Simultaneous Removal of Ammonia and Phosphorus from Wastewater in a Continuous Flow Vertical Bioreactor

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public

Abstract

The objective of this PhD thesis was to study, develop and analyze an effective bioreactor and biological process to simultaneously remove nitrogen and phosphorus compounds from wastewater with minimum requirements for energy and footprint. A novel biological nutrient removal process was developed in a vertically configured pilot-scale bioreactor. The bioreactor set-up and its biological process were undertaken in the Water Technologies Laboratory at Ryerson University from November 2012 to December 2013. The bioreactor consisted of three consecutive vertical stages including Anoxic 1, Anoxic 2 and Aerobic stages. The reactor was aligned with an Anaerobic Lateral Unit (ALU) which provided a strict anaerobic condition to cultivate and promote the growth of phosphorus accumulating organisms (PAOs). The unique features of the bioreactor are the foundations for development of a simultaneous nitrification-denitrification-biological phosphorus removal process (SNDP). This thesis reveals that SNDP is the main pathway for the removal of ammonia, nitrate, nitrite and phosphorus. The SNDP process shows high level of nitrogen and phosphorus compounds removal with over 95% phosphorous and nitrogen removal efficiencies during one (1) year of laboratory operations. The co-existence of microorganisms in the SNDP process was highly influenced by the actions of all three redox conditions, 1) anaerobic, 2) anoxic, and 3) aerobic zones. The redox variations were influenced by the bioreactor configuration, HRTs, SRTs and nutrient concentrations.

The biomass samples from the bioreactor were studied rigorously using advanced molecular biology techniques such as genomic sequencing. Microbial structure, diversity and interactions in the SNDP were studied in details. The results obtained in this work proved the presence of a new genus of microorganism known as *Saprospiracae* which occupied more than 67% of the biomass in the Anoxic stages and 48% of the biomass in the Aerobic stage of the

bioreactor. The second largest group belonged to the genus of *Zoogloea* with over 11% and 36% in the Anoxic and Aerobic stages respectively. The vertical continuous flow bioreactor developed and operated in this research created a unique habitat for the growth of these microorganisms. To optimize the SNDP process, a series of experiments were performed on the bioreactor by varying three important parameters including:

1) Dissolved Oxygen (DO) concentration in the Aerobic stage ranging from 0-0.5 to 5.5-6 mg/L;

2) COD concentration in the Anaerobic stage ranging from 1000 to 1400 mg/L and,

3) NH_3-N concentration in the inlet ranging from 49 to 120 mg/L.

The experimental results showed that DO in the range of 2.5-3 mg/L were the optimum concentration for the SNDP process. The COD concentrations fed to the ALU could be lowered to 1000mg/L at SRT of approximately 50 days. Moreover, the intracellular PHAs in the biomass was found to be a great asset for the SNDP process as COD addition to the ALU could be lowered from 1400 mg/L to 1000 mg/L without having any negative impact on the process. The inlet NH_3-N concentration of 49 mg/L was found to be the optimum level for the SNDP process. Biological phosphorus uptake was negatively affected when NH_3-N concentration in the inlet was increased from 49 mg/L to 120 mg/L. The negative effects of high NH_3-N were likely caused by increase in NO_2^- concentration and accumulation in the bioreactor which inhibited the activities of the PAOs. Both the SNDP process developed in this research and the vertical continuous flow bioreactor are innovations in the area of water/wastewater treatment. Indeed, the microbial distributions in anoxic and aerobic environment have not been found in any laboratory scale nor large scale BNR plant to date.

Acknowledgement

The concept of a vertical bioreactor in wastewater engineering belongs to my supervisor and mentor, Dr. Manuel Alvarez Cuenca. This idea manifested through his broad industrial and academic expertise, open mind and thirst for new discoveries. In the Fall 2010, he generously handed the idea to me to see how far I could advance it. I was honored to be given this opportunity and have been grateful ever since.

The results obtained from this PhD work transformed the idea of vertical bioreactor design in wastewater into a solid reality. To complete this work, I faced numerous challenges, unpredictable oppositions and unbelievable degree of hostility. But, I completed the task at hand with hard work, intellectual integrity and perseverance.

Of course, this work could not be accomplished without the continuous support and trust of my Supervisor, Dr. Cuenca.

I would like to thank Dr. Joo-Hwa (Andrew) Tay of the University of Calgary for his thorough and fair evaluation of my PhD thesis. I appreciate very much his academic honesty. Also, I'd like to extend my acknowledgement to Dr. Josh D. Neufeld of the University of Waterloo. The microbial ecology presented in Chapter 4 stems from Dr. Neufeld's knowledge of the advanced techniques in microbial analysis.

Furthermore, the work presented in this PhD thesis could not be accomplished without the emotional support of my family especially my mother, Hosnieh and my three beloved sisters, Faranak, Bahar and Mona.

Thank you all!

Maryam

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List of Abbreviations

ANAMMOX	Anaerobic Ammonium Oxidizing Bacteria
ADP	Adenosine Di-phosphate
ALU	Anaerobic Lateral Unit
AOB	Ammonium Oxidizing Bacteria
ASM	Activated Sludge Model
ATP	Adenosine Triphosphate
BOD	Biological Oxygen Demand
BPR	Biological Phosphorus Removals
COD	Chemical Oxygen Demands
DNA	Deoxyribonucleic Acid
DO	Dissolved Oxygen
DPAO	Denitrifying Phosphorus Accumulating Organisms
GAO	Glycogen Accumulating Organisms
HRT	Hydraulic Residence Time
MLE	Modified Ludzack-Ettinger
MLSS	Mixed Liquor Suspended Solids
MLVSS	Mixed Liquor Volatile Suspended Solids
NIR	Nitrite Reductase
NOB	Nitrite Oxidizing Bacteria
NOR	Nitric Oxide Reductase
OTU	Operational Taxonomic Unit
PAO	Phosphorus Accumulating Organism
PCR	Polymerization Chain Reaction
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PHV	Polyhydroxyvalerate
P_i	Phosphorus
PP	Poly-phosphate
RAS	Returned Activated Sludge
SND	Simultaneous Nitrification-Denitrification
SNDP	Simultaneous Nitrification-Denitrification-Phosphorus Removal
TCA	Tri-Carboxylic Acid
TP	Total Phosphate
TSS	Total Suspended Solids
SBR	Sequencing Batch Reactor
SRT	Solids Residence Time
VFA	Volatile Fatty Acids
WWTP	Wastewater Treatment Plant

List of Symbols

Symbol	Description	Units
pH	pH value	[--]
T	Temperature	[^{o}C]
X	Biomass Concentration	[mg/L]
S	Substrate Concentration	[mg/L]
X_{PAOs}	PAOs Biomass Concentration	[mg/L]
X_{DPAOs}	Denitrifying PAOs Biomass Concentration	[mg/L]
X_{AOB}	Ammonium Oxidizing Biomass Concentration	[mg/L]
X_{NOB}	Nitrite Oxidizing Biomass Concentration	[mg/L]
μ	Microbial Specific Growth Rate	[hr^{-1}]
μ_{max}	Maximum Specific Growth Rate	[hr^{-1}]
$\mu_{max,\ AOBs}$	Maximum Specific Growth Rate of AOBs	[hr^{-1}]
$\mu_{max,\ PAOs}$	Maximum Specific Growth Rate of PAOs	[hr^{-1}]
$\mu_{max,\ DPAOs}$	Maximum Specific Growth Rate of DPAOs	[hr^{-1}]
S_{NH3-N}	Ammonia-Nitrogen Concentration	[mg/L]
S_{NO3-N}	Nitrate-Nitrogen Concentration	[mg/L]
S_{NO2}	Nitrite Concentration	[mg/L]
S_{TP}	Total Phosphorus Concentration	[mg/L]
S_{COD}	COD Concentration	[mg/L]
S_{BOD}	BOD Concentration	[mg/L]
K_{NH3-N}	Half Saturation Ammonia-Nitrogen Concentration	[mg/L]
K_{NH-AOB}	Half Saturation Ammonia-Nitrogen Concentration for AOBs	[mg/L]
$K_{COD-AOB}$	Half Saturation COD Concentration for AOBs	[mg/L]
$K_{TP-PAOs}$	Half Saturation Total Phosphorus Concentration for PAOs	[mg/L]
K_{DPAOs}	Half Saturation Total Phosphorus Concentration for DPAOs	[mg/L]
$K_{DO-PAOs}$	Half Saturation Oxygen Concentration for PAOs	[mg/L]
$K_{NO3-N-DPAOs}$	Half Saturation Nitrate-Nitrogen Concentration for DPAOs	[mg/L]
ΔG	Change in Gibbs Free Energy	[J/mole]

Chapter 1- Introduction

1.1 Significance of Biological Nutrient Removal

Nutrient pollution in natural water sources has become an overwhelming problem for many countries around the world. Accumulation of nutrients in our water bodies expedites the growth of toxic algae and other aquatic species in lakes, rivers and coastal waters causing eutrophication and hypoxia. Human illness, losses of fishery stocks, negative economic impact on tourism and recreational industries, and property losses are a few examples of the impact of eutrophication. Although the economic cost of eutrophication is complex, to establish the combined losses caused by eutrophication in U.S freshwater have been estimated in $2.2 billion per year (Dodds et *al.*, 2009). Excessive use of fertilizers in agriculture and inadequate industrial and domestic wastewater treatment are the main sources of nutrients (i.e. phosphorus and nitrogen). Nutrient discharges into the water bodies significantly increase the growth of algae. Excessive growth of algae and other aquatic plants imposes a great demand for oxygen for both their growth and decay. The biodegradation of algae by microorganisms requires great amounts of oxygen. According to Randall et *al.* (1992), it has been estimated that one (1) kilogram of soluble phosphorus (P) can produce 111 kilograms of algae which consequently produces 138 kilograms of chemical oxygen demand (COD). Similarly, one (1) kilogram of nitrogen (N) could potentially produce 16 kilograms of algae with a COD equivalency of 20 kilograms. Therefore, phosphorus and nitrogen discharges can produce large amounts of COD. This COD accumulation caused by excess of nutrients is detrimental to aquatic life. This global problem can be mitigated with both better environmental regulations and environmental technologies/processes for nutrient removal from water/wastewater.

Nitrogen and phosphorus are the two most influential elements in nature. Thus, there is a strong urge to learn about the mechanisms that process, transport and store these elements in the

atmosphere, hydrosphere, geosphere and biosphere. Nitrogen and phosphorus can be transformed into simple or complex compounds in water/wastewater. The biochemical processes involved in nitrogen transformation include ammonification, nitrification, denitrification, nitritation and anaerobic ammonium oxidation. To date, the only known biological phosphorus process is the utilization of some specialized organisms called phosphorus accumulating organisms (PAOs) capable of storing poly-phosphate. Phosphorus can also be removed from wastewater using certain chemical coagulants (i.e. aluminum sulphide, iron chloride).

Biological Nutrient Removal (BNR) is widely used to reduce the impact of point source nutrient discharges on the oxygen content of receiving waters. BNR has proven to be the most economic and environmentally beneficial path to combat nutrient pollution. The overall energy requirement of a BNR process can be reduced drastically by implementing both biological phosphorus removal (BPR) and biological nitrogen removal. Combined phosphorus and nitrogen compounds removal results in COD stabilization in the anaerobic and anoxic zones of wastewater treatment plants. For example, nitrification does not require carbon sources (COD), but consumes dissolved oxygen (DO) and alkalinity (carbonate alkalinity as CO_3^{-2}) to convert ammonia (NH_3) to nitrate (NO_3^-). On the other hand, denitrification utilizes COD and produces alkalinity up to 50% of what was consumed during nitrification. Recovery of the alkalinity destroyed during nitrification is a huge benefit for poorly buffered wastewater. In addition, the aeration required for nitrification can be recovered (approximately 50%) using the influent COD for denitrification (Randall et *al.,* 1998).

The BPR process involves the accumulation of short-chain fatty acids (SCFA) in the microbial cells under anaerobic conditions as polyhydroxyalkanoates (PHAs). Intracellular PHAs are subsequently used for energy and growth under anoxic and aerobic conditions where

sufficient electron acceptors are available. During this step, the phosphorus is taken up by microorganisms to store excess energy required for growth and cell maintenance. The use of nitrate rather than oxygen has the following advantages:

- Organic substrates in wastewater are usually limited. Denitrifying PAOs (DPAOs), utilize their intracellular PHAs to remove both phosphorus and nitrate. Therefore, the same amount of organics (used in denitrification) can be used for nitrate and phosphorus removal with no additional COD requirements.
- This double use of carbon source will result in reduced sludge production.
- The use of nitrate as electron acceptor reduces the aeration demands of BNR processes (Kuba et *al.*, 1996).

The microbial populations in large BPR plants often differ from those seen in laboratory reactors. For instance, *Rhodocyclus* related organisms have been found as one of the dominating PAOs in laboratory reactors whereas *Actinobacteria* have been found as major and important PAOs in full-scale plants (Beer et. *al.*, 2006). Figure 1-1 shows the distribution of major BNR organisms in a Danish wastewater treatment plant (Nielsen, et *al*., 2010).

