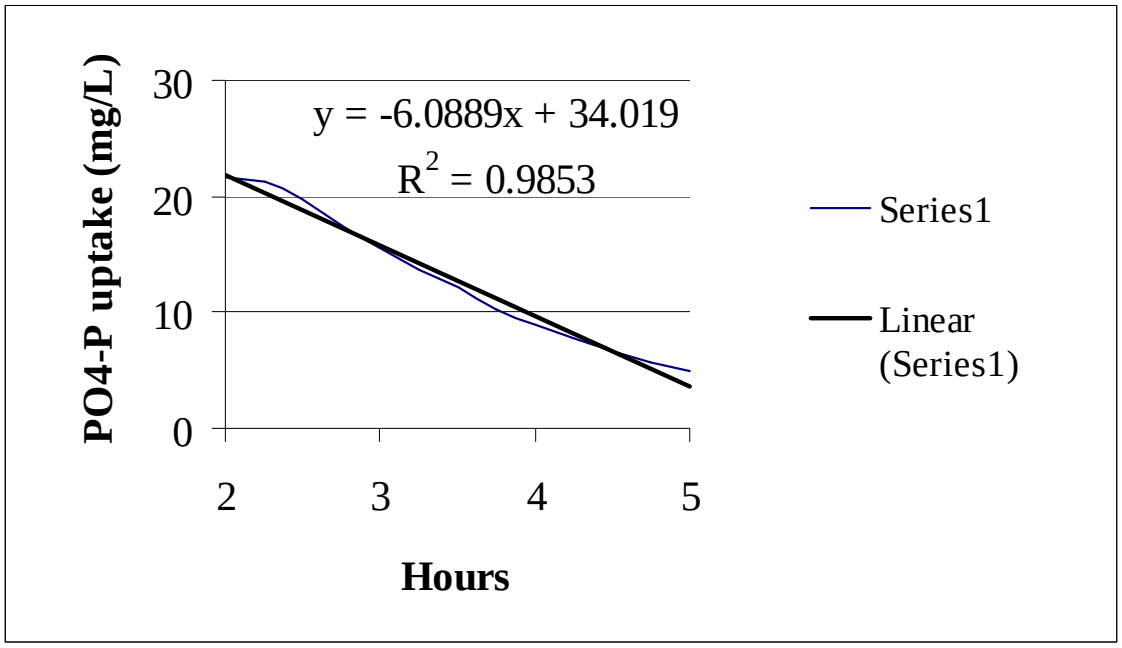

Figure B.18 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 5 (MBNR (Biological) system mixed liquor)

B.5.2 MBNR (Chemical) System

Table B.10 Data for batch test number 5 with MBNR (Chemical) system mixed liquor

Date: 26/1/2011	Time	Hours	ORP (mV)	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	11:30 AM	0	155	5.557	0.856		12.562	1.267
Target DO = 2-3 mg/L	12:30 PM	1	113	0.217	1.056			
100 mg/L of acetate before anaerobic phase	1:30 PM	2	80	0.105	0.651		14.83	0.903
	2:30 PM	3	42	0.099	0.964			
	3:30 PM	0	5	0.088	2.223	76.30	16.918	2.299
		0.25	-75	0.192	7.581	84.42	19.442	1.162
		0.5	-136	0.106	13.24	88.83	17.208	1.449
		0.75	-162	0.099	14.736	81.20	16.356	2.04
		1	-177	0.077	16.551	81.76	20.62	3.398
		1.25	-184	0.104	18.135	74.06	21.08	4.698
		1.5	-191	0.102	19.015	80.99	26.3	5.196
		1.75	-194	0.094	19.498	65.58	24.34	5.733
		2	-197	0.111	20.599	70.84	24.62	6.075
		2.25	37	1.111	19.945		29.22	6.583
		2.5	57	1.134	19.712	3.46	22.52	5.133
		2.75	70	1.26	17.852		23.56	5.096
		3	75	1.349	15.697	1.07	22.5	4.922
		3.25	80	1.109	13.523		24.96	4.878
		3.5	83	1.242	11.734	0.69	19.604	3.87
		3.75	86	1.101	9.899		22.36	3.791
		4	87	1.079	8.434	0.91	21.48	3.257
		4.25	93	1.165	7.114		20.52	2.94
		4.5	98	1.186	5.942	2.98	21.4	3.652
		4.75	96	1.137	4.867		21.12	3.609
	8:30 PM	5	97	1.128	4.119	0.37	21.68	2.98

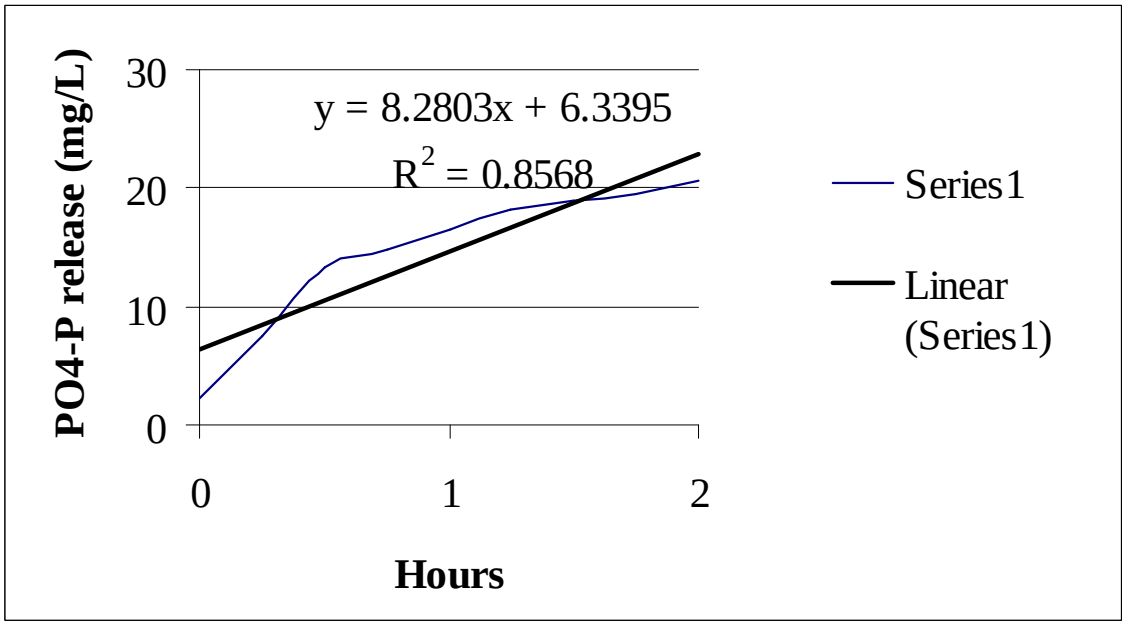

Figure B.19 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 5 (MBNR (Chemical) system mixed liquor)

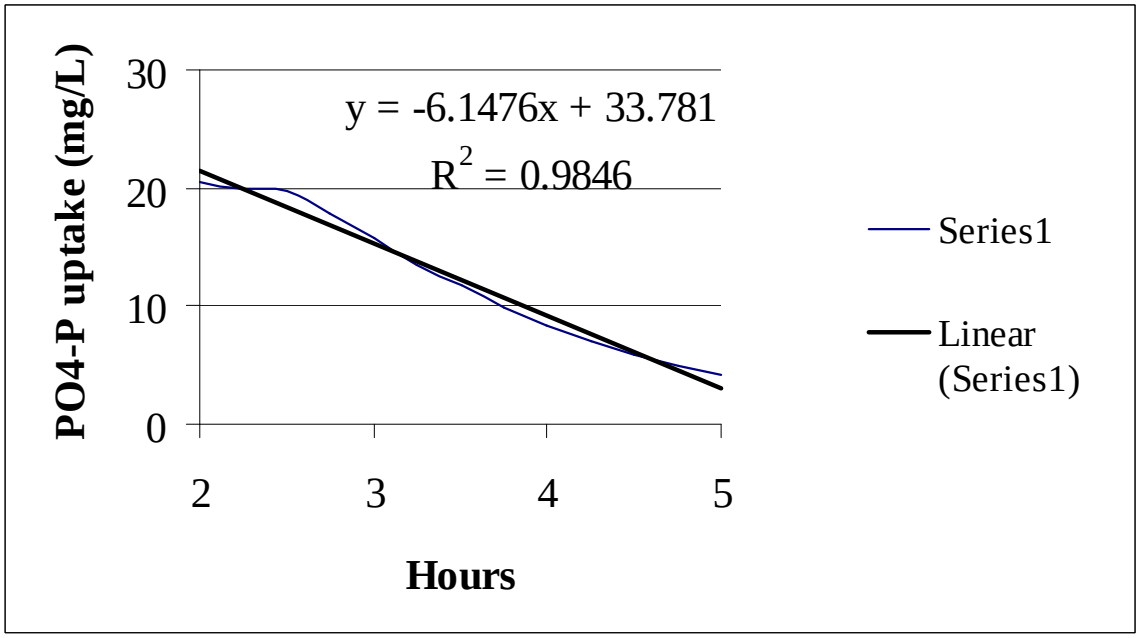

Figure B.20 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 5 (MBNR (Chemical) system mixed liquor)

B.6 Batch Test Number 6

B.6.1 MBNR (Biological) System

Table B.11 Data for batch test number 6 with MBNR (Biological) system mixed liquor

Date: 8/2/2011	Time	Hours	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	11:00 AM	0	9.083	2.145			
Target DO = 2-3 mg/L	3:00 PM	0	0.649	12.429	100.00		
100 mg/L of acetate before anaerobic phase		0.5	0.601	17.683	82.75		
		1	0.531	21.464	76.83		
		1.5	0.597	23.323	70.00		
		2	0.584	23.654	65.04		
		2.5	1.085	21.72	3.90		
		3	1.027	17.047	1.98		
		3.5	1.024	13.219	0.85		
		4	0.976	9.957	1.39		
		4.5	0.973	7.607	1.20		
	8:00 PM	5	1.128	5.162	1.84		

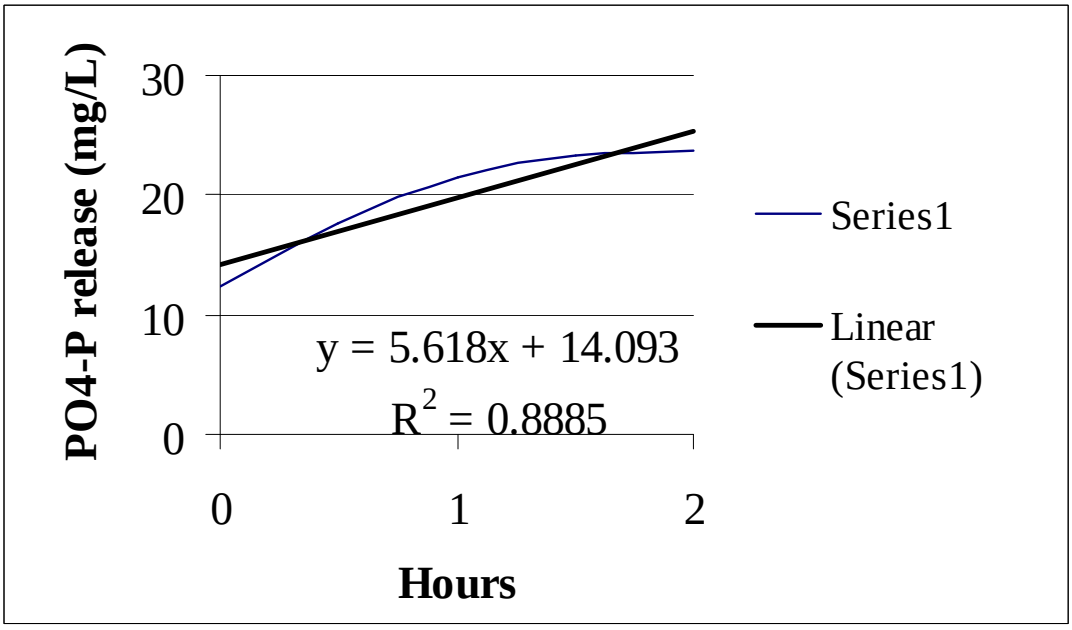

Figure B.21 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 6 (MBNR (Biological) system mixed liquor)

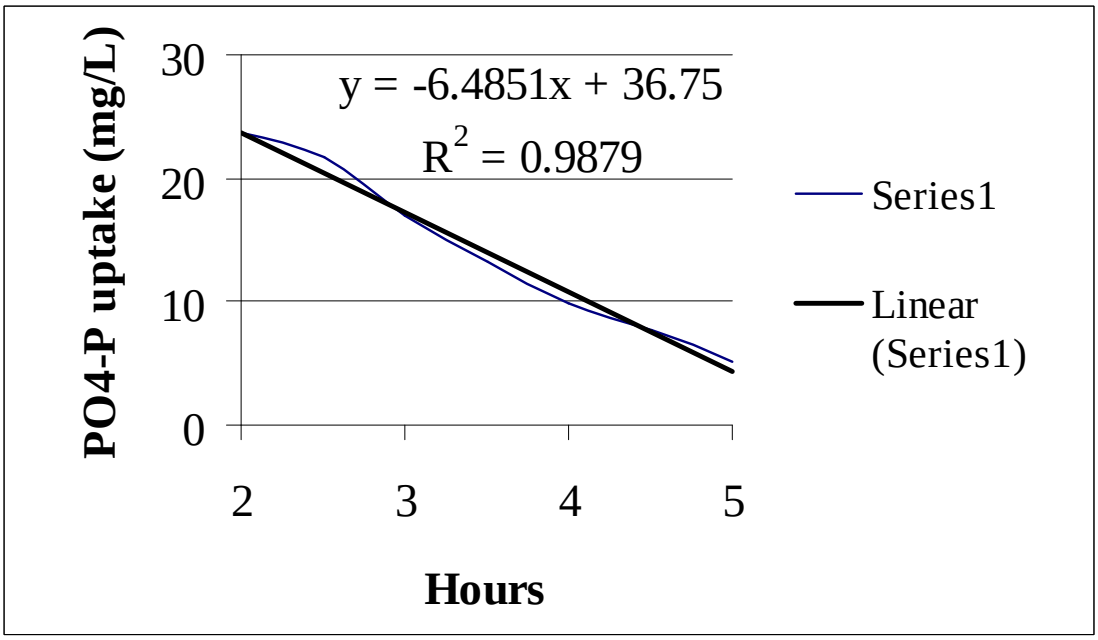

Figure B.22 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 6 (MBNR (Biological) system mixed liquor)

B.6.2 MBNR (Chemical) System

Table B.12 Data for batch test number 6 with MBNR (Chemical) system mixed liquor

Date: 8/2/2011	Time	Hours	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	11:00 AM	0	7.638	1.09			
Target DO = 2-3 mg/L	3:00 PM	0	0.591	9.436			
100 mg/L of acetate before anaerobic phase		0.5	0.617	15.679			
		1	0.542	19.103			
		1.5	0.602	20.776			
		2	0.531	21.839			
		2.5	1.168	18.713			
		3	1.086	14.325			
		3.5	1.257	10.644			
		4	1.028	7.211			
		4.5	1.072	5.047			
	8:00 PM	5	1.059	3.134			

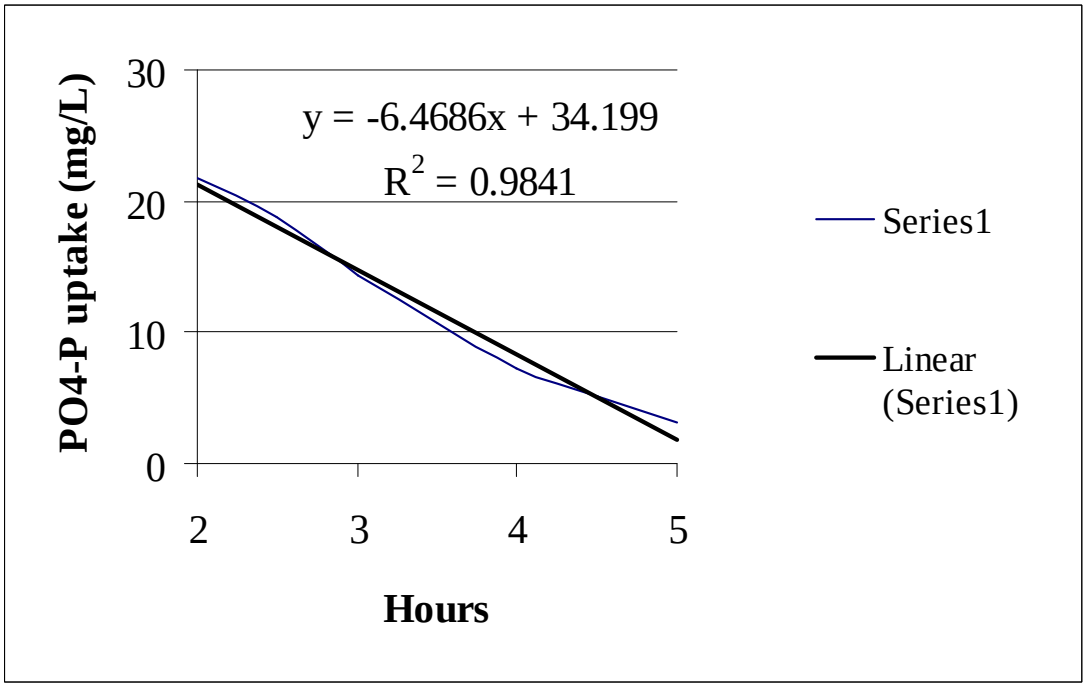

Figure B.23 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 6 (MBNR (Chemical) system mixed liquor)

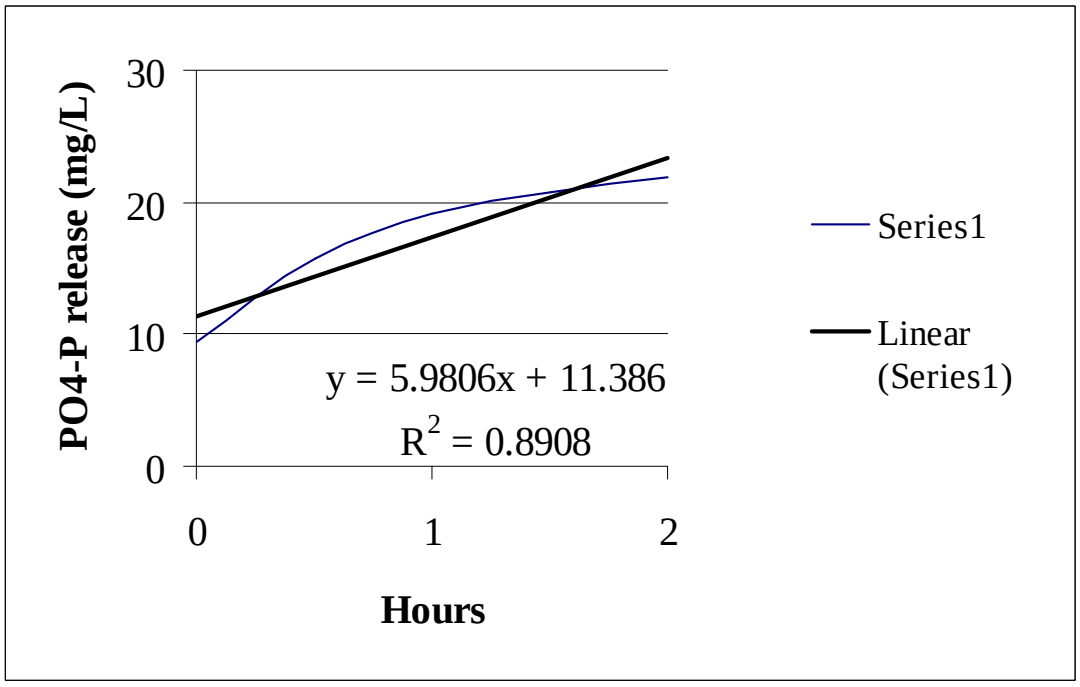

Figure B.24 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 6 (MBNR (Chemical) system mixed liquor)

B.7 Batch Test Number 7

B.7.1 MBNR (Biological) System

Table B.13 Data for batch test number 7 with MBNR (Biological) system mixed liquor

Date: 22/2/2011	Time	Hours	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	3:00 PM	0	0.22	10.151	100.0	15.934	3.122
Target DO = 2-3 mg/L		0.5	0.071	16.361	76.0	16.696	4.124
100 mg/L of acetate before anaerobic phase		1	0.178	19.57	73.1	18.208	5.072
		1.5	0.059	22.426	74.7	20.44	5.801
		2	0.069	24.17	76.1	21.38	6.115
		2.5	0.541	21.236	2.3	19.168	5.53
		3	0.556	16.429	1.7	17.322	4.709
		3.5	0.449	13.162	1.4	16.538	3.572
		4	0.305	10.39	0.9	15.78	3.156
		4.5	0.176	7.697	1.2	14.088	2.674
	8:00 PM	5	0.121	6.385	1.2	14.282	2.452