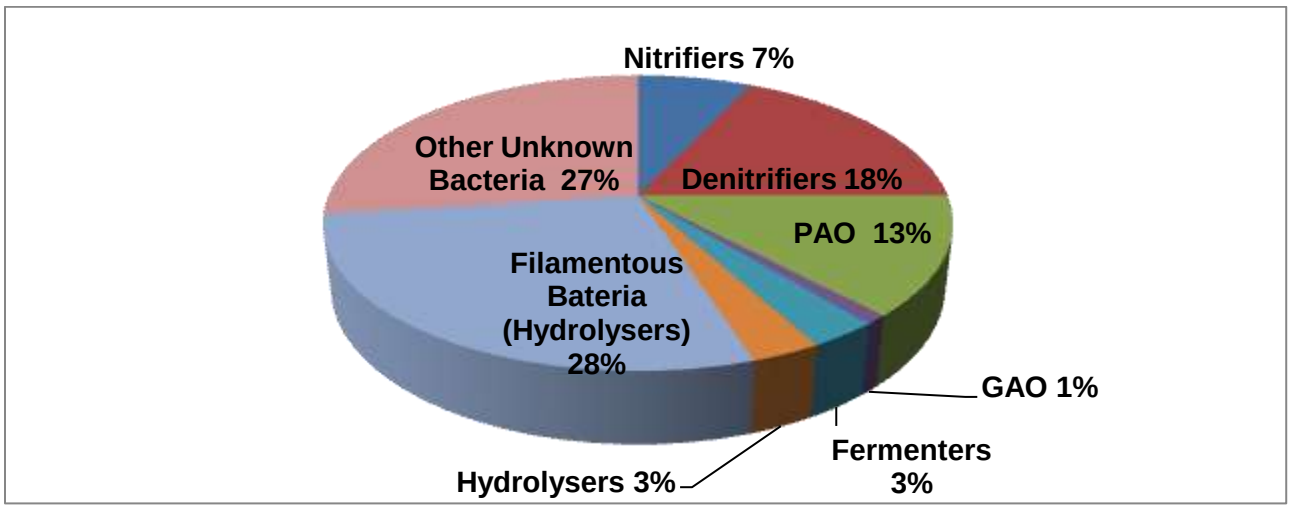


Figure 1-17 Distribution of major functional groups in Danish BPR plants

(Nielsen, et *al*., 2010)

Figure 1-1 shows that nutrient removal organisms form (40%) less than half of the microbial population in wastewater. More than 27% of them are unclassified and still unknown. Thus, there are great needs for advanced bio-processes, and unknown pools of microorganisms in wastewater provide far-reaching research opportunities.

1.2 Objectives of the Present Research

The main objective of the research presented in this dissertation was to study, design, develop and analyze an effective process which can simultaneously remove ammonia and phosphorus from wastewater with minimum requirements for energy and construction foot print. To develop this process, an innovative bioreactor was designed. Extensive study of the fundamentals of biochemical reactions in wastewater, reaction kinetics and reactor design were the three corner stones of this work. Combining the fundamentals of chemical engineering such as fluid dynamics, process control and thermodynamics were essential in developing the idea of a vertical bioreactor. In conjunction with the study of both conventional and advanced processes in wastewater treatment, this researcher designed the experimental method, including numerous modifications and data collection and analyses.

The performance of the bioreactor and ultimately the effectiveness of the process have been analyzed with the help of recent scientific techniques and instrumentation in the field of molecular biology. The results obtained throughout the course of this PhD research have been analyzed in two parallel phases:

- Macro-level (bioreactor design and performance)
- Micro-level (microbial structure identified in the process)

Both macro and micro-level analyses of the results helped the author to understand that the dominant processes were simultaneous nitrification, denitrification and biological phosphorus removal.

Macro-Level Analysis

Bioreactor configuration is a fundamental parameter in successful BNR processes. The reactor structure allows for the creation of the environmental conditions leading to the formation of ecosystems that favor the growth of new microbial species as suggested by Littleton et.*al.* (2003). The most appropriate reactor configuration for a BNR process depends on factors such as the target effluent quality, influent quality, process control, biological process complexity, and available foot print. Construction space limitation is one of the problems facing municipal wastewater treatment facilities. Many of the existing BNR processes take place in plane, horizontal basins with large foot print. The multistage vertical bioreactor used in the present work was capable of producing and governing an effective BNR process. The importance of the bioreactor designed and used in this work includes:

- Construction flexibility and lower land requirements due to the cylindrical symmetry and vertical configuration;
- Superior mixing and mass transfer because of the circular cross section which avoids the stagnancies normally seen in rectangular bioreactors;
- Fewer pumping requirment due to the vertical alignment of the stages which provides smooth gravity flow of water from one stage to the other;
- Fewer number of pumps and mixers significantly reduce the energy consumption and consequently the operational and maintenance costs.

Micro-Level Analysis

The microbial process developed in this bioreactor was named "simultaneous nitrification denitrification-BPR" (SNDP). The SNDP process was ecologically sound as it produced less sludge and required both less oxygen and less organic carbon than conventional BNR processes. One of the important parts of this PhD research was to determine, quantitatively in the reactor, the microbial population involved in phosphorus and nitrogen compounds removal. The micro-level analysis was used to determine the phylogenetic affiliations and dynamics of the bacteria involved in the SNDP process. The genetic sequencing of the samples provided knowledge on microbial distribution and structure at the single cell level. A new species that belongs to the phylum of *Saprospirasae* was found using advanced molecular biology techniques. *Saprospirasae* and *Zoogloea* were two dominant organisms in the biomass samples and their symbiotic relationship may have been the key to the successful SNDP process performance.

The overall community dynamics combined with the identification of the main bacterial populations were important tools to optimize the SNDP process. This knowledge can be used to evaluate the feasibility of such process in industrial scale application.

1.3 Dissertation Structure

Figure 1-2 lays out the structure of this thesis. Chapter 1 and 2 include introduction and literature review. Chapter 3 provides bioreactor design and process results that were obtained throughout the course of this PhD research. Chapter 4 gives detailed results of the microbial analysis of the samples from the bioreactor. The proposed behavior and physiology of the microorganisms found in the samples were discussed using available scientific literature. Chapter 5 delivers a more specific approach to evaluate the performance of the bioreactor and the SNDP

process. Three unstructured empirical models, predicting different process design parameters, have been proposed in chapter 6. The models adequately represent experimental data for nitrification, denitrification and BPR. Chapter 7 shows the results and discusses the impacts of variation in DO, NH_3-N and COD concentrations on both NH_3-N and phosphorus uptake rates in the Anoxic and Aerobic stages of the bioreactor.

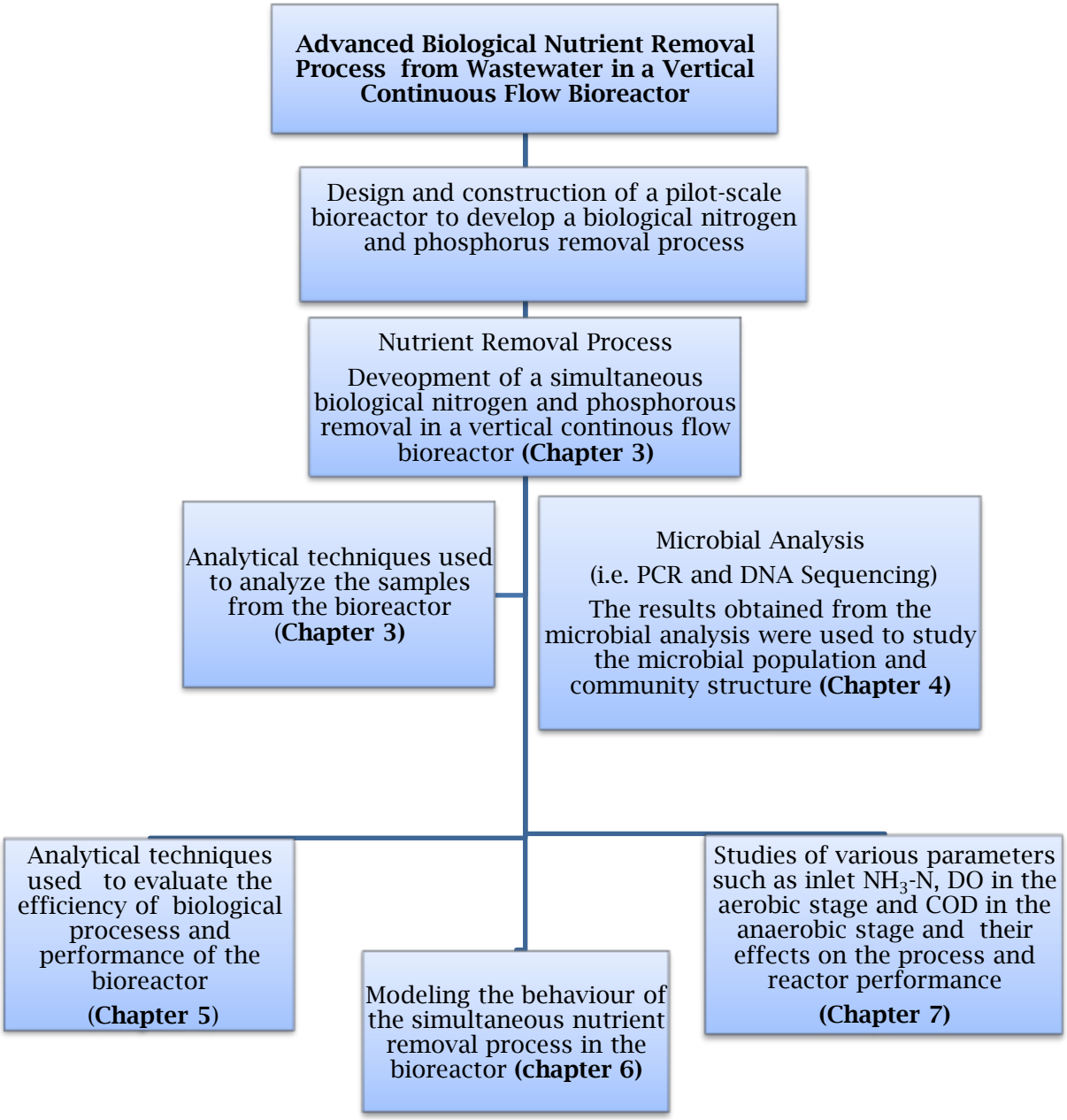


Figure 1-18 Diagrams of the phases of the research

Chapter 2 - Literature Review

2.1 Nitrogen and Phosphorus Cycles

Nitrogen is an essential element of life. Living organisms contain up to 14% nitrogen in the forms of protein and DNA. In the environment, nitrogen is transformed into various compounds as a result of processes of nitrogen fixation, nitrogen assimilation, dissimilation, nitrification and denitrification. Industrial nitrogen fixation (i.e. Haber process) and increased urbanization have led to the distortion of the natural nitrogen balance. Therefore, there is an increasing accumulation of nitrogenous compounds in rivers, lakes, aquifers and other water bodies (Wong et. *al.*, 2003). Nitrogen compounds exist in water as organic nitrogen, ammonia/ammonium, nitrite and nitrates. Other forms of nitrogen compounds include nitrous oxide, nitric oxide, and nitrogen dioxide. Figure 2-1 illustrates a full nitrogen cycle showing the production and transformation of nitrogen compounds in the atmosphere, soil and aquifers.

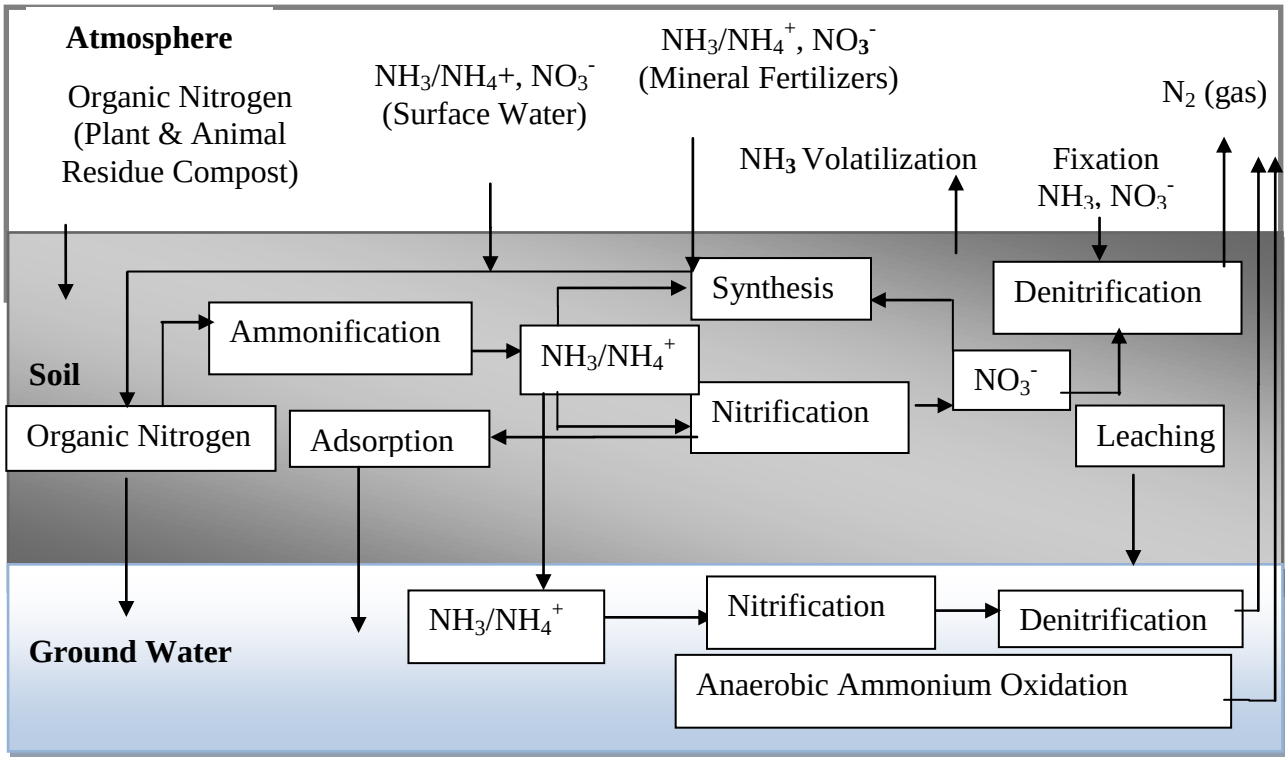


Figure 2–1 Nitrogen Cycle in the Environment

(US EPA, 1993)

Nitrogen compounds exist in all three phases (gas, liquid, solid). Free nitrogen or nitrogen gas (N_2) has an extremely strong triple bond (N≡N) and is very stable whereas its single bond is rather weak. The strength of a carbon single bond (C-C) is 347 kJ/mol but nitrogen single bond (N-N) strength is only 160 kJ/mol. The low energy in N-N bond results in natural formation of unstable and highly reactive nitrogen compounds. Table 2-1 lists the thermodynamic properties of nitrogenous compounds in wastewater (Jetten, 2009).