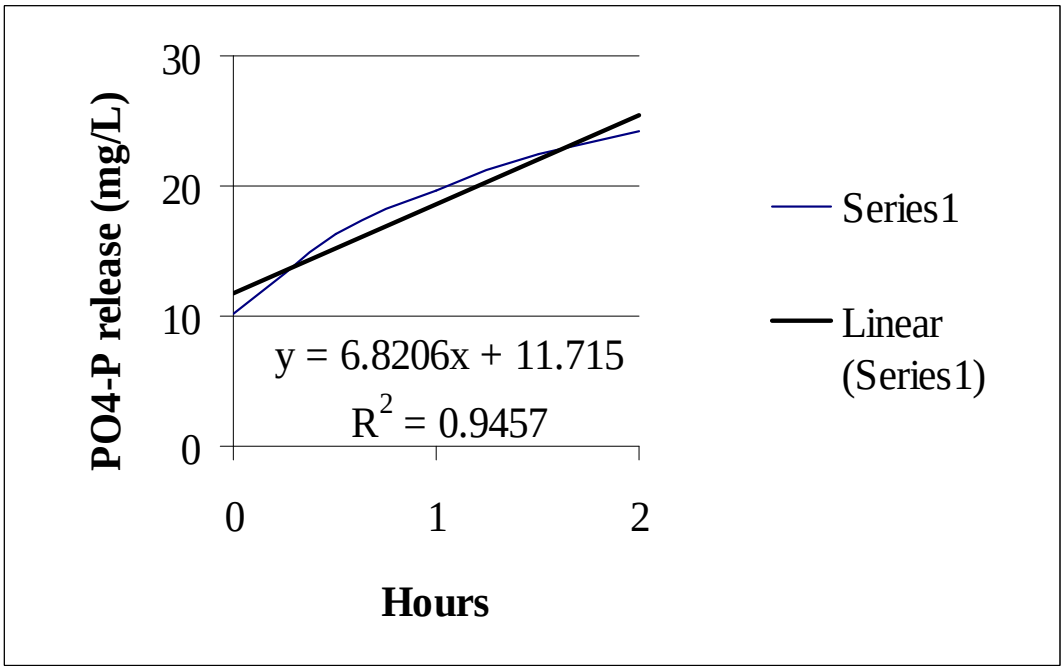

Figure B.25 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 7 (MBNR (Biological) system mixed liquor)

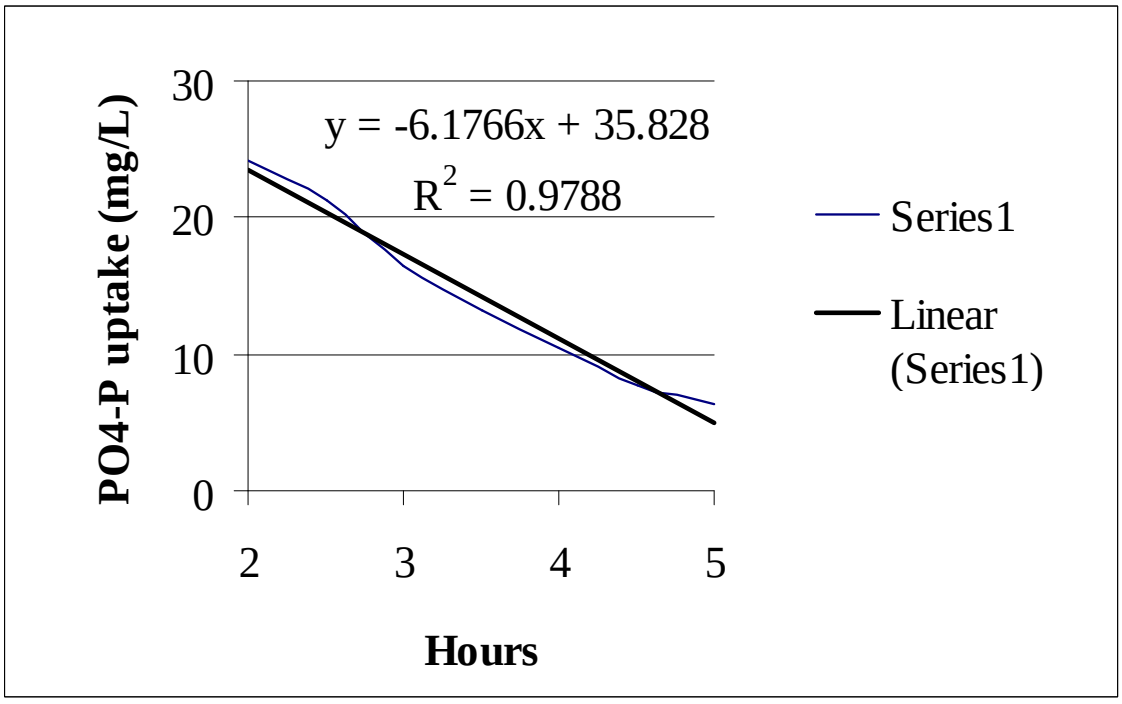

Figure B.26 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 7 (MBNR (Biological) system mixed liquor)

B.7.2 MBNR (Chemical) System

Table B.14 Data for batch test number 7 with MBNR (Chemical) system mixed liquor

Date: 22/2/2011	Time	Hours	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	3:00 PM	0	0.115	7.399	100.0	14.854	3.032
Target DO = 2-3 mg/L		0.5	0.056	14.771	74.6	17.5	4.527
100 mg/L of acetate before anaerobic phase		1	0.12	18.964	58.6	18.634	5.15
		1.5	0.089	21.098	59.1	19.116	5.93
		2	0.019	23.247	60.1	20.28	6.63
		2.5	1.332	18.808	1.6	17.224	4.656
		3	1.336	13.832	1.5	15.482	3.66
		3.5	1.313	9.558	1.4	14.114	3.031
		4	1.349	6.564	0.9	13.596	2.486
		4.5	1.428	4.319	1.3	12.948	2.136
	8:00 PM	5	1.481	2.893	1.1	12.334	1.916

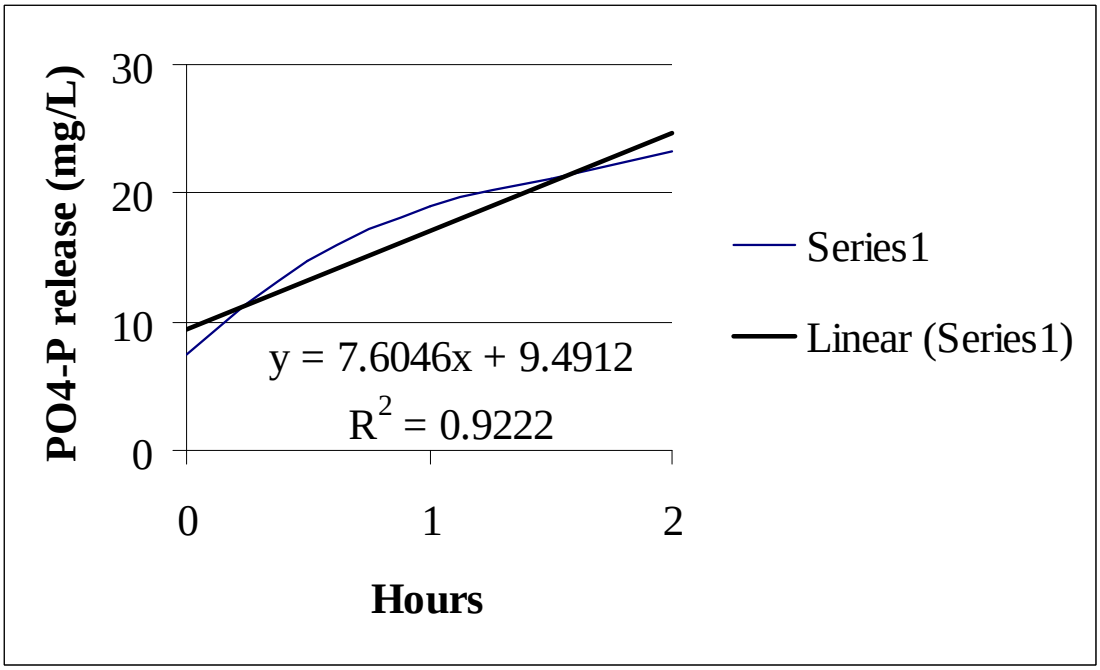

Figure B.27 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 7 (MBNR (Chemical) system mixed liquor)

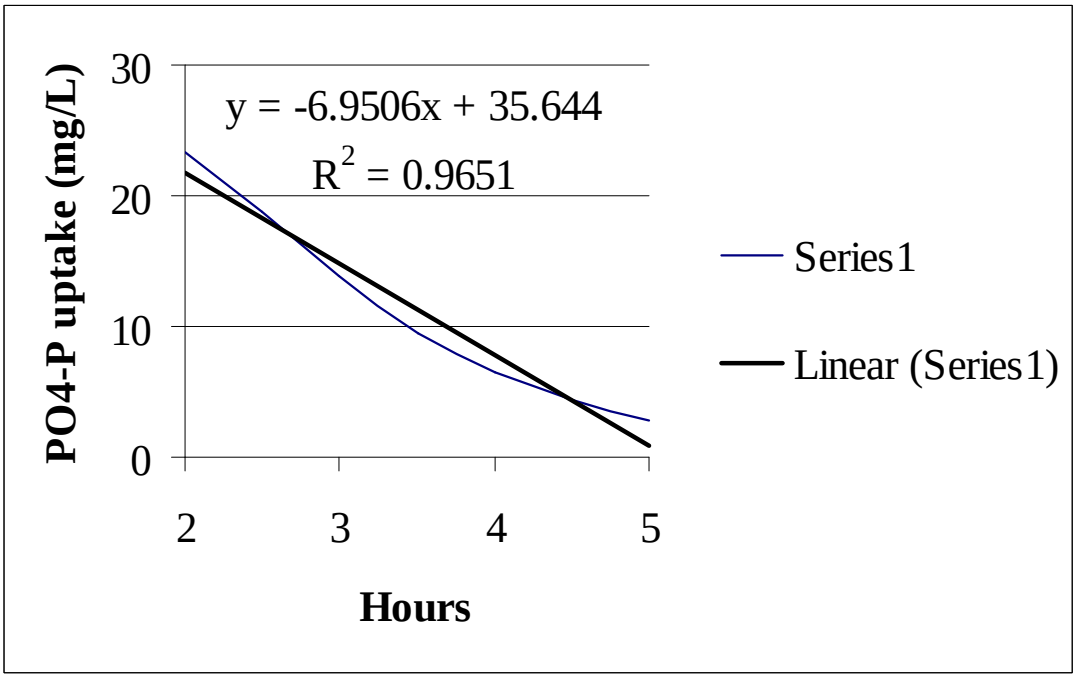

Figure B.28 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 7 (MBNR (Chemical) system mixed liquor)

B.8 Batch Test Number 8

B.8.1 MBNR (Biological) System

Table B.15 Data for batch test number 8 with MBNR (Biological) system mixed liquor

Date: 07/3/2011	Time	Hours	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	3:00 PM	0	0.115	7.399	100.0	14.854	3.032
Target DO = 2-3 mg/L		0.5	0.056	14.771	74.6	17.5	4.527
100 mg/L of acetate before anaerobic phase		1	0.12	18.964	58.6	18.634	5.15
		1.5	0.089	21.098	59.1	19.116	5.93
		2	0.019	23.247	60.1	20.28	6.63
		2.5	1.332	18.808	1.6	17.224	4.656
		3	1.336	13.832	1.5	15.482	3.66
		3.5	1.313	9.558	1.4	14.114	3.031
		4	1.349	6.564	0.9	13.596	2.486
		4.5	1.428	4.319	1.3	12.948	2.136
	8:00 PM	5	1.481	2.893	1.1	12.334	1.916