Table 2-1 Thermodynamic Properties of Inorganic Nitrogen Compounds

(Wong et *al.*, 2003)

Compound Formula	Oxidation State	$\Delta H_f°$ (kJ mol^{-1})	$\Delta G_f°'$ (kJmol^{-1})	S° (J mol^{-1}K)	pK
Ammonium, NH_4^+	-3	133.1	-79.4	713	9.2
Hydrazine, N_2H_4 (aq)	-2	34.4	128.5	-316	6.1
Hydroxylamine, NH_2OH (aq)	-1	-98.7	-22.9	-254	6.0
Dinitrogen gas, N_2 (g)	0	0	0	0	-
Nitrous oxide, N_2O (g)	+1	82.4	104.6	-74	-
Nitric oxide, NO (g)	+2	90.6	86.9	12	-
Nitrite, NO_2^-	+3	-105.0	-37.4	-227	3.3
Nitrogen dioxide, NO_2 (g)	+4	33.3	51.5	-61	-*
Nitrate, NO_3^-	+5	-208.2	-111.7	-324	-1.5

° refers to standard conditions (pH 0, 25°C)
°' to physiological conditions (pH 7, 25°C).
*Nitric dioxide reacts in water to nitrite and nitrate
$\Delta H_f°$ (kJ mol^{-1}): Standard-State Enthalpy Change in fluid phase
$\Delta G_f°'$(kJmol^{-1}): Standard-State Gibbs Function Change
S° (J mol^{-1} K): Entropy
pK: logarithmic measure of the acid dissociation constant which is a quantitative measure of the strength of an acid in solution

In nature (i.e. marine settings) the supply of NH_4^+, NO^-_2 and NO^-_3 is often the limiting growth. Hence, it is crucial to understand the nitrogen cycle. As dead algae are being decomposed, nitrogen is released as NH_4^+. In the oxic zone, the uppermost part of the ocean, this is converted to NO_2^- and eventually to NO_3^- by nitrification catalyzed by nitrifying bacteria. On

the one hand the released nitrate can then be taken up by growing phytoplankton. On the other hand it may be used as a terminal electron acceptor for the anaerobic oxidation of organic matter. Wastewater generated from municipalities, industrial facilities, landfill leachate, farms and other sources are very rich in nitrogen compounds. Nitrogen compounds are produced, assimilated, absorbed, released and transferred from one phase to the other through the natural nitrogen cycle. Processes responsible for nitrogen transformations include:

- Nitrogen Fixation
- Ammonification
- Bacterial Synthesis

Nitrogen Fixation is the conversion of free nitrogen (N_2) into nitrogen compounds that can be assimilated by green plants. Nitrogen fixation can occur biologically, through lightning or the biological processes:

Biological: $N_2 \rightarrow$ Organic-N

Lightning: $N_2 \rightarrow NO_x$

In industry, nitrogen fixation takes place through the Haber-Bosch process. N_2 and H_2 are combined under high pressure and temperature in the presence of a nickel catalyst.

$$N_2\,(g) + 3H_2\,(g) \xrightarrow{\text{Temperature: } 400\text{-}500^{o}C;\ \text{Pressure: } 200\text{-}300\ \text{atm}} 2\,NH_3\,(g) \qquad \text{[Eq. 2-1]}$$

A century ago, there was insufficient reactive nitrogen to feed the growing human population. So, all industries including the food industry depended on fossil nitrogen and manure. The invention of the Haber-Bosch process created a revolution as humans could make cheap reactive nitrogen from an inexhaustible supply of atmospheric N_2. Unfortunately, the excessive production of reactive nitrogen is causing unprecedented changes to the global

nitrogen cycle by doubling the total fixation of nitrogen. Human alteration of the natural nitrogen cycle has caused major transformations in water, air and soil (Sutton, 2011).

Ammonification is the biochemical degradation of organic-N into NH_3 (ammonia) or NH_4^+ (ammonium) by heterotrophic bacteria using organic carbon as energy source for bacterial reproduction, cellular reconstruction and growth. Heterotrophs can transform organic nitrogen either in the presence of oxygen (aerobic conditions) or without oxygen (anaerobic conditions).

$$\text{Organic-N} + \text{Microorganisms} \rightarrow NH_3/\ NH_4^+ \qquad \text{[Eq. 2-2]}$$

Cellular Synthesis is the biochemical mechanism in which NH_4^+ or NO_3^- is converted into plant protein (Organic-N) (WEF, 1998):

$$NH_4^+ + CO_2 + \text{green plants} + \text{sunlight} \rightarrow \text{Organic-N} \qquad \text{[Eq. 2-3]}$$

$$NO_3^- + CO_2 + \text{green plants} + \text{sunlight} \rightarrow \text{Organic-N} \qquad \text{[Eq. 2-4]}$$

Phosphorus Cycle

Phosphorus is an essential element for microbial growth and there is abundant concentration of phosphorus in soil due to the presence of microorganisms. The global cycle of phosphorus (shown in Figure 2.2) starts from the earth's crust where it erodes and passes through the water cycle to our ecosystem. Phosphorus does not have a rapid global cycle like the Carbon (C) or Nitrogen (N) cycles. Due to its slow natural cycle, low solubility in water and rapid transformation to insoluble forms, phosphorus has become the growth-limiting nutrient in nature. Human interference in the phosphorus cycle occurs by exploitation of the phosphate rocks, overusing fertilizers and insufficient phosphorus removal from wastewater (Smil, 2000).

Economically exploitable phosphate rock, the major source of industrial phosphorus, is estimated to be depleted in 50–100 years (Steen, 1998). Furthermore, eutrophication is caused by excess phosphorus concentrations in surface waters which is known as a major environmental concern worldwide (McDowell, 2004).

Figure 2-2 shows biological, chemical, biochemical and biogeochemical transformation of phosphorus in nature. This literature survey focuses on the biological transformation of phosphorus in water bodies.

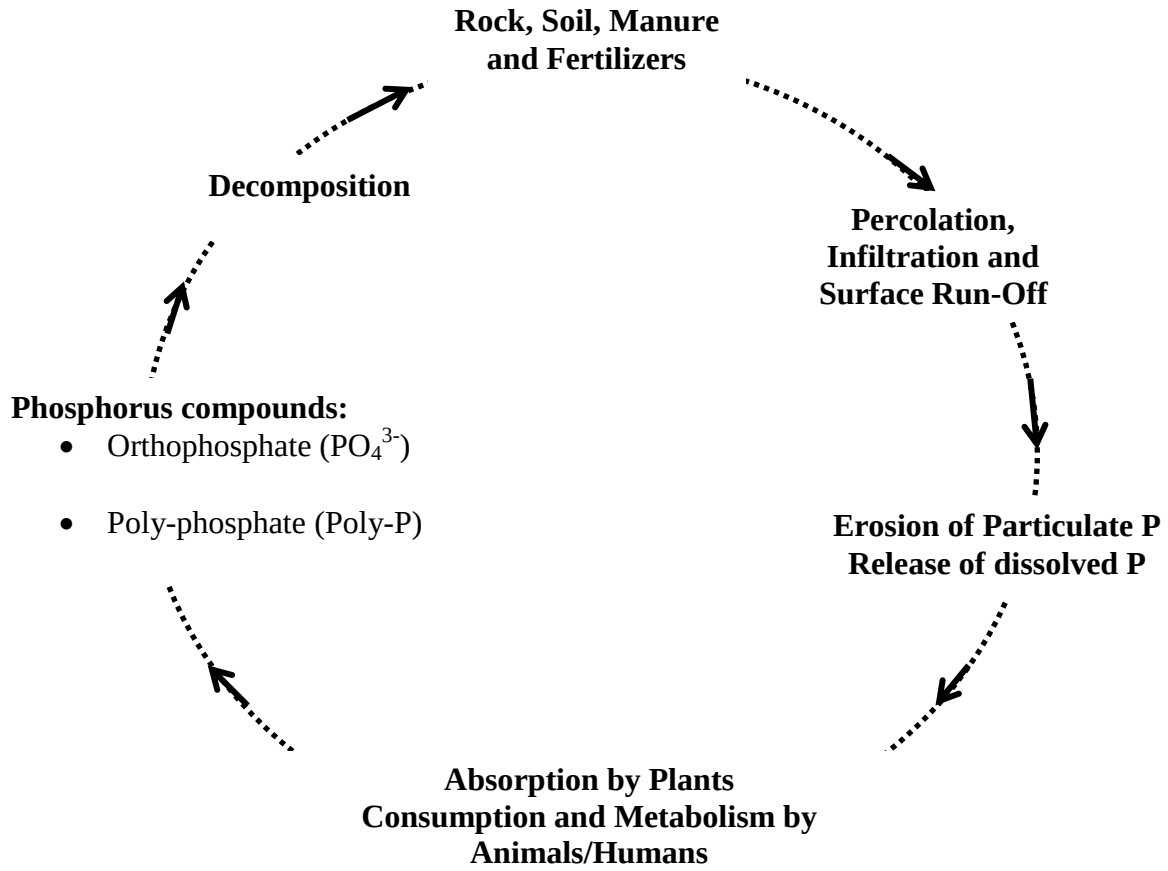


Figure 2–2 Phosphorus Cycle

(McDowell, 2004)

2.2 Biological Treatment of Nitrogen Compounds in Wastewater

Biological nutrient removal and recovery have become important parts of water/wastewater engineering in the last three decades. Chemical nutrient removal has been a rudimentary and problematic method widely adopted by water pollution control plants. Indeed, due to the high costs of chemicals and large volume of precipitating sludge, chemical nutrient removal is considered an expensive and labor intensive method. Whereas, processes such as biological ammonium, nitrates/nitrite and phosphorus removals have shown superiority over conventional chemical methods and are far more cost effective and environmentally friendly (Tchobanoglous, 2003). There are two biological processes in wastewater treating nitrogen compounds into free nitrogen gas.

- The first process is Simultaneous Nitrification-Denitrification (SND) (section 2.2.4)
- The second process is Anaerobic Ammonium Oxidation (ANAMMOX) (section 2.2.3)

Both processes comprise a number of important steps. These steps involve a variety of specialized microorganisms some of them identified in the last decades and many of them still unknown. Fortunately, the advancement in molecular biology techniques allows scientists to understand better the microbial processes in our environment or engineered ecosystem. Some of these microbial analysis techniques include i) denaturant gradient gel electrophoresis (DGGE), ii) polymerization chain reaction (PCR), iii) fluorescence *in situ* hybridization (FISH) with DNA probes, iiii) DNA sequencing, iv) cloning and the creation of a gene library. As a result, biological nitrogen removal from wastewater provides new opportunities and possibilities for scientific research.

2.2.1 Nitrification

In wastewater treatment, nitrification involves two phylogenetically unrelated groups of bacteria. Ammonium oxidizing bacteria (AOBs) convert NH_4^+ to NO_2^- and Nitrite Oxidizing Bacteria (NOB) oxidize NO_2^- to NO_3^- (Knapp, 2007). Therefore, the two steps of nitrification include:

- NH_4^+ oxidation is performed by a wide group of AOBs.
- NO_2^- oxidation is the 2nd step in nitrification and is performed by NOB.

The biochemical reaction is shown below:

$$NH_4^+ + 3/2O_2 \rightarrow NO_2^- + 2H^+ + H_2O \qquad \text{[Eq. 2-5]}$$

NOBs complete the second step in nitrification:

$$NO_2^- + 1/2O_2 \rightarrow NO_3^- \qquad \text{[Eq. 2-6]}$$

The ammonium oxidation to hydroxylamine is catalyzed by ammonia monooxygenase (AMO) as shown in Eq. 2-7. Next, is the oxidation of hydroxylamine to nitrous acid is catalyzed by hydroxylamine oxidoreductase (HAO) as shown in Eq. 2-8.

$$NH_4^+ + O_2 + 2H^+ + 2e^- \xrightarrow{AMO} NH_2OH + H_2O \quad \Delta G = -120 \text{ kJ/mol} \qquad \text{[Eq. 2-7]}$$

$$NH_2OH + + H_2O \xrightarrow{HAO} HNO_2 + 4H^+ + 4e^- \quad \Delta G = -114 \text{ kJ/mol} \qquad \text{[Eq. 2-8]}$$

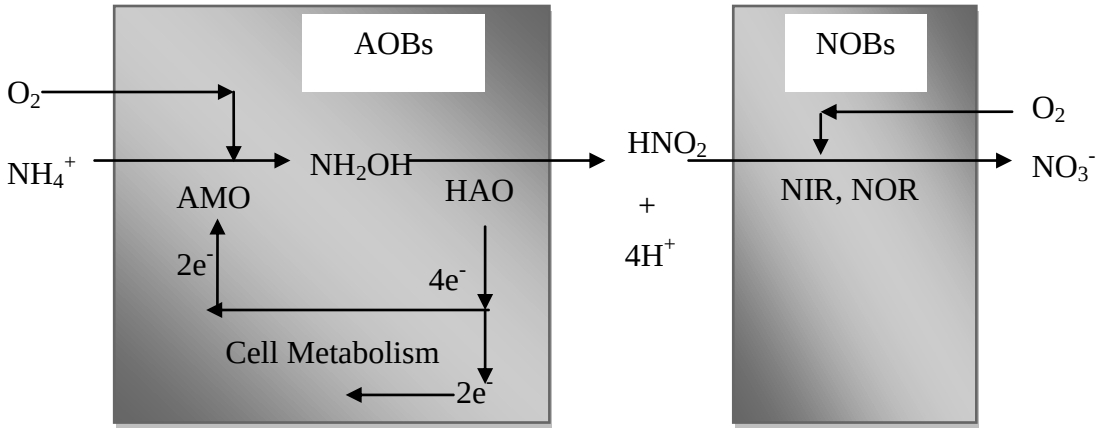


Figure 2–3 Enzyme Reactions involved in Ammonium Oxidation by AOBs and Nitrite Oxidation by NOBs

(Arp, et *al*. 2003)

In Figure 2-3, nitrite reductase (NIR) and nitric oxide reductase (NOR) are enzymes involved in the second step of nitrification. The ammonium oxidation is the rate-limiting step of nitrification because of the slow growth rates and sensitivity of the AOBs (Wagner, 1996). Most of the ammonium in the cell is oxidized and converted into nitrite and only a small fraction is assimilated by the biomass for cell metabolism. In general, AOBs are a subclass of the β-proteobacteria. Some of the well-known AOBS are *Nitrosomonas oligotropha* and *Nitrosospira* clusters found in low ammonium loading wastewater. Members of the *Nitrosomonas europaea* and *Nitrosococcus mobilis* clusters are found in systems with high ammonium loads (Mobarry et. *al*., 1996). The threshold of ammonium loading that shifts the community structure of AOBs in wastewater is still unclear.

2.2.2 Denitrification

The denitrification process is performed by facultative anaerobic prokaryotes (Knowles, 1982). It reduces nitrate in the following steps: $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$.

The nitrate (NO_3^-) formed by nitrification is used by plants as either a nitrogen source for cellular synthesis or is reduced to N_2 through the process of denitrification by heterotrophic bacteria (US EPA, 1993). NO_3^- can, however, contaminate groundwater if it is not used for synthesis or reduced through denitrification. Heterotrophic bacteria utilize the oxygen attached in the NO_3^- molecule in the absence of free oxygen in water. The reaction by denitrifying bacteria is shown below:

$$NO_3^- + \text{Organic Matter} \xrightarrow{\text{bacteria}} N_2 + CO_2 + OH^- + H_2O \qquad \text{[Eq. 2-9]}$$

Denitrification is very similar to the biological oxidation of organic matter except that it occurs without the presence of oxygen. Heterotrophic bacteria under aerobic conditions oxidize organic compounds as it is shown in Eq. 2-10.

$$O_2 + \text{Organic Matter} \rightarrow CO_2 + OH^- + H_2O \quad \text{[Eq. 2-10]}$$

If oxygen is dissolved in water, microorganisms will preferentially use oxygen rather than NO_3^- to oxidize the organic matter. When this occurs, NO_3^- accumulates and passes into both surface and ground water. Thus, the anoxic phase plays a very important role in wastewater treatment where NO_3^- is used as an electron acceptor. It is also critical that sufficient carbon source be available to serve as the electron donor for denitrification. Denitrifiers use nitrate/nitrites as electron acceptors in the absence of molecular oxygen. Autotrophic denitrification is also possible with either elemental sulfur or hydrogen gas used as the electron donor. Autotrophic denitrification is not a significant process in the treatment of wastewater. Denitrification typically involves the sequential reduction of NO_3^-, NO_2^-, NO, N_2O, and finally N_2. This pathway is catalyzed by metallo-enzymes (reductases). In biological processes, the main function of metals is to serve as electron transfers in the form of electrophiles or nucleophilic groups. Incomplete denitrification leading to N_2O accumulation may occur due to lack of the key metal cofactors such as copper, and when insufficient electron donor is available for full denitrification. In addition, the presence of inhibitors, such as dissolved oxygen (DO) and hydrogen sulfide (H_2S) as well as acidic environment can result in incomplete denitrification (Bergaust, 2010). Denitrification can be carried out independently or in conjunction with biological phosphorus removal (Grady, 1999). Figure 2-4 shows the nitrification-denitrification processes in biological wastewater treatment. The following reaction shows the conversion of nitrate to free nitrogen.

$$NO_3^- + \text{Organic Matter (i.e. BOD)} \rightarrow N_2\,(\text{gas}) + CO_2 + H_2O + OH^- + \text{New Cells} \quad \text{[Eq. 2-11]}$$

Organic matter is used by denitrifying bacteria as carbon and energy source. The type and amount of organic matter are important factors in the rate of denitrification. Organic compounds

(carbon source) include methanol (CH_3OH) and Volatile Fatty Acids (VFA) such as acetic acid, propionic acid and butyric acid (Jeyanayagam, 2005).

2.2.3 Anaerobic Ammonium Oxidation (ANAMMOX Process)

Anaerobic ammonium oxidation or Deammonification consists of two steps:

- Partial Nitrification
- Anaerobic Oxidation of Ammonia

1. <u>Partial Nitrification</u>

Partial NH_4^+ oxidation in which ammonium oxidizing bacteria (AOBs) consume the NH_4^+ in water and produce NO_2^- is shown in Eq. 2-12 below. Because of the inhibitory nature of excess NO_2^-, its generation by AOBs can be controlled by selecting the optimum conditions of temperature, pH, SRT and HRT.

The first step is partial nitrification of NH_4^+ by AOBs.

$$4NH_4^+ + 3O_2 \rightarrow 2NH_4^+ + 2NO_2^- + 4H^+ + 2H_2O \qquad \text{[Eq. 2-12]}$$

2. <u>Anaerobic Oxidation of Ammonia by Anammox</u>

The second step is a process in which NH_4^+ and NO_2^- are converted to N_2 by Anammox bacteria. Anammox bacteria utilize NH_4^+ and NO_2^- as electron acceptors and produce N_2. In the Anammox process, hydrazine (N_2H_4) and hydroxylamine (NH_2OH) are formed as process intermediates. The oxidation of NH_4^+ is mediated by the enzyme hydroxylamine oxidoreductase (HAO). The proposed mechanism involves the combination of NH_4^+ and NH_2OH to form N_2H_4 which is finally converted to N_2 (Jetten, 1997).

$$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O \qquad \text{[Eq. 2-13]}$$

Partial nitrification and Anammox can take place in one reactor where two guilds of bacteria form compact granules. Equation 2.14 illustrates a combined partial nitrification and Anammox process.

$$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- + 0.13H^+ \rightarrow 0.26NO_3^- + 1.02N_2 + 0.066\ CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad \text{[Eq. 2-14]}$$

Deammonification was a staggering scientific discovery at Delft University, the Netherlands in 1989. This process happens spontaneously in the environment (oceans, lakes) and plays a major role in the nitrogen cycle. Deammonification involves the oxidation of NH_4^+ to N_2 using NO_2^- as electron acceptor. Figure 2-4 shows a complete nitrogen cycle and half a cycle through the nitrogen removal pathways.

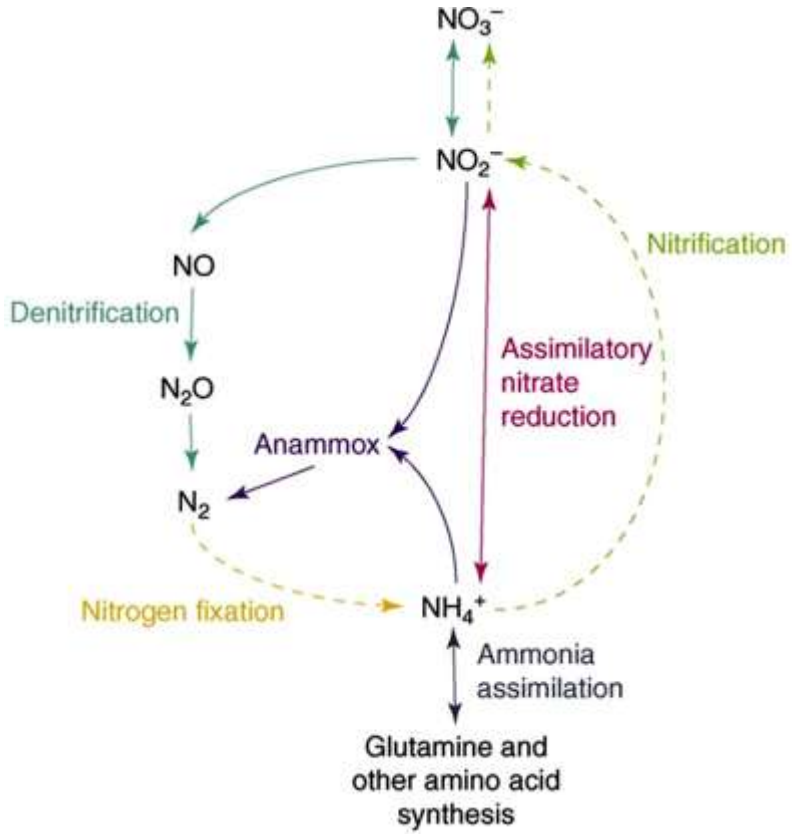


Figure 2-20 Nitrification, Denitrification & Anammox Process in the Nitrogen Cycle

Nitrogen Removal in Wastewater Treatment

(Richardson et *al.*, 2009)

2.2.4 Simultaneous Nitrification Denitrification (SND)

Simultaneous nitrification-denitrification (SND) is an important microbial process through which ammonium (NH_4^+) is converted directly to NO_2^- and NO_3^- and ultimately to N_2 with a mixed culture of nitrifiers and denitrifiers. SND relies on concurrent aerobic NH_4^+ oxidation and anaerobic denitrification under identical operating conditions. As NH_4^+ oxidation is a relatively slow process, SND requires a slowly degradable carbon substrate such that reducing power is available for denitrification throughout the NH_4^+oxidation process. As it can be seen from Figure 2-5, denitrification completes the nitrogen cycle by producing N_2.

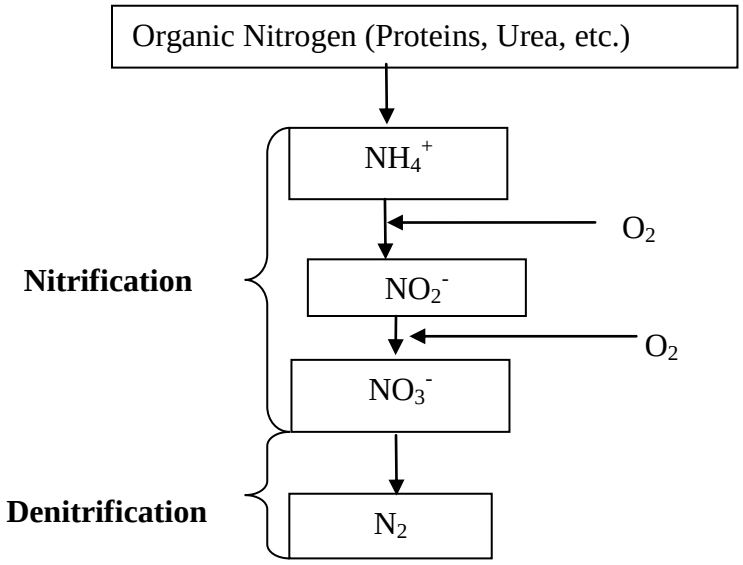


Figure 2-21 Conventional Nitrification and Denitrification

The SND process is feasible in a single compartment by controlling the DO concentration. Compared to conventional nitrification and denitrification, SND offers several advantages listed below (Seifi, 2012):

- The SND process eliminates the need for either two separate stages operated in series, or intermittent aeration in a single tank, thus continuous effluent output can be achieved with a smaller footprint;

- It utilizes 22–40% less carbon source and reduces sludge yield by 30%;
- Neutral pH and less demand for alkalinity can be accomplished in the reactor; because alkalinity is consumed during nitrification but produced during denitrification;
- It consumes less energy due to the reduction in aeration requirement.