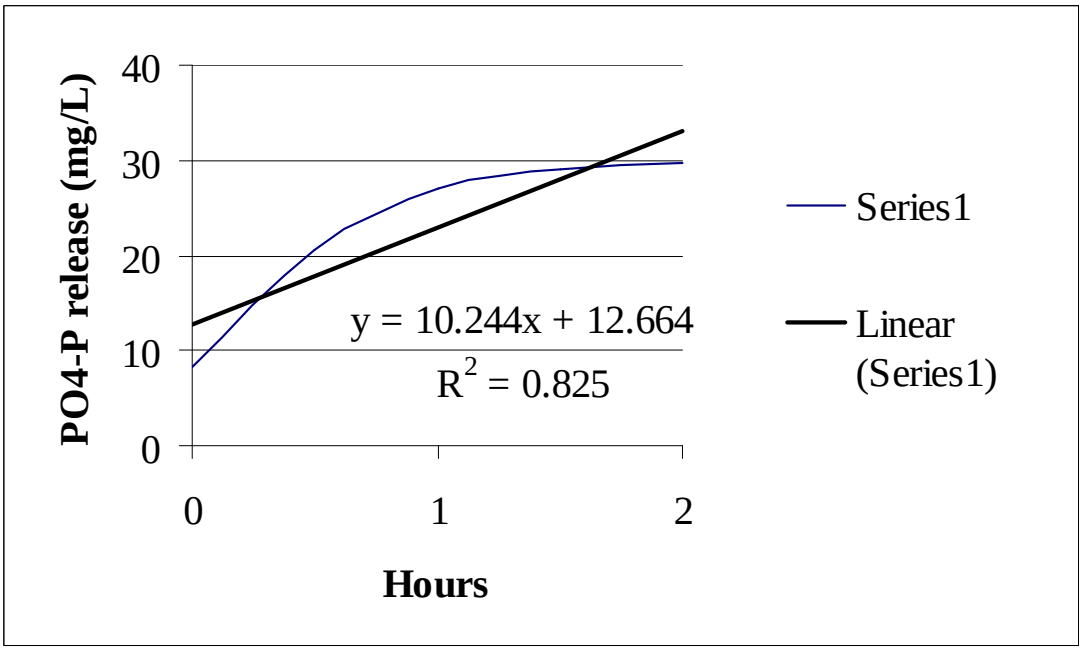

Figure B.29 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 8 (MBNR (Biological) system mixed liquor)

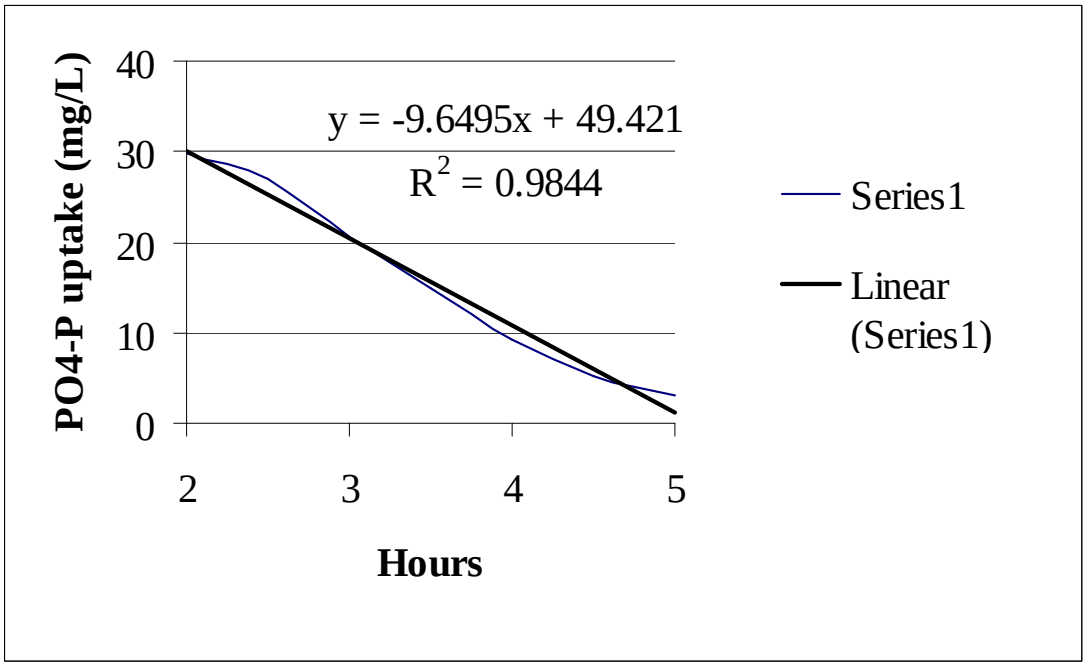

Figure B.30 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 8 (MBNR (Biological) system mixed liquor)

B.8.2 MBNR (Chemical) System

Table B.16 Data for batch test number 8 with MBNR (Chemical) system mixed liquor

Date: 07/3/2011	Time	Hours	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	3:00 PM	0	0.023	14.899	100.00	14.02	2.116
Target DO = 2-3 mg/L		0.5	0.091	25.65	60.60	15.974	3.283
100 mg/L of acetate before anaerobic phase		1	0.121	29.438	58.35	18.178	4.478
		1.5	0.014	30.658	53.72	19.738	5.239
		2	0.107	31.056	54.59	21.5	5.822
		2.5	3.399	29.475	1.41	17.394	3.815
		3	3.484	25.194	1.32	16.074	2.675
		3.5	3.61	19.38	0.0	15.062	2.054
		4	3.574	13.004	0.0	14.278	1.687
		4.5	3.843	6.673	0.0	14.002	1.51
	8:00 PM	5	3.973	2.038	0.0	13.97	1.363

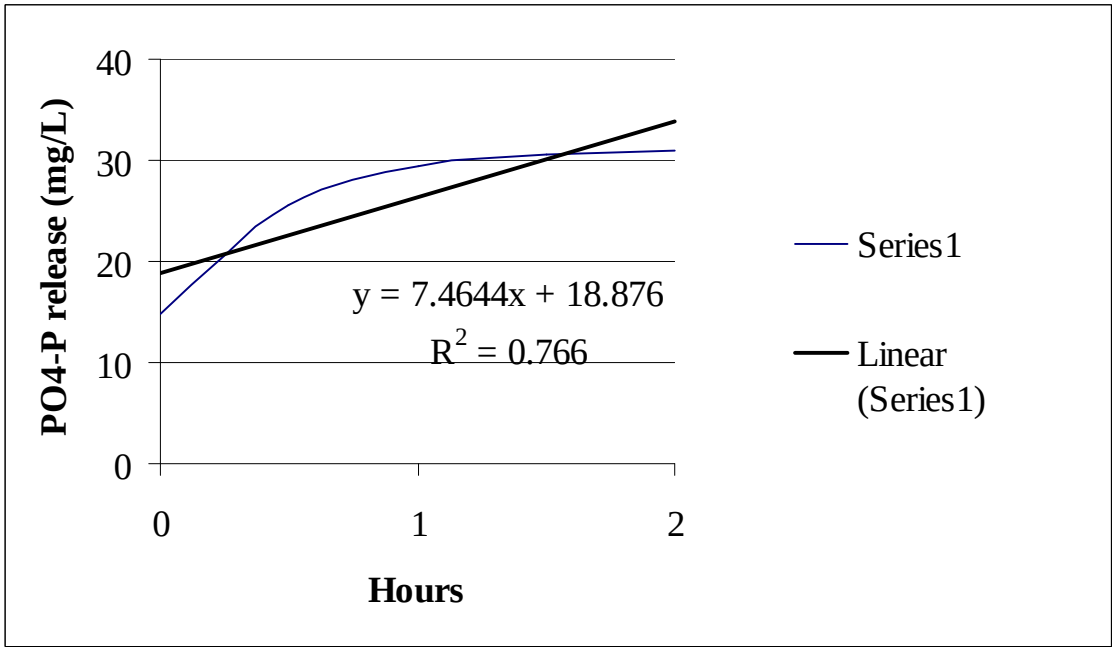

Figure B.31 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 8 (MBNR (Chemical) system mixed liquor)

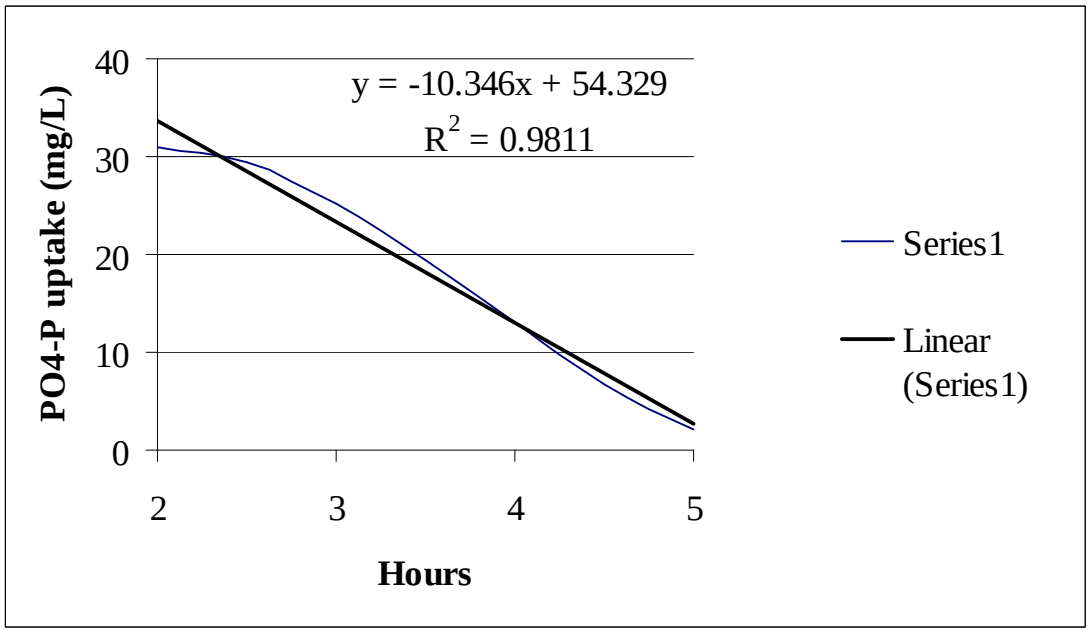

Figure B.32 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 8 (MBNR (Chemical) system mixed liquor)

B.9 Batch Test Number 9

B.9.1 MBNR (Biological) System

Table B.17 Data for batch test number 9 with MBNR (Biological) system mixed liquor

Date: 18/4/2011	Time	Hours	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	2:00 PM	0	0.00	5.109	100.00	13.74385	2.234692
Target DO = 2-3 mg/L		0.5	0.00	14.086	61.57	15.63079	5.199054
100 mg/L of acetate before anaerobic phase		1	0.00	20.85	52.97	16.48084	5.112586
		1.5	0.01	25.279	45.09	18.36329	5.907941
		2	0.00	29.088	45.90	20.48644	6.759867
		2.5	2.41	22.364	0.00	18.00245	5.650692
		3	2.68	17.942	0.00	15.30034	4.564772
		3.5	2.42	13.659	0.00	14.16505	3.769049
		4	2.59	10.81	0.00	13.54652	3.29447
		4.5	2.82	8.141	0.00	13.1713	2.807592
	7:00 PM	5	3.00	5.474	0.00	12.03037	3.582184