The SND process depends on floc forming characteristics of the biomass. In many studies the efficiency of the SND process was found to be directly proportional to the floc size of the biomass. As well, efficiency of the process was found to decrease with reduction in the floc size. The flocs or granules create a unique micro-environment including aerobic and anoxic zones for both nitrifying and denitrifying bacteria as shown in Figure 2-6 (Liu, et *al.*, 2007). A more detailed description of the SND process is presented in section 4.3.3 and Fig. 4-3.

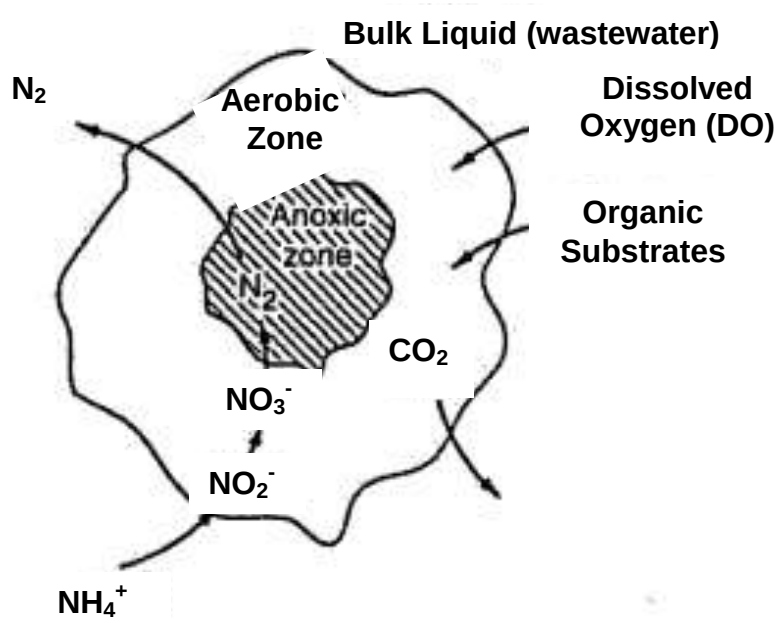


Figure 2-22 Bio-floc (granule) in SND Process

A more detailed description of the SND process in section 4.3.3 and Fig. 4-3.

2.3 Biological Phosphorus Removal (BPR) in Wastewater Treatment

The concept of phosphate necessity in living cells has been used to develop biological processes and eliminate the excess phosphorus from the environment, particularly from surface waters. In 1970s, James Barnard in South Africa had a breakthrough while investigating the process later known as Biological Phosphorus Removal (BPR). He improved this process over the years and patented several other processes for both separate and combined nitrogen and phosphorus removal. Based on those findings, he proposed three zones for BPR processes: anaerobic, aerobic and anoxic zones (US EPA, 2010). Wastewater contains phosphorus as either particulate or dissolved matter. Particulate phosphorus which is insoluble in water is part of the living and dead plankton. Dissolved phosphorus includes inorganic phosphorus (PO_4^{-3}, and poly-phosphate) and organic phosphorus. The average concentration of phosphorus both inorganic and organic in domestic wastewater is approximately 5 to 20 mg/L (Scheer, 1996). Microorganisms use small amounts of soluble phosphorus for their cellular maintenance and operation which is approximately 1.9% of their dry cell weight. However, there are special types of bacteria called phosphorus accumulating organisms (PAOs) which have the ability to uptake phosphorus in excess of their biological requirements and form poly-phosphate granules within their cells when they are subjected to anaerobic and then aerobic conditions. PAOs store phosphorus from 5% to 38% of their cell mass (Rustrian, 1997).

The common forms of phosphorus in water are orthophosphate (PO_4^{-3}), poly-phosphate which is a polymer of phosphoric acid, and organically bound phosphate (i.e. proteins). PO_4^{-3} and poly-phosphate come from the decomposition of organic matter and can be removed through biological or chemical processes. The amount of phosphorus in wastewater relative to the quantities of nitrogen and carbon is greater than the necessary amount required for biological

synthesis. Thus, conventional wastewater treatment can remove only 20 to 40% of the phosphorus concentration in wastewater. To achieve higher phosphorus removal (to above 95%) an advanced phosphorus removal process is required.

Biological phosphorus removal is a hypersensitive process that is affected by external disturbances such as high rainfall, excessive nitrate loading to the anaerobic reactor and many other important factors such as pH, temperature and lack of carbon source. Therefore, stability and reliability of BPR must be maintained and monitored through instrumentation and control.

Over the past two decades, various technologies for biological phosphorus removal have been developed, modified and used in the wastewater treatment industry. They all consist of anaerobic, aerobic as well as anoxic stages if phosphorus removal and denitrification are combined. In the anaerobic stage, with sufficient carbon source, PAOs uptake carbon (i.e. acetate) and convert it intracellularly into a special type of polymer called polyhydroxyalkanoates (PHAs). Under aerobic conditions, PAOs uptake phosphorus from the wastewater for the reconstruction of cell structure as well as for growth and reproduction. The following diagram (Figure 2-7) of a PAO cell shows the uptakes of volatile fatty acids (VFAs) (1), and forms intracellular PHAs (2) and releases phosphorus into the wastewater in the anaerobic phase (3). This figure also shows, in the subsequent aerobic phase, how a PAO cell utilizes internal PHAs (for energy) (4) uptakes phosphorus and forms poly-phosphate (poly P) (5).

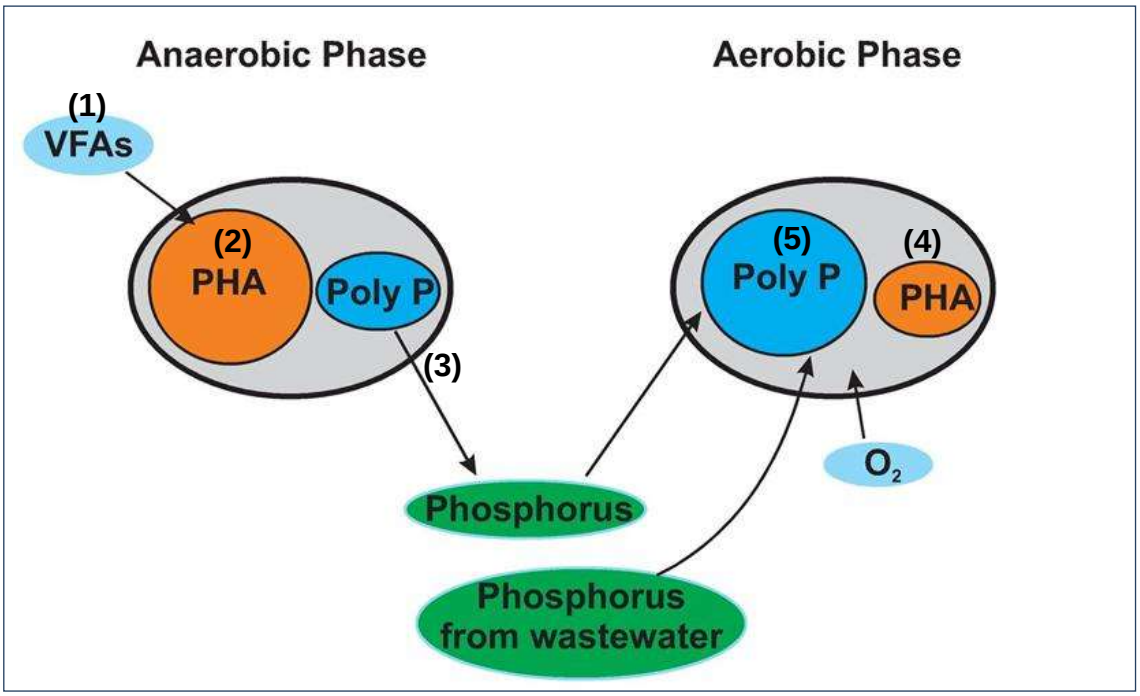


Figure 2-23 VFAs and Phosphate Uptake by PAOs under Anaerobic/Aerobic Conditions

There is a relationship between the absorbed concentration of acetate and the concentration of released phosphate into the liquid in the anaerobic phase as suggested by Wentzel *et al* (1988) which is 0.5 mgP/mgCOD. The amount of readily biodegradable substrate in wastewater is essential for the enhancement of the BPR process. Facultative bacteria ferment readily biodegradable materials into short chain volatile fatty acids (VFAs) and subsequently acetate which can be utilized by PAOs. PAOs cannot do this fermentation themselves. Uptake and utilization of VFAs is a relatively rapid process but the fermentation of biodegradable organic matter is rather slow. Fermentation can be a rate limiting reaction in the anaerobic phase if only a small concentration of VFA is present in the wastewater (Grady, 1999).

Biological phosphorus removal is accomplished by creating conditions favorable for the growth of PAOs. As it was discussed previously, the Anaerobic stage provides selective advantages for the PAOs to dominate the heterotrophic bacterial community. Due to the lack of oxygen and nitrate in this zone, PAOs cannot oxidize the organic matter instead they produce

and store intercellular PHAs. Proton motive force (PMF) is generated via the efflux of phosphorus from surrounding wastewater through the cell membrane. When PAOs arrive into the Aerobic stage, they oxidize these carbon polymers providing the energy source to take up phosphorus from the wastewater. PAOs use a small portion of the internal phosphorus to build up their cell structure and to grow. The remaining phosphorus is intracellularly accumulated as poly-phosphates. Two important parameters in the microbial kinetics of PAOs is their growth and decay rate. The decay and growth rates of PAOs are significantly slower than those of normal heterotrophic bacteria. The decay and growth rates of PAOs have been experimentally found to be 0.04/day and 0.04/h respectively (Kortstee1, 2000). Therefore, it is expected to achieve a stable and efficient phosphorus removal process after a continuous long term operation. BPR processes have been implemented in numerous wastewater treatment plants around the world. BPR processes are inherently advantageous over chemical phosphorus removal. These advantages include:

- Less sludge production
- No chemical costs
- Good sludge settling due to lower filamentous bacterial growth
- Easier resource recovery

2.3.1 Microbiology of Phosphorus Accumulating Organisms (PAOs)

PAOs have a competitive advantage over other heterotrophic organisms because of their capabilities to uptake organic matter and form biodegradable polymers (PHAs) in the Anaerobic phase. In the subsequent Aerobic phase, PAOs take up orthophosphates (PO_4^{3-}) from wastewater and form intracellular poly-phosphate. PAOs have been found to accumulate phosphorus up to 38% of their cell mass which is much greater than the amounts of phosphorus required for

normal cell growth and repair. In comparison, normal heterotrophic bacteria are only able to store phosphorus to about 2.5% which is considerably lower than the amount stored by PAOs (Mino, 1998). PAOs often develop the ability of phosphorus removal when they are subjected to alternating Anaerobic phase and subsequent Aerobic and/or Anoxic phases. This is partially due to their higher energy requirement to accomplish the cyclical chain reactions compared to normal heterotrophic bacteria. During the Anaerobic phase, PAOs take up and store easily biodegradable organic matters and convert them into polymers like PHAs mainly in the form of Polybetahydroxybutyrate (PHBs), and Polybetahydroxyvalerate (PHV). The energy to uptake acetate and convert and store it as PHAs is obtained partially from the breakdown of glycogen as well as from the hydrolysis of energy-rich polyphosphates into orthophosphate. In the Anaerobic phase PAOs release phosphorus into the liquid (wastewater) due to intracellular phosphorus concentration gradient. Therefore, the phosphorus concentration increases in the liquid bulk under Anaerobic conditions and then decreases to a very low level in the Aerobic phase. In the Anaerobic stage, PAOs release phosphorus into the bulk solution (Thus increasing the phosphorus concentration in the wastewater) while taking up all the acetate from wastewater. In this stage, PAOs store the acetate (or VFAs) as PHAs inside their cells while using the cell's glycogen storage (reducing glycogen concentration). After wastewater enters the Aerobic stage, PAOs oxidize all the stored PHAs to grow, reproduce and rebuild their cellular structure. Meanwhile, they take up PO_4^{3-} from the bulk solution and store them internally as polyphosphates.

The sequential hydrolysis of Adenosin Triphosphate (ATP) to Adenosin Diphosphate (ADP) and Adenosin Monophosphate (AMP) is another energy source for the PAOs. The hydrolysis of ATP and ADP are shown in the following equations (Baetens, 2001):

$$ATP + H_2O \rightarrow ADP + P_i + H+ + energy \quad \Delta G^o = -310\ kJ/mol \qquad [Eq.\ 2\text{-}15]$$

$$ADP + H_2O \rightarrow AMP + P_i + H^+ + energy \quad \Delta G^o = -318\ kJ/mol \qquad [Eq.\ 2\text{-}16]$$

In the above equations ΔG^o is denoted as Gibbs free energy or available energy which is released by hydrolysis of the ATP and ADP.

PHAs Formation and Storage

The morphological characteristics of PAOs are described as non-motile rods or cocci, usually exist in clusters and contain positive staining PHAs, cellular granules. As mentioned earlier, in the anaerobic conditions, PAOs take up easily biodegradable matter and convert it into PHAs (mainly as copolymers such as PHB and PHV) with concurrent release of PO_4^{3-} into the surrounding water. The release of PO_4^{3-} is due to the phosphorus concentration gradient between the inside and outside PAO's cellular membrane. The concentration of phosphorus is high inside the PAO cell due to ATP hydrolysis and polyphosphate breakage compared to lower phosphorus concentration in the water surrounding the cell. When PAOs leave the Anaerobic stage and enter the Aerobic stage, they oxidize and utilize their cellular stored PHAs and uptake phosphorus from water (both phosphorus content in the influent and phosphorus that is being released under Anaerobic conditions). A small portion of the phosphorus taken up by PAOs is used for their cellular growth, reconstruction and reproduction. However, the rest of the phosphorus is converted and stored in the form of polyphosphate inside their cells. Figure 2-8 is a microscopic image of PHA granules in a microbial cell.

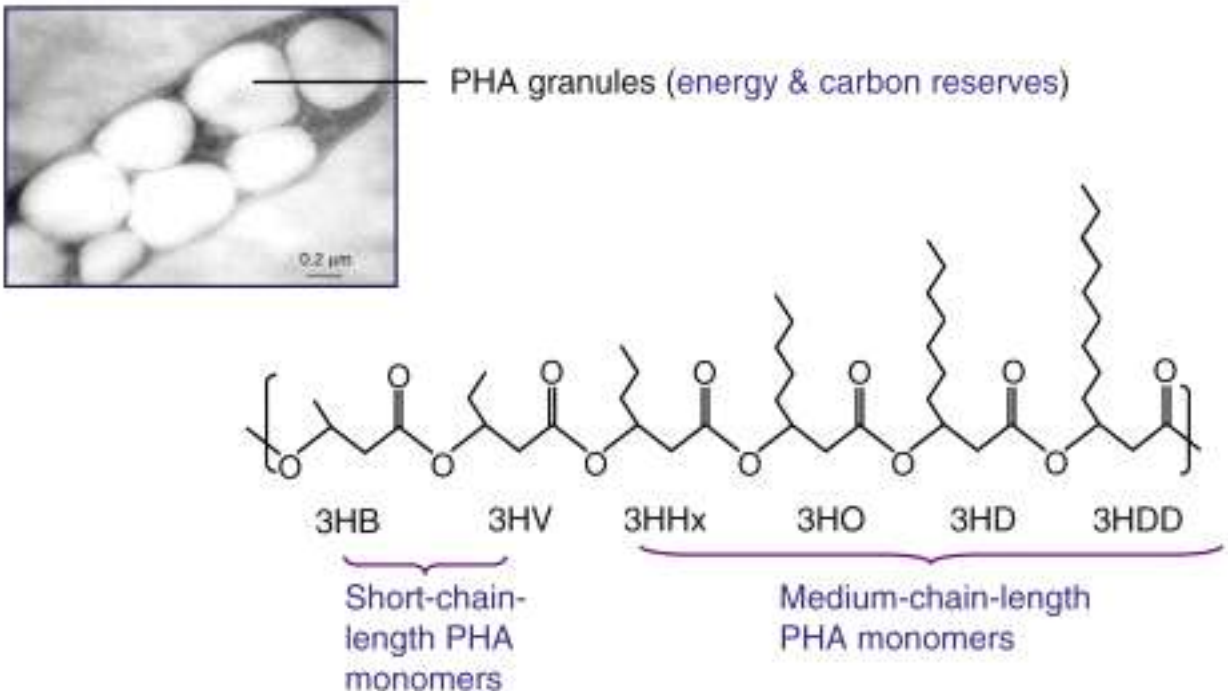


Figure 2-24 Some of the Most Common PHAs

(Chen, 2011)

There are more than 150 types of PHA polymers (Chen, 2011) however only several of these PHAs have been discovered in PAOs.

Polyphosphate Formation and Storage

Poly-phosphates exist in the cytoplasm, periplasm, in the cell membrane and on the cell surface of microorganisms. PAOs accumulate excess phosphorous and store them as inert intracellular polyphosphate granules. Some of the well-known PAOs include *Candidatus Accumulibacter phosphate, Acinetobacter, Microlunatus phosphovorus strain NM-1, Pseudomonas sp, Propionibacter pelophili*. The polyphosphate granules stored by the PAOs are usually located in the nucleoplasmic region or in association with multi-enzyme complexes, i.e. RNA polymerase and ribosomes (Kusano and Ishihama, 1997). Polyphosphates (poly-P) are

made of orthophosphates linear chains linked together by energy-rich phosphoanhydride bonds as shown in Figure 2-9.

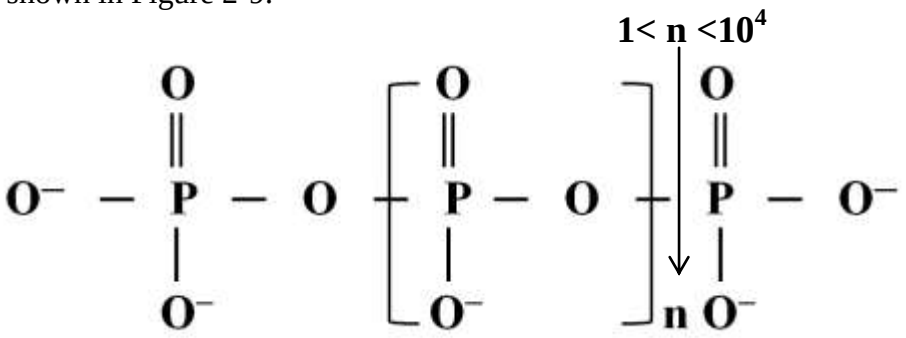


Figure 2-25 Chemical Structure of Intracellular Poly-phosphate (Poly-P) Compounds

Inorganic poly-P chains are important sources of energy for microorganisms. The length of intracellular poly-P chains vary from few units (n=2 or 3) to $n=10^4$ units (Dawes, 1985). A polyphosphate chain needs counter ions to neutralize the negative charge. The most important counter ions are Mg^{2+}, Ca^{2+} and K^{2+}, and to a lesser extent cations like Mn^{2+}, Al^{3+}, Fe^{3+} (Schönborn et *al.*, 2001). According to Smolders et *al.* (1994), transport of the positive ions (Mg^{2+}, K^{2+}) through the cell membrane does not require energy. These ions are used by the cells for polyphosphate synthesis. Phosphate transport across the cell membrane is a process requiring energy. Phosphate is negatively charged and has to be taken up against an electrical potential difference.

Stored polyphosphates play an important role as energy source for the generation of ATP and source of phosphorus for metabolic processes such as both nucleic acid and phospholipid synthesis (Kulaev and Vagabov, 1983). PAOs can store more than one poly-P granule per cell. The number of granules in a cell is directly related to the microbial age (Streichan et *al.* 1990). Researchers including Roinestad and Yall (1970) have found several poly-P granules in *Zoogloea* a sub-class of betaproteobacteria. In the present research, *Zoogloea* is the second

dominant microbial group in the bioreactor. More details about *Zoogloea* are discussed in chapter 4.

Anoxic vs. Aerobic Phosphorus Uptake in BPR

Early research on PAOs suggested that the micro-organisms responsible for BPR could only grow and accumulate phosphate under aerobic conditions. This idea was supported by the observations that nitrate entering the anaerobic phase adversely affected the BPR efficiency. Barker and Dold (1996) found secondary phosphorus release rather than phosphorus uptake under anoxic conditions. More investigations, however, proved that a fraction of the PAOs can uptake phosphorus and accumulate poly-phosphate under anoxic conditions (Kuba et *al.*, 1993). The denitrifying capacity of PAOs was proved by Vlekke et *al.* (1988) operating Sequencing Batch Reactors (SBR) using an anaerobic-anoxic sequence. Further research on denitrifying PAOs (DPAOs) and comparative studies between DPAOs and normal PAOs showed that the phosphorus uptake rate was significantly lower in the anoxic stage than in the aerobic stage, i.e. 10 and 70 mg P/g VSS.h respectively (Kuba et *al*., 1993; Filipe and Daigger, 1999).

Secondary Phosphorus Release Phenomenon

Secondary phosphorus release is a phenomenon in BPR associated with the release of phosphorus with no subsequent uptake of VFAs and no formation of intercellular PHAs. The phosphorus released in this manner usually is not taken up by the PAOs which results in a higher phosphorus concentration in the liquid phase, and a reduced phosphorus removal efficiency. The primary causes of secondary phosphorus release include (Chen, et *al.*, 2004):

- Long hydraulic residence time (HRT) in the anaerobic stage;
- Long residence time (SRTs) of the biomass in the clarifier;

- Long HRTs in the aerobic or anoxic stages cause cell lysis and phosphorus release;
- Addition of nitrate into the anaerobic stage;
- Blending BPR biomass with other sludges (i.e primary or secondary).

2.3.2 Proposed Biochemical Models in BPR

Various biochemical mechanisms have been postulated to define PAO's behavior in the Anaerobic and Aerobic phases. Wentzel et *al.* (1990) pointed out two possible biochemical models, the Comeau-Wentzel and the Mino model. These two models form the basis for most publications on phosphorus removal.

Comeau-Wentzel Model

According to the Comeau-Wentzel Model, acetate which is formed as a result of the fermentation by heterotrophic microorganisms under Anaerobic conditions passes through the PAO's cell membrane and gets activated to acetyl-CoA (Molecular formula: $C_{23}H_{38}N_7O_{17}P_3S$). The energy for acetate uptake and acetyl-CoA formation is provided by ATP hydrolysis to ADP as shown in Eq. 2-15 and 2-16. PAOs respond to the decrease in ATP/ADP ratio by re-synthesizing ATP from the internal polyphosphates breakage. About 90% of the Acetyl-CoA is stored as PHB or PHV. The remainder of Acetyl-CoA is metabolized through the Tri-Carboxylic Acid (TCA) cycle to provide the reducing power ($NADH^+ + H^+$) for the synthesis of PHB/PHV (Grady, 1999). Figure 2-10 illustrates the Comeau-Wentzel model under Anaerobic condition.

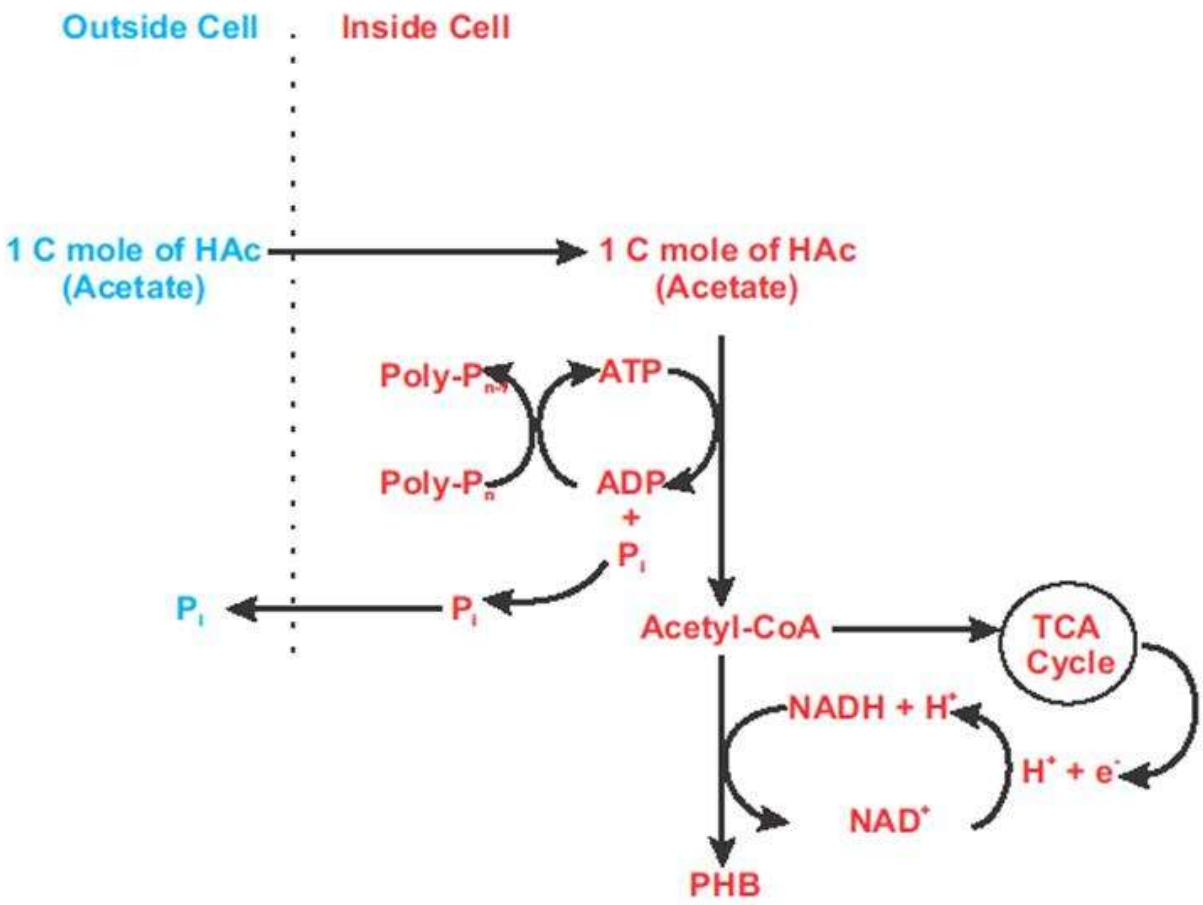


Figure 2-26 Comeau-Wentzel Model for the PAO's Anaerobic Metabolism

(Wentzel, 1990)

Under Aerobic conditions, PAOs oxidize the stored PHBs to obtain energy from the utilization of Acetyl-CoA through the TCA cycle. The energy released from the TCA cycle is then used to transport phosphorus across the cell membrane. Figure 2-11 illustrates the proposed Comeau-Wentzel model under Aerobic conditions.