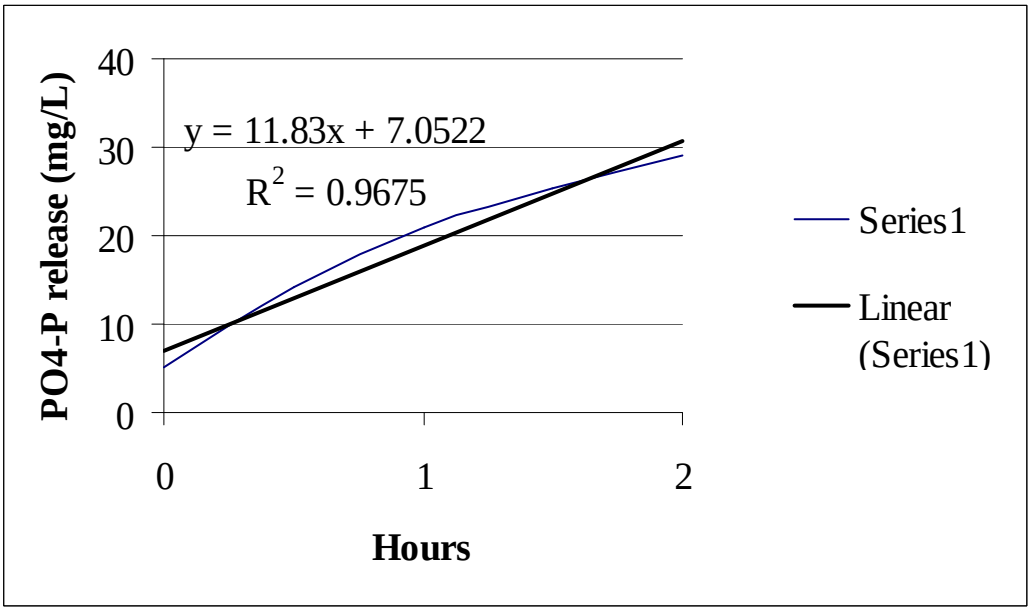

Figure B.33 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 9 (MBNR (Biological) system mixed liquor)

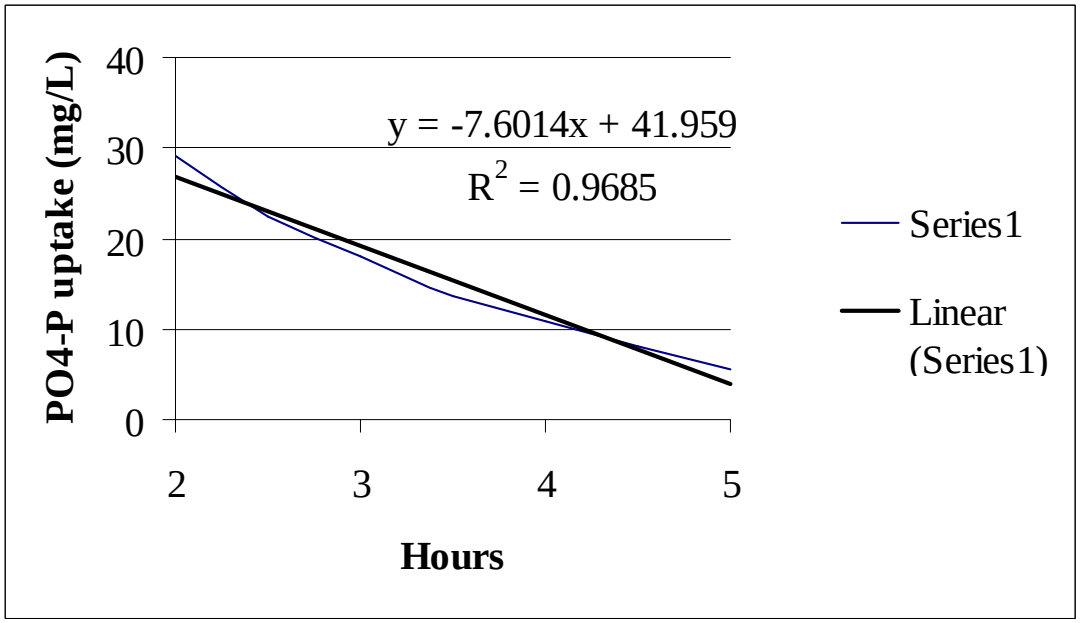

Figure B.34 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 9 (MBNR (Biological) system mixed liquor)

B.9.2 MBNR (Chemical) System

Table B.18 Data for batch test number 9 with MBNR (Chemical) system mixed liquor

Date: 18/4/2011	Time	Hours	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	2:00 PM	0	0.13	1.791	100.00	13.64338	2.08642
Target DO = 2-3 mg/L		0.5	0.00	10.76	53.16	15.86598	3.564256
100 mg/L of acetate before anaerobic phase		1	0.00	18.038	42.40	20.33295	6.709559
		1.5	0.00	21.68	37.44	22.21725	6.448926
		2	0.00	24.239	34.12	22.69775	7.147339
		2.5	2.51	18.658	0.00	19.29951	4.983419
		3	2.39	13.067	0.00	17.04671	3.542473
		3.5	2.50	9.446	0.00	16.31892	3.35452
		4	2.64	5.999	0.00	14.22689	2.760265
		4.5	2.87	3.564	0.00	13.31697	2.00
	7:00 PM	5	3.00	1.912	0.00	12.9	1.864377

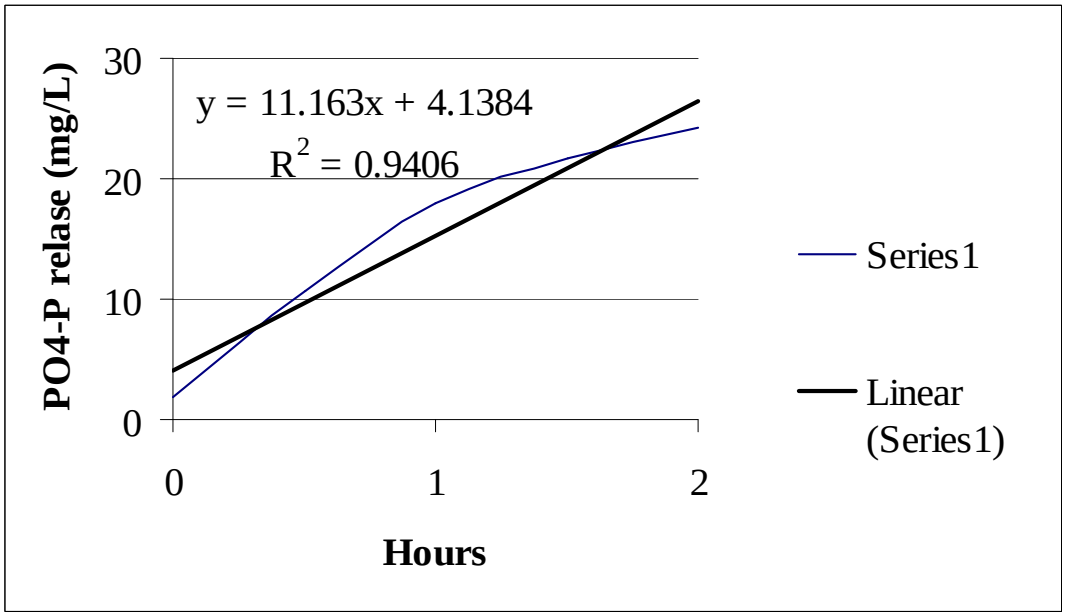

Figure B.35 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 9 (MBNR (Chemical) system mixed liquor)

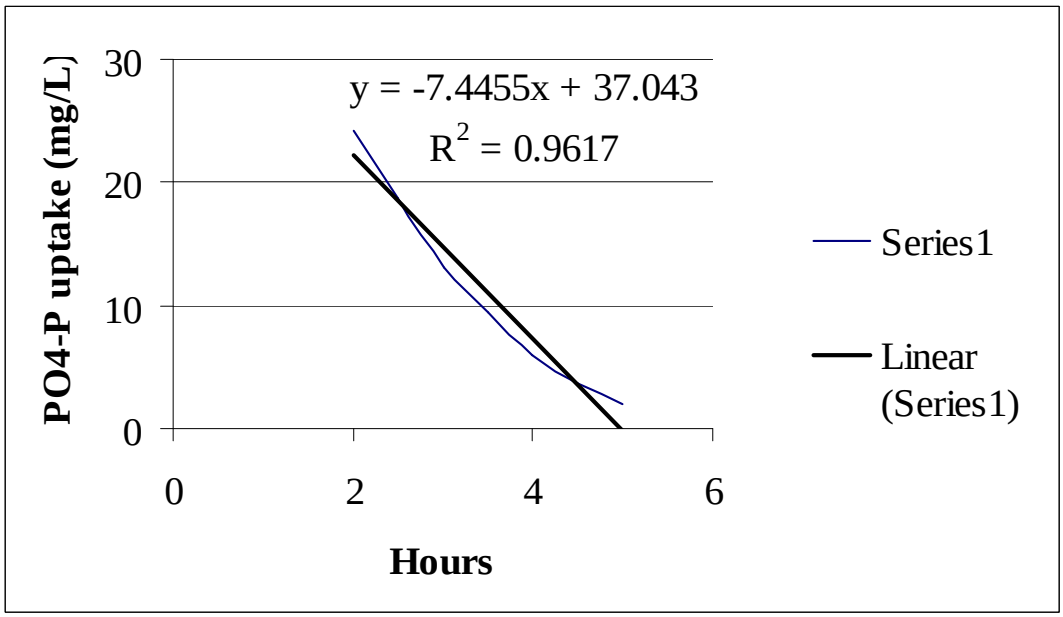

Figure B.36 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 9 (MBNR (Chemical) system mixed liquor)

B.10 Batch Test Number 10

B.10.1 MBNR (Biological) System

Table B.19 Data for batch test number 10 with MBNR (Biological) system mixed liquor

Date: 17/5/2011	Time	Hours	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	1:00 PM	0	0.02	4.932	61.16	14.89616	2.738179
Target DO = 2-3 mg/L		0.5	0.07	15.5	54.20	17.24857	4.710405
100 mg/L of acetate before anaerobic phase		1	0.09	23.014	51.26	19.65868	6.319245
		1.5	0.05	28.21	44.12	21.18397	7.684081
		2	0.02	32.617	41.69	22.81708	8.565048
		2.5	1.84	27.066	3.35	19.03879	6.746379
		3	1.83	20.354	0.00	17.21536	5.320634
		3.5	1.97	15.997	0.0	14.66017	4.285616
		4	2.11	10.762	0.0	13.89981	3.671015
		4.5	1.96	7.3	0.0	13.05799	2.992497
	6:00 PM	5	2.59	6.207	0.0	12.2	2.5

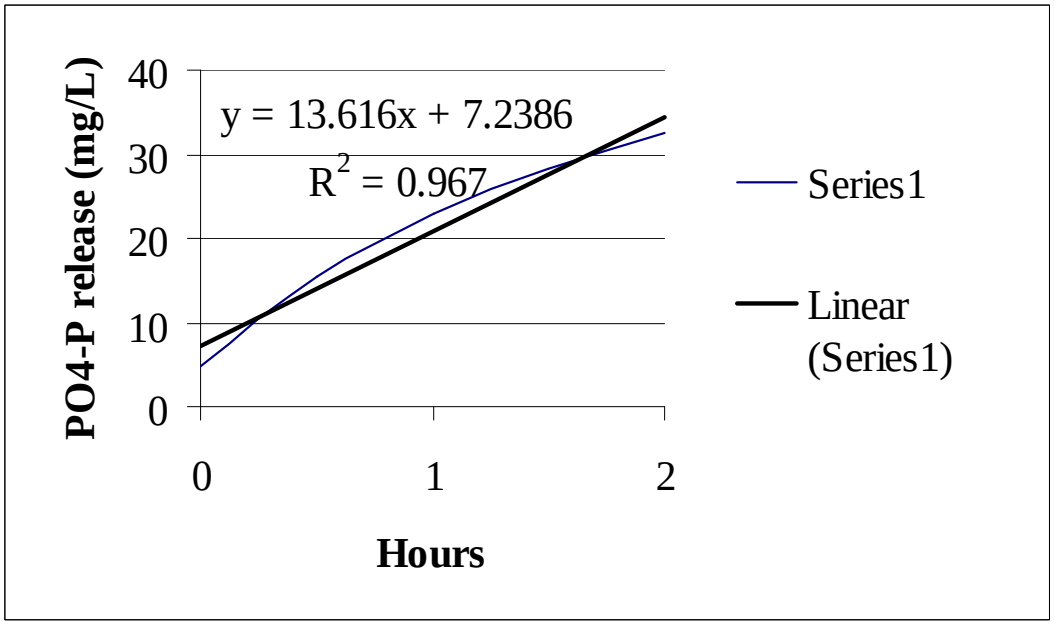

Figure B.37 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 10 (MBNR (Biological) system mixed liquor)

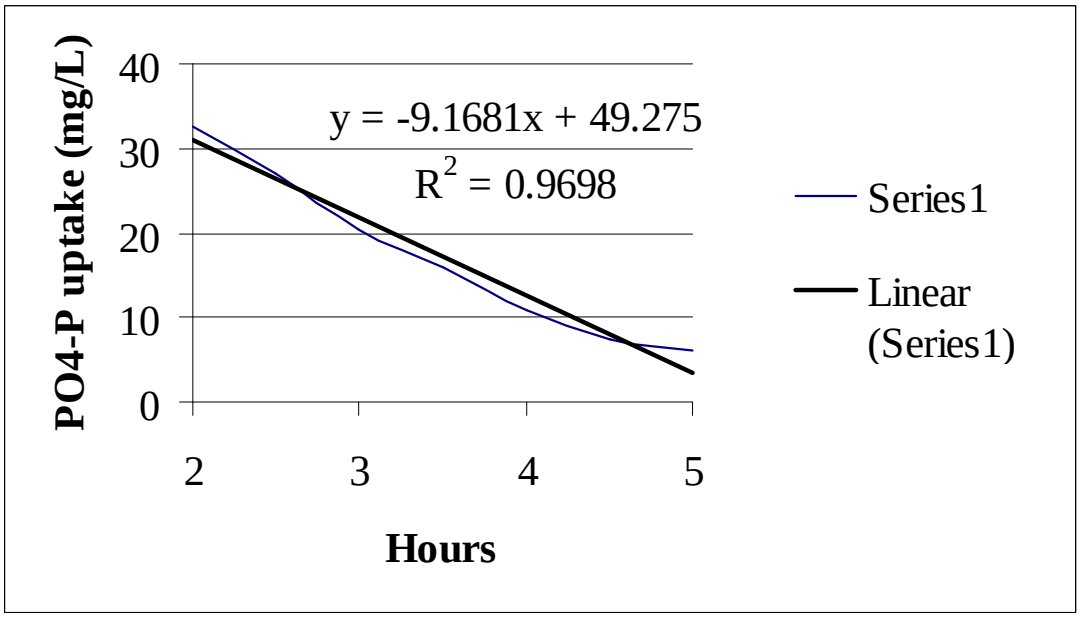

Figure B.38 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 10 (MBNR (Biological) system mixed liquor)

B.10.2 MBNR (Chemical) System

Table B.20 Data for batch test number 10 with MBNR (Chemical) system mixed liquor

Date: 17/5/2011	Time	Hours	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	1:00 PM	0	0.00	1.232	100.00	13.72	2.67
Target DO = 2-3 mg/L		0.5	0.03	5.815	56.05	15.32	3.74
100 mg/L of acetate before anaerobic phase		1	0.00	8.703	46.89	16.97	4.61
		1.5	0.02	10.143	43.44	17.77	4.94
		2	0.00	10.999	44.28	18.27	4.99
		2.5	2.51	7.033	2.73	16.00	5.65
		3	4.76	2.783	0.00	12.13	2.62
		3.5	4.85	0.756	0.0	11.40	2.37
		4	5.04	0.226	0.0	11.50	2.28
		4.5	4.69	0.081	0.0	11.38	2.16
	6:00 PM	5	5.38	0.06	0.0	11.00	2.00

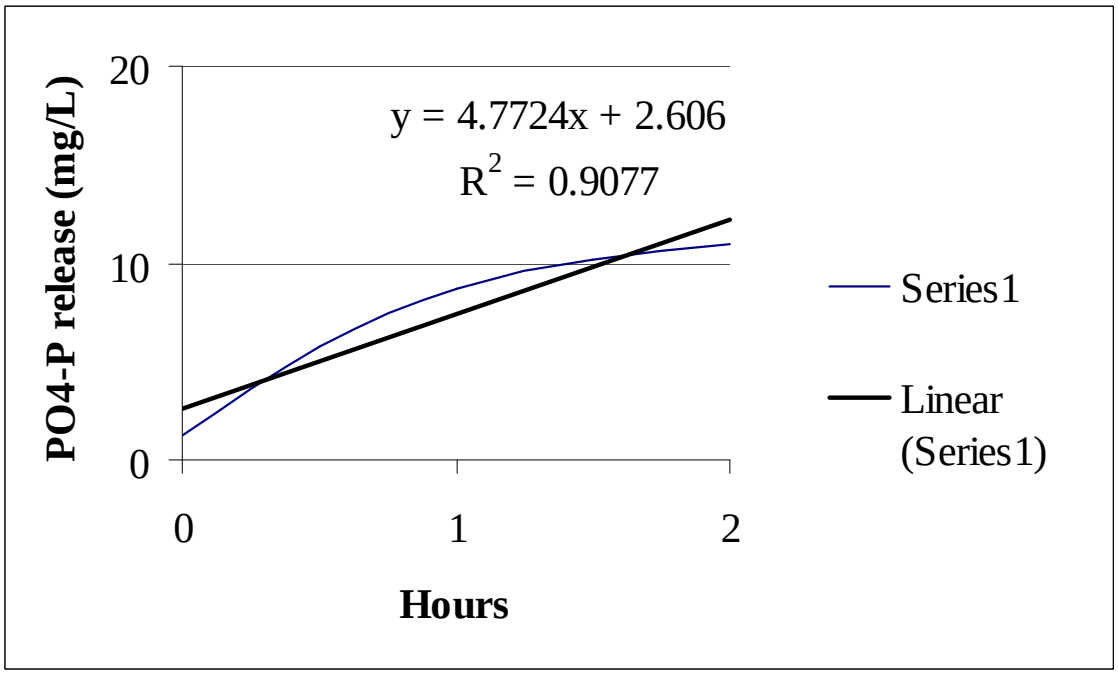

Figure B.39 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 10 (MBNR (Chemical) system mixed liquor)

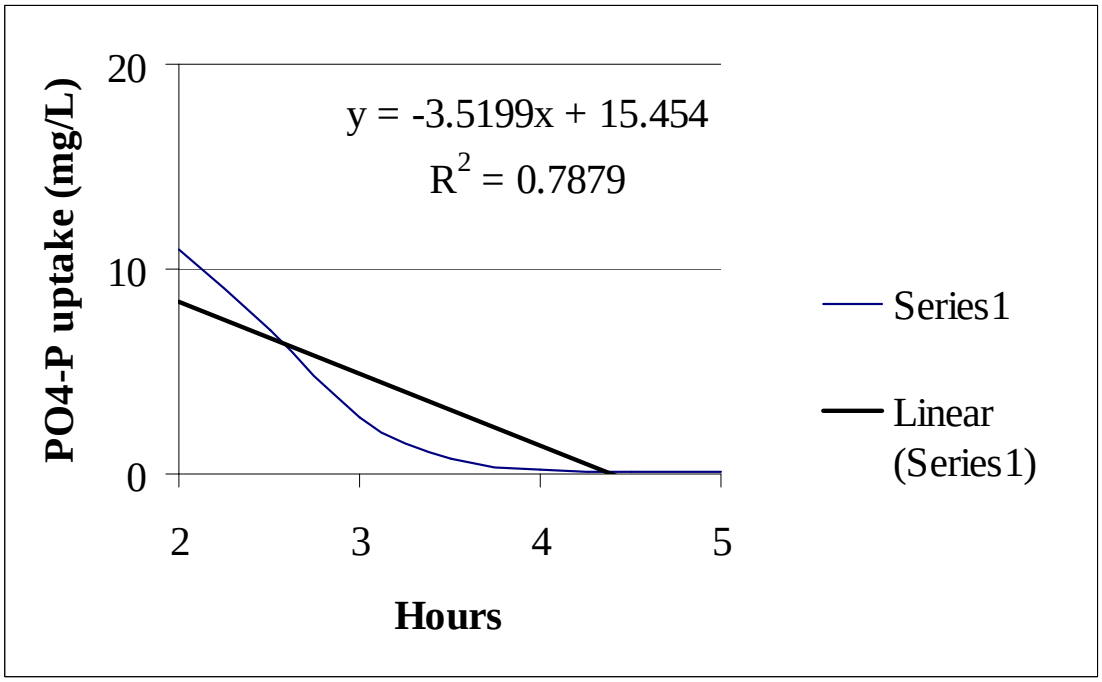

Figure B.40 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 10 (MBNR (Chemical) system mixed liquor)

B.11 Batch Test Number 11

B.11.1 MBNR (Biological) System

Table B.21 Data for batch test number 11 with MBNR (Biological) system mixed liquor

Date: 02/6/2011	Time	Hours	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	2:00 PM	0	3.063	5.231	100.00	13.93062	2.079716
Target DO = 2-3 mg/L		0.5	0.104	16.056	26.90	17.0265	3.675883
100 mg/L of acetate before anaerobic phase		1	0.1	24.45	20.17	20.24088	5.626079
		1.5	0.161	27.756	11.97	21.84723	6.873969
		2	0.101	32.208	8.95	23.11492	7.696573
		2.5	1.299	25.516	0.14	19.08724	5.816656
		3	0.857	19.446	0.00	15.99782	4.52368
		3.5	0.21	16.816	0.0	16.31305	3.990807
		4	0.124	12.489	0.0	14.49307	3.159651
		4.5	0.222	9.286	0.0	13.43677	2.559441
	7:00 PM	5	0.293	6.582	0.0	12.47984	2.128057