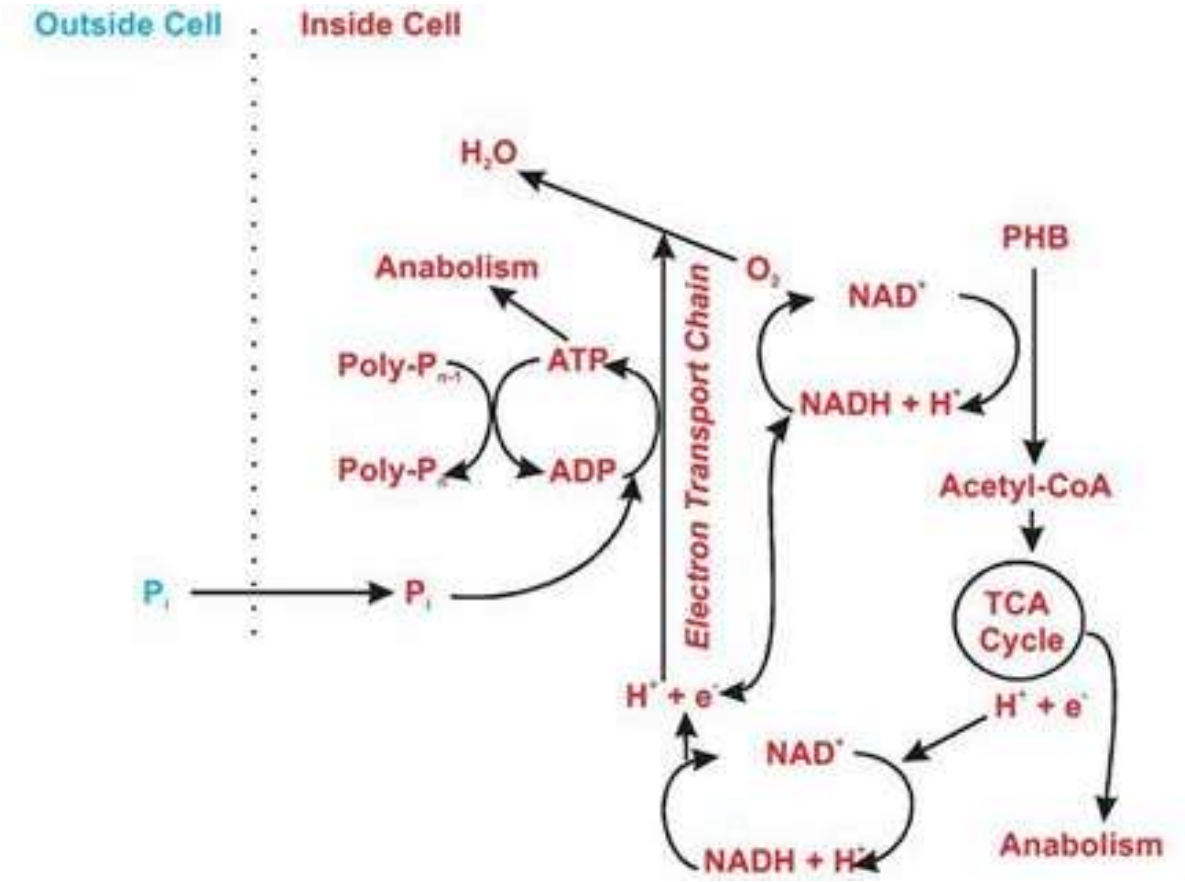


Figure 2-27 Comeau-Wentzel Model for the PAO's Aerobic Metabolism

(Wentzel, 1990)

Mino Model

This model is very similar to the Comeau-Wentzel model, with the major difference being the role of glycogen inside the cell. Figure 2-12 illustrates the Mino model in the Anaerobic phase. Based on this model, the reducing power required for the synthesis of PHB from acetyl-CoA comes from the metabolism of the glucose released from the glycogen not by the TCA cycle. Glucose is oxidized to pyruvate through Entner-Doudorof (ED) or Embden-Meyerhof-Parnas (EMP) pathway, thereby providing some of the ATP required to convert acetate to acetyl-CoA as well as some of the reducing power needed for PHB synthesis (Wentzel, 1990).

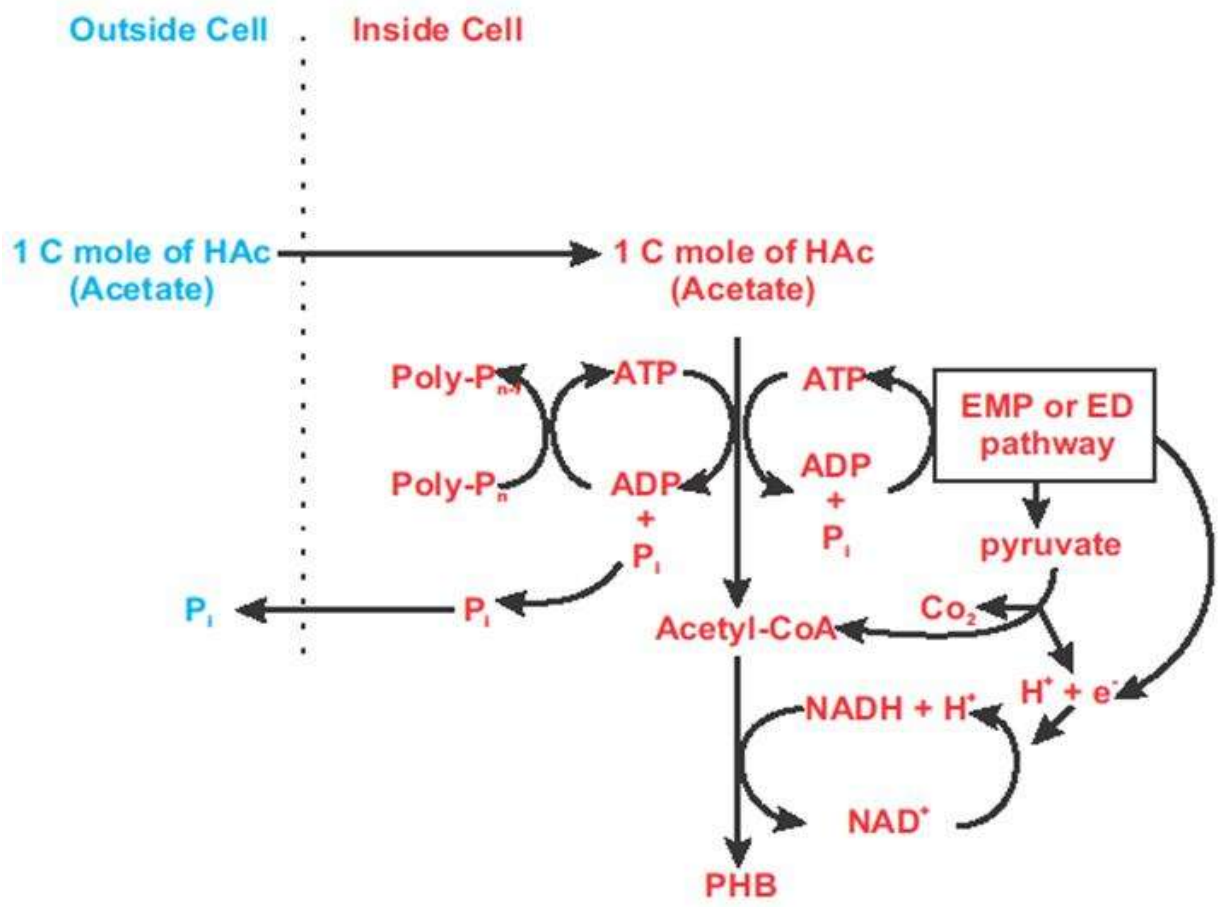


Figure 2-28 Mino Model for the PAO's Anaerobic Metabolism

(Smolders, 1995)

The main difference between Comeau-Wentzel and Mino models are mainly cellular metabolisms under the Anaerobic stage. Based on these two models, PHAs are broken down in the Aerobic phase for biomass synthesis, PO_4^{3-} uptake & polyphosphate storage. In addition, the Mino model (Figure 2-13) suggests that in the Aerobic zone, PHBs are used to replenish the cellular glycogen.

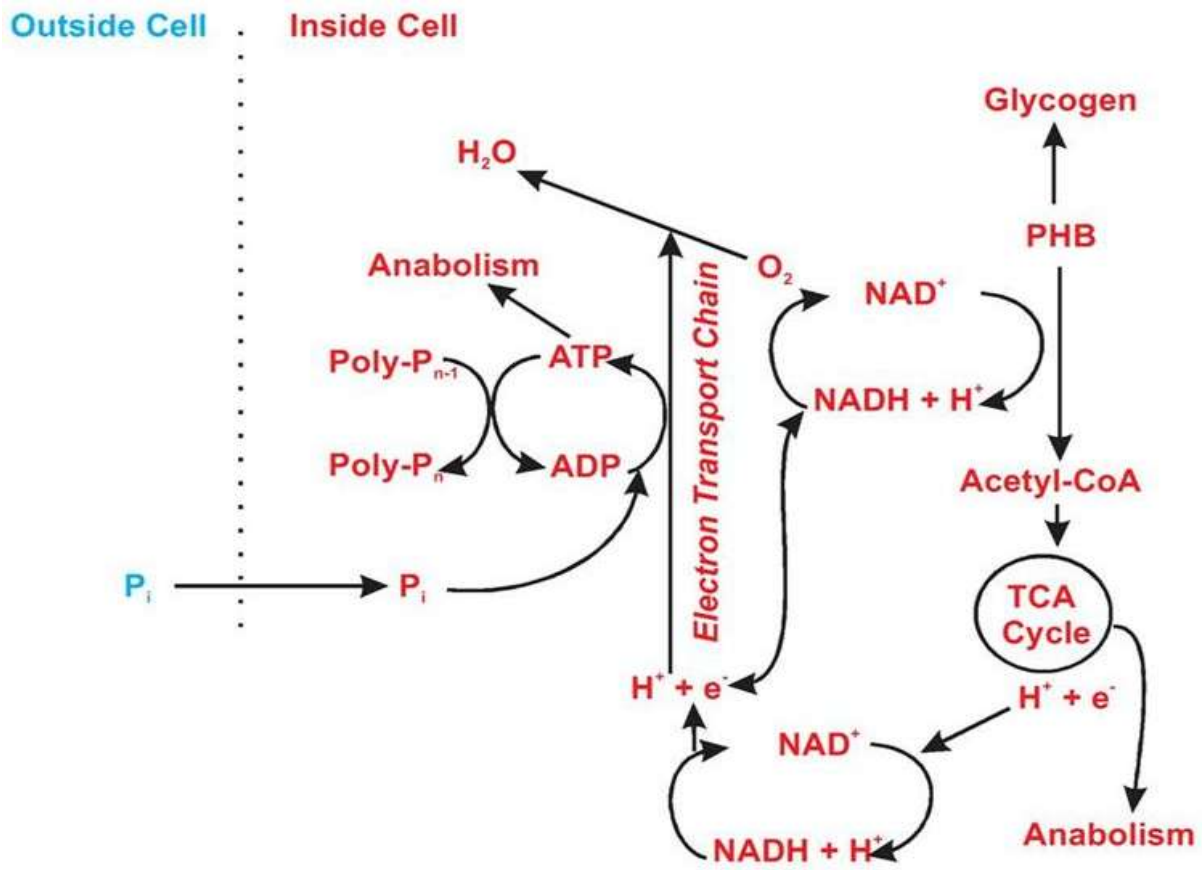


Figure 2-29 Mino Model for the PAO's Aerobic Metabolism

(Smolders, 1995)

Both the Comeau-Wentzel and the Mino models have proven to be partially valid. However, a combination of either models or a completely different biochemical model is needed to explain truly the Anaerobic/Aerobic phenomena in the BPR process. Although, this concept has been investigated by researchers for a long time still no generally accepted model exists.

2.4 Simultaneous Nitrogen and Phosphorus Removal

By providing anaerobic, anoxic and aerobic conditions for microorganisms, biological nutrient removal (BNR) can be successfully achieved in wastewater treatment plants (WWTP). In response to their specific environment, microorganisms carry out cellular metabolism under

these redox conditions. Water/wastewater under anoxic condition has high NO_3^- concentrations as electron acceptors with very low or zero DO concentrations. In conventional WWTP, the removal of nutrients occurs after the secondary treatment. That is, after the elimination of most of the BOD_5 and NH_4^+. These processes are followed by both nitrification and denitrification to eliminate the nitrates and phosphates.