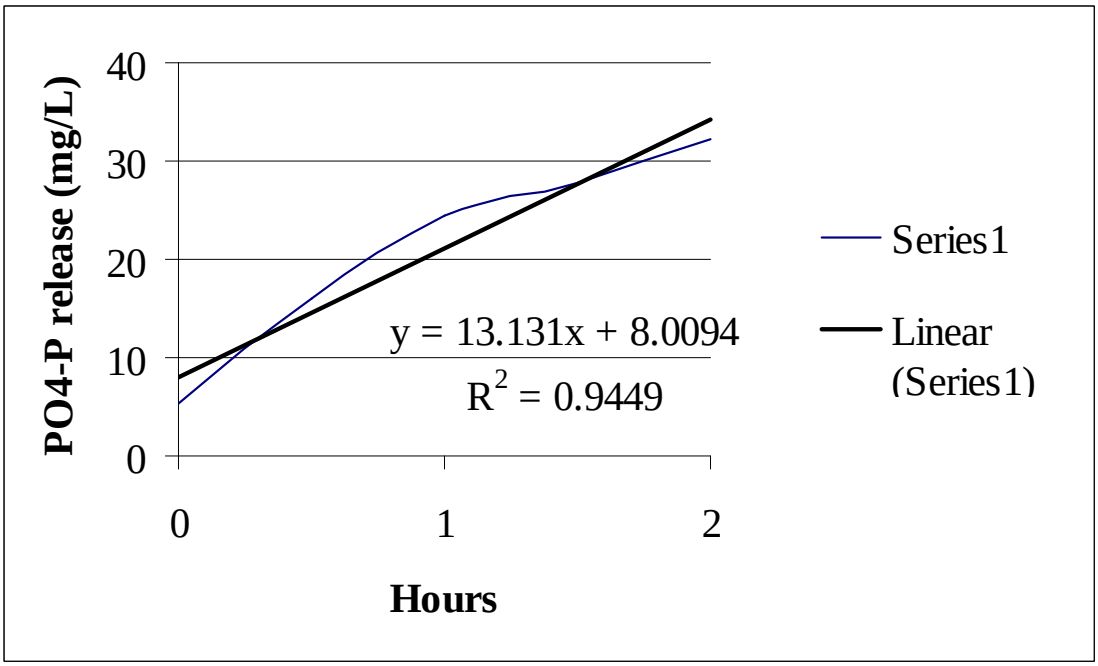

Figure B.41 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 11 (MBNR (Biological) system mixed liquor)

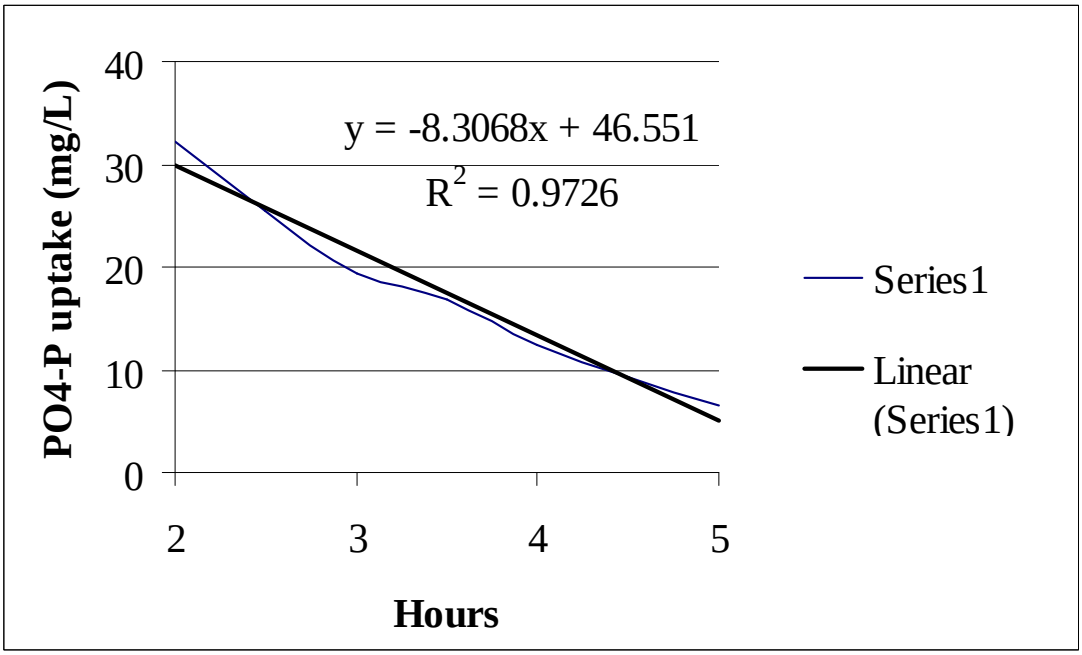

Figure B.42 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 11 (MBNR (Biological) system mixed liquor)

B.11.2 MBNR (Chemical) System

Table B.22 Data for batch test number 11 with MBNR (Chemical) system mixed liquor

Date: 2/6/2011	Time	Hours	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	2:00 PM	0	0.087	0.889	100.00	13.10375	2.085116
Target DO = 2-3 mg/L		0.5	0.083	3.522	24.01	14.63597	2.917675
100 mg/L of acetate before anaerobic phase		1	0.083	5.384	59.68	15.32436	3.272401
		1.5	0.089	6.564	59.02	15.5176	3.513803
		2	0.092	7.308	57.10	15.6099	3.511471
		2.5	1.641	4.806	2.90	14.3696	2.524794
		3	1.59	2.145	0.00	13.18256	2.111074
		3.5	1.618	0.841	0.0	13.02981	1.976526
		4	1.732	0.413	0.0	11.36721	1.52114
		4.5	1.786	0.249	0.0	10.75549	1.373808
	7:00 PM	5	1.88	0.219	0.0	10.1	1.34526

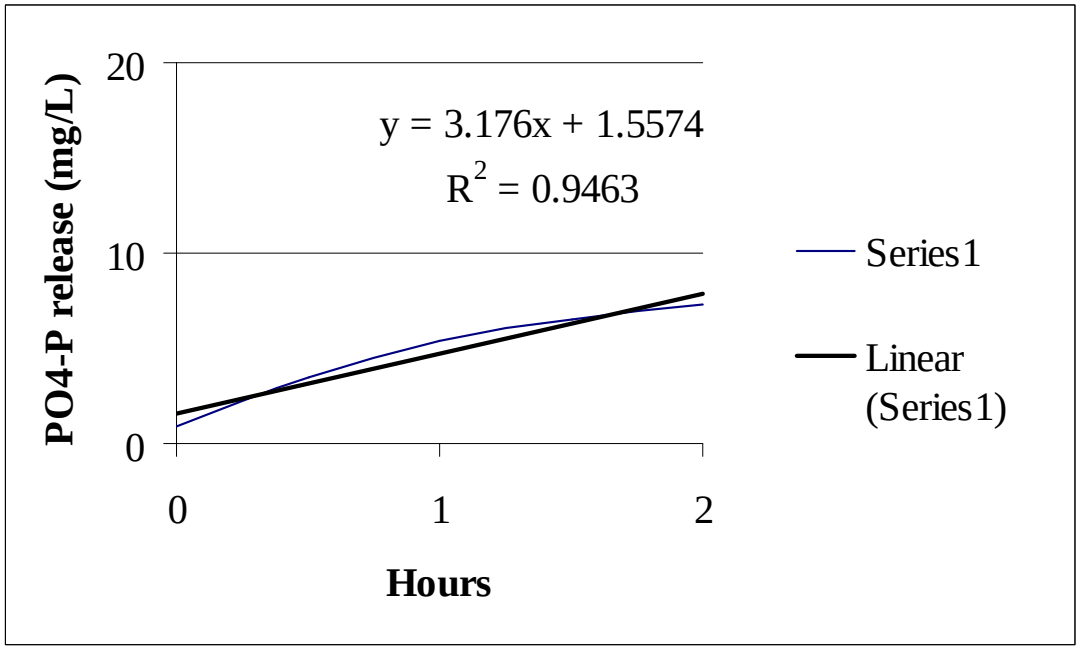

Figure B.43 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 11 (MBNR (Chemical) system mixed liquor)

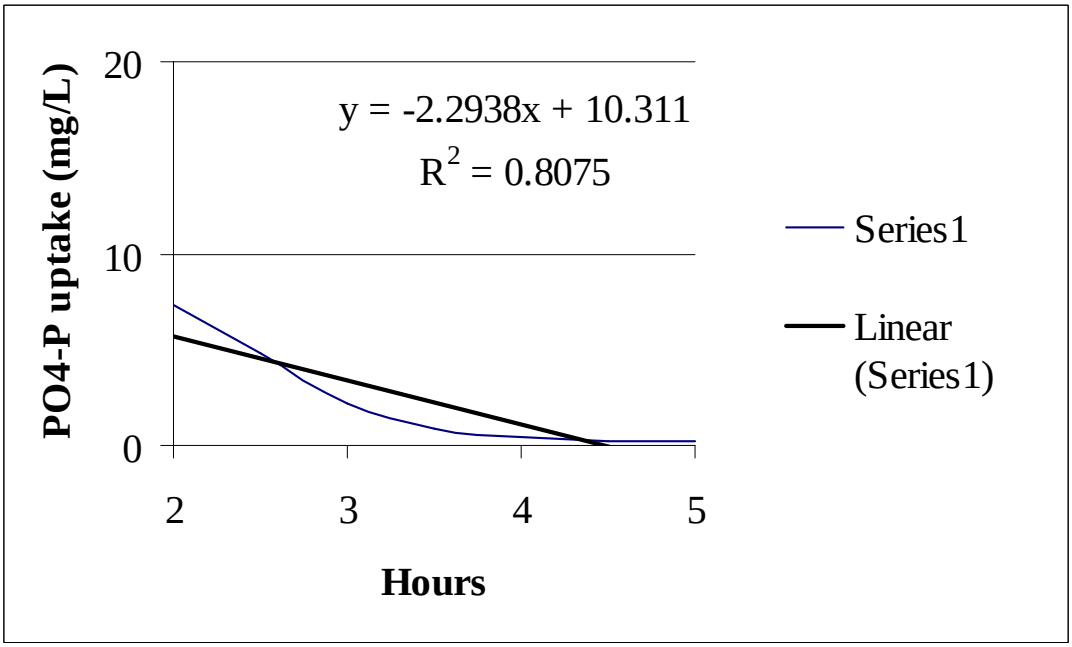

Figure B.44 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 11 (MBNR (Chemical) system mixed liquor)

B.12 Batch Test Number 12

B.12.1 MBNR (Biological) System

Table B.23 Data for batch test number 12 with MBNR (Biological) system mixed liquor

Date: 16/6/2011	Time	Hours	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	2:00 PM	0	0.138	5.03	100.0	16.08479	2.093229
Target DO = 2-3 mg/L		0.5	0.118	14.416	1.0	18.0497	3.721816
100 mg/L of acetate before anaerobic phase		1	0.142	21.093	29.3	19.70514	5.088615
		1.5	0.129	25.302	0.7	21.99964	6.446785
		2	0.162	29.025	46.9	24.77621	8.014369
		2.5	1.434	24.494	0.5	21.3084	6.2508
		3	1.474	19.163	0.0	17.77026	4.389072
		3.5	1.424	13.789	0.0	14.66669	3.12981
		4	1.411	9.331	0.0	15.2721	2.654099
		4.5	1.645	6.086	0.0	14.2755	2.135432
	7:00 PM	5	1.648	3.553	0.0	12.51727	1.632162

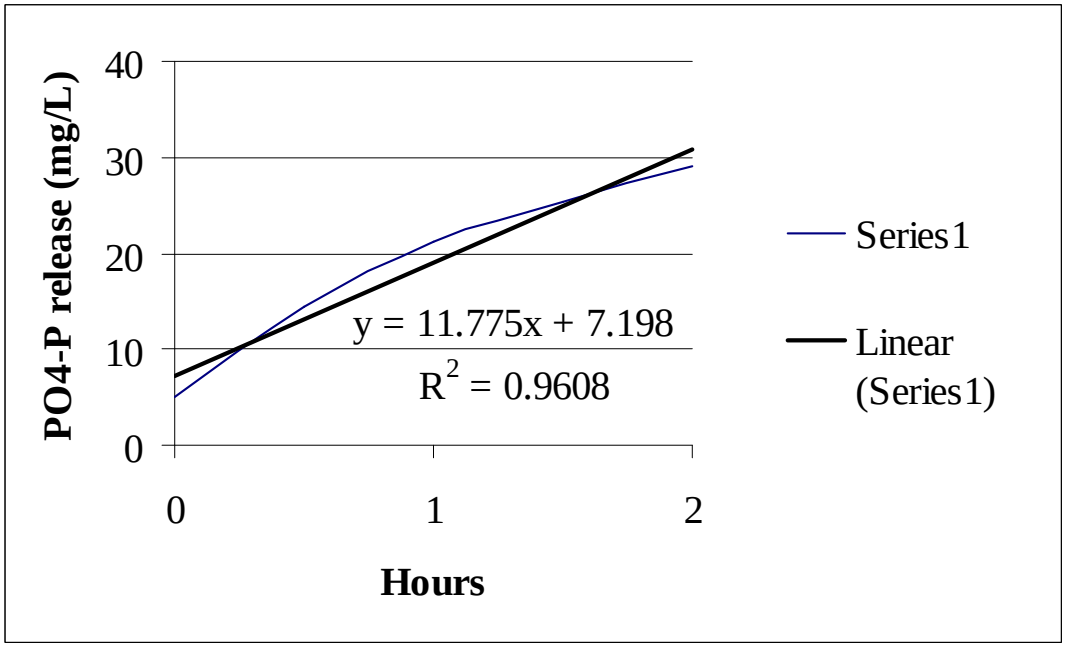

Figure B.45 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 12 (MBNR (Biological) system mixed liquor)

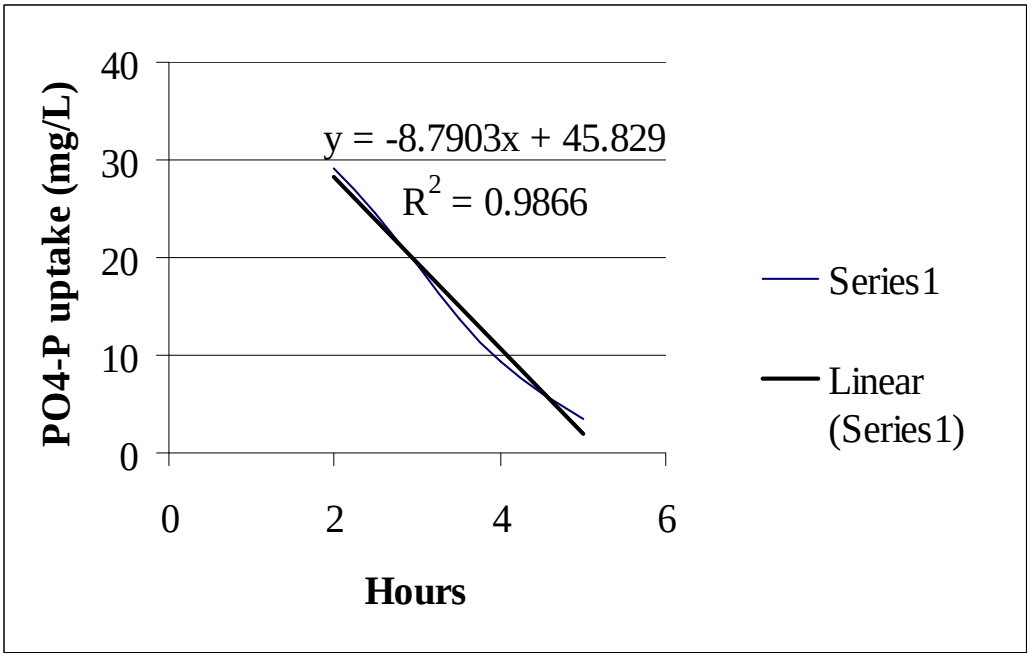

Figure B.46 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 12 (MBNR (Biological) system mixed liquor)

B.12.2 MBNR (Chemical) System

Table B.24 Data for batch test number 12 with MBNR (Chemical) system mixed liquor

Date: 16/6/2011	Time	Hours	NO_3-N (mg/L)	PO_4-P (mg/L)	Acetate (mg/L)	K^{+1} (mg/L)	Mg^{+2} (mg/L)
4 hrs for denitrification	2:00 PM	0	1.228	0.452	100.0	14.62367	1.832968
Target DO = 2-3 mg/L		0.5	0.176	2.3	66.9	13.20086	2.57371
100 mg/L of acetate before anaerobic phase		1	0.154	3.842	34.1	14.14907	3.612554
		1.5	0.67	4.581	4.3	15.99835	2.931998
		2	0.115	5.034	0.8	16.81265	3.05726
		2.5	2.176	3.548	1.6	14.13399	1.939348
		3	2.139	2.24	0.00	14.01142	1.550173
		3.5	2.153	1.612	0.0	12.89292	1.228567
		4	2.148	1.071	0.0	12.65098	1.104894
		4.5	2.266	0.868	0.0	12.29731	1.008923
	7:00 PM	5	2.41	0.638	0.0	11.58184	1.191554

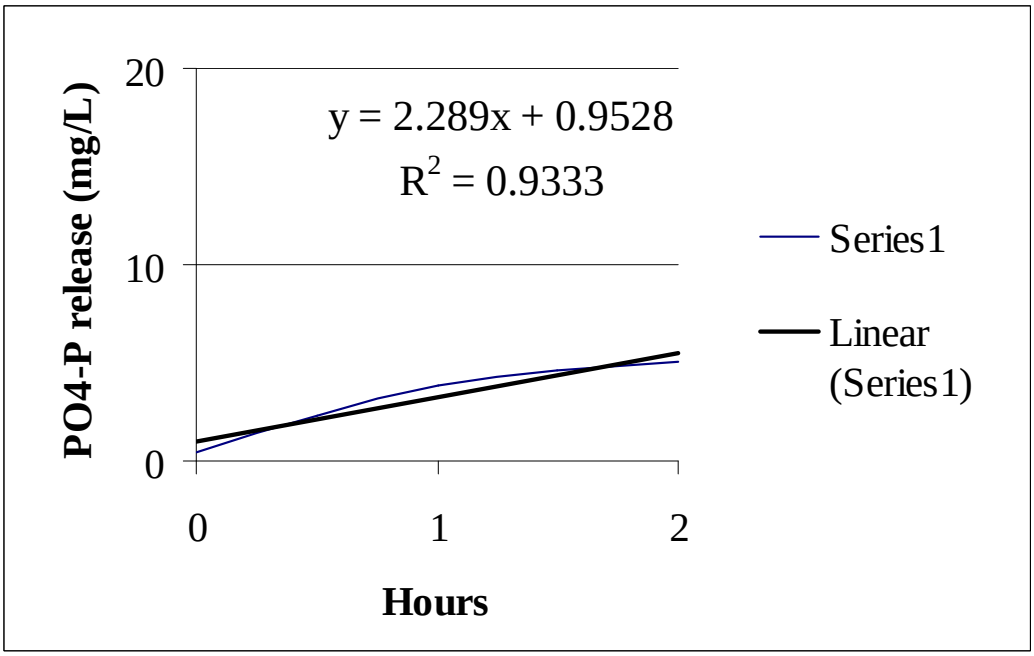

Figure B.47 Specific PO_4-P release (mg/L·hr) in anaerobic phase of batch test number 12 (MBNR (Chemical) system mixed liquor)

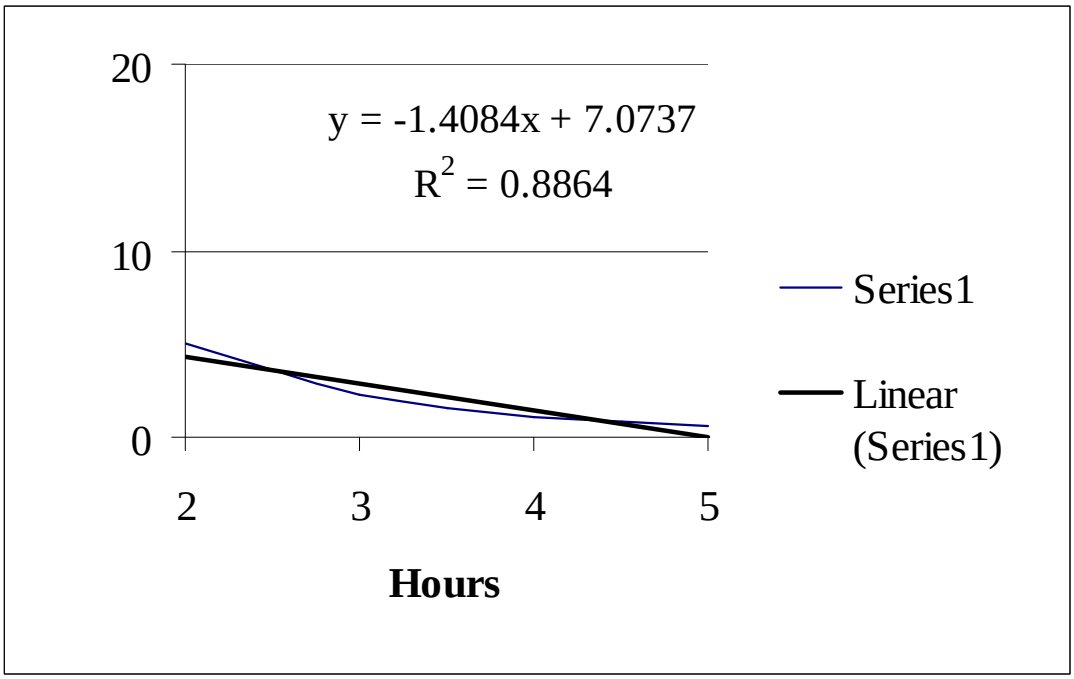

Figure B.48 Specific PO_4-P uptake (mg/L·hr) in aerobic phase of batch test number 12 (MBNR (Chemical) system mixed liquor)