Simultaneous nitrogen and phosphorus removal can be achieved by incorporating anaerobic, aerobic and anoxic stages and recycling the biomass and the wastewater. Introducing the feed to the bioreactor and separation of biomass from the treated wastewater can result in a substantial reduction in energy, construction materials, and space requirement.

As mentioned earlier, combined biological nitrogen and phosphorus removal is more economic and environmentally friendly than conventional activated sludge treatment plants with chemical or physical nutrient removal processes. The aeration required for BPR can be reduced by denitrifying phosphorus uptake using nitrate as the sole electron donor in the Anoxic stage. Aeration required for nitrification can also be reduced by incorporating the Anaerobic stage with optimum COD concentration. Under anaerobic conditions, many nitrifying and denitrifying organisms form intracellular PHAs which are utilized in the Anoxic and Aerobic stages. Therefore, nitrification/denitrification can take place with microbial PHAs with no need for additional carbon source.

2.4.1 Existing BNR Processes/Reactors

This section is a brief perspective of the bioreactors most frequently installed in wastewater treatment plants.

Modified Ludzak-Ettinger Process (MLE)

MLE process represents one of the simplest systems within which both nitrification and denitrification take place in different stages. In this process, both wastewater and recycled biomass enter the anoxic stage with a very low DO and high NO_3^- concentrations as shown Figure 2-14. The MLR to influent flow ratio ranges from 100:1 to 300:1 (MLR:Influent) depending on the extent required of denitrification (Grady et *al.*, 1999).

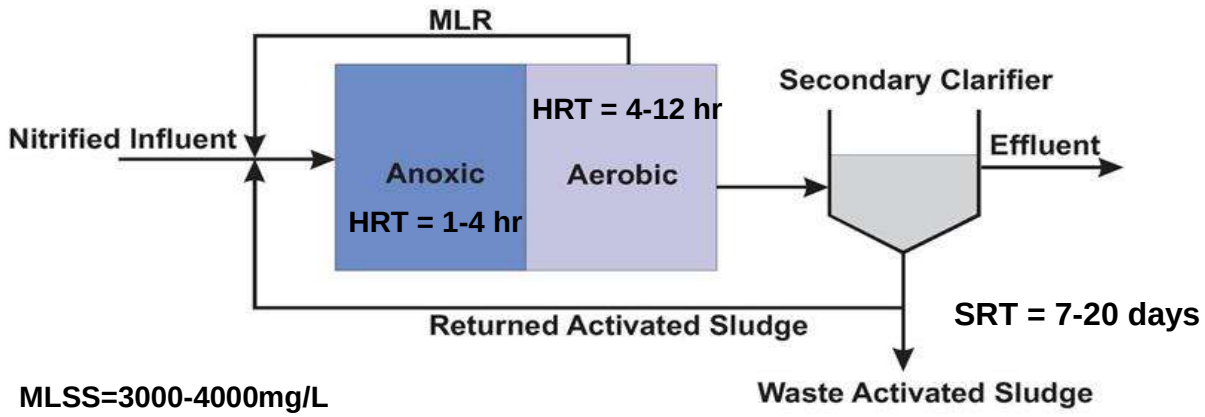


Figure 2-30 Modified Ludzak-Ettinger Process

Phoredox (A/O)

The term A/O stands for anaerobic and aerobic (oxic) which represent the sequence of these phases in the process. This is the basic process configuration for biological phosphorus removal first developed by Barnard in 1974 and then patented by Air Products and Chemicals Inc. (Grady et *al.*, 1999). Some of the qualitative characteristics of A/O process include:

- Simple operation and short HRT;
- Low BOD/P ratio;
- Good phosphorus removal;
- Unstable phosphorus removal if nitrification occurs;

- Commercially established.

Figure 2-15 illustrates the A/O reactor sequence.

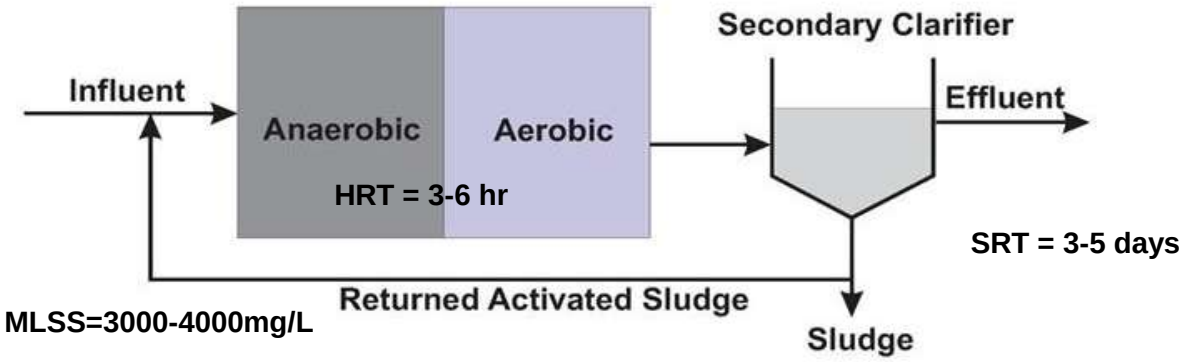


Figure 2-31 Phoredox (A/O)

A^2/O Process

The term A^2/O stands for anaerobic, anoxic and aerobic bioreactors in sequence. It is combination of the MLE process for nitrogen removal and the A/O process for phosphorus removal. The nitrogen removal capability of this process is very similar to MLE. However, the phosphorus removal efficiency is lower than A/O (Grady et *al.*, 1999).

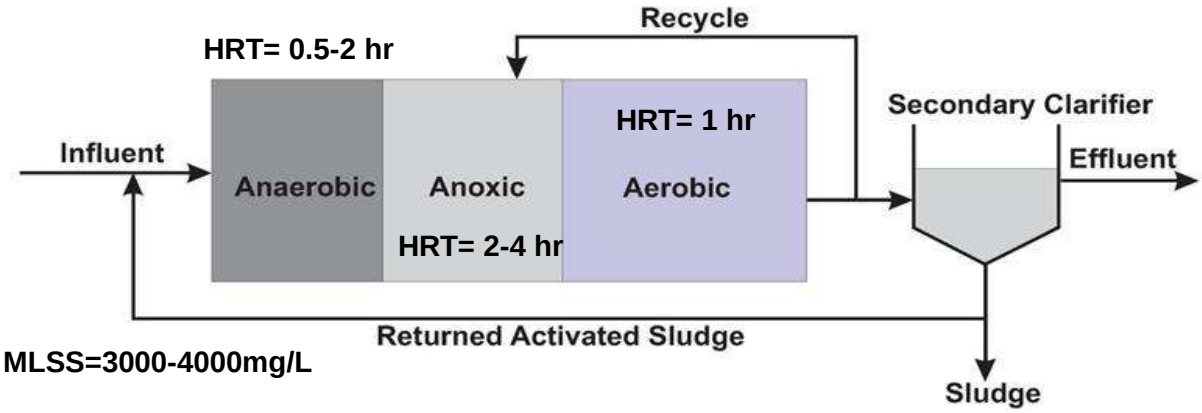


Figure 2-32 A^2/O Process

These are some of the main features of this process:

- Produces good settling sludge;

- Simple operation;
- Ammonia removal is limited by internal recycle ratio;
- Needs higher BOD/P ratio than the A/O process;
- Moderate TP removal efficiency.

Modified Bardenpho Process

The modified Bardenpho process is very similar to the 4-stage Bardenpho process with an anaerobic stage added to achieve phosphorus removal. The Modified Bardenpho consists of five stages in series including: anaerobic, anoxic, aerobic, anoxic, and aerobic. This configuration allows for the removal of phosphorus, nitrogen and carbon. This configuration allows for the removal of phosphorus, nitrogen and carbon compounds (Grady et *al.*, 1999). The sequences of these stages are shown in Figure 2-17.

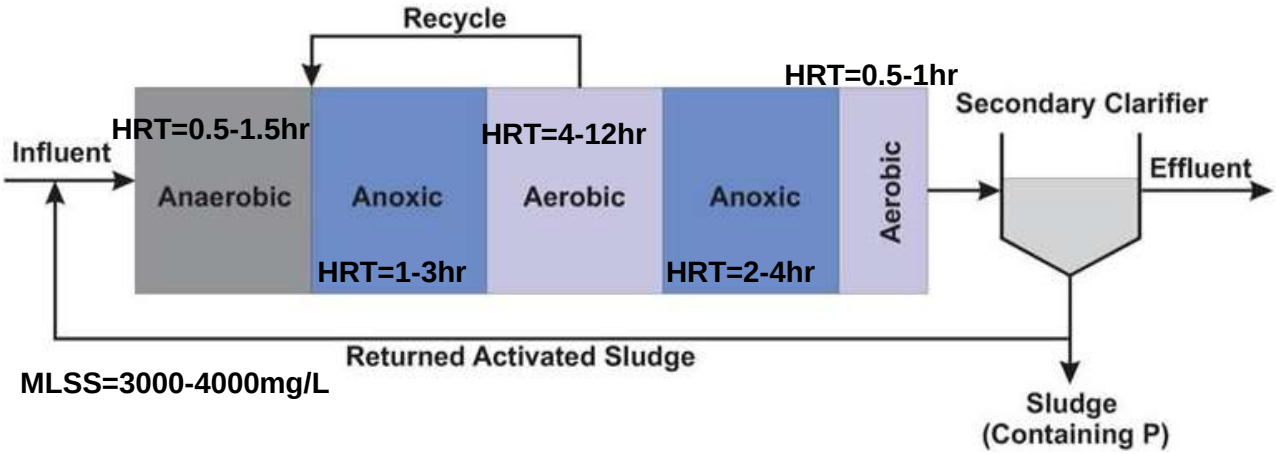


Figure 2-33 Modified Bardenpho Process

This process has several limitations including high construction surface requirement, complicated design and control as well as moderate phosphorus removal capability. Other characteristics of this process include:

- good settling sludge;
- 3 to 5 mg/L TN concetratopms in unfiltered effluent;
- Less TP removal efficiency than A/O or A^2/O;
- Long HRT of up to 22 hours.

UCT (Standard and Modified)

The UCT process stands for the University of Cape Town process where it was developed (Figure 2-18). The standard UCT process is very similar to the A^2/O process with two exceptions (Grady et *al.* 1999):

- The returned activated sludge (RAS) is recycled to the anoxic stage instead of the anaerobic stage;
- The internal recycle is from the anoxic stage to the anaerobic stage.

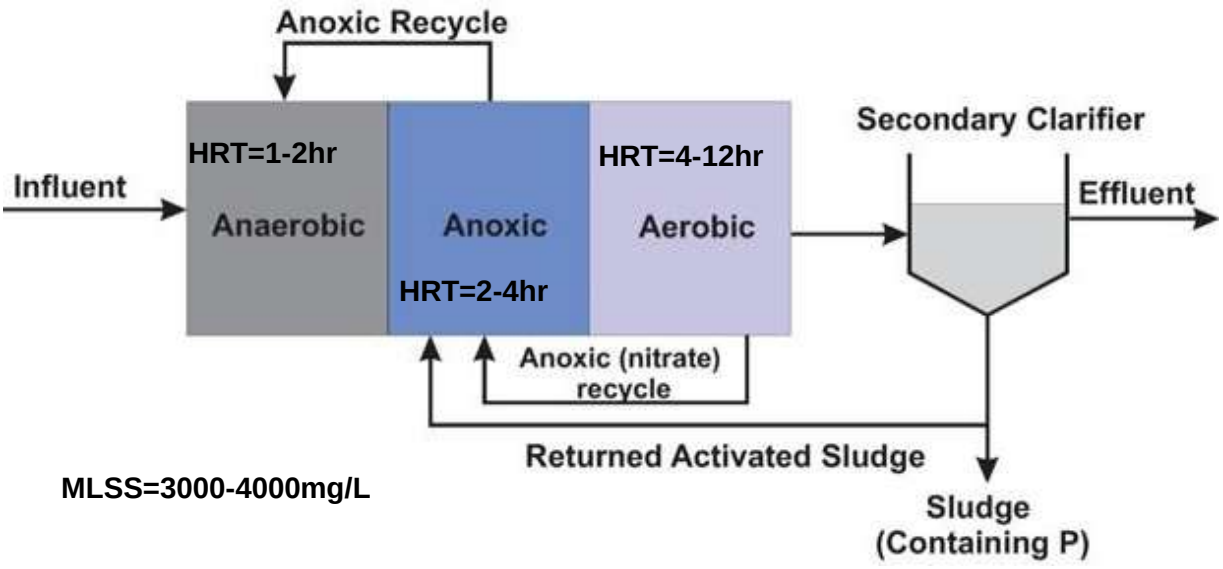


Figure 2-34 Standard UCT Process

In the modified UCT process shown in Figure 2-19, the RAS is directed to an anoxic stage that does not receive internal nitrate recycle flow. The second anoxic stage receives

recycled internal nitrate from the aerobic zone to achieve a better denitrification. The modified UCT has high nutrient removal efficiency, however the process is very difficult to monitor and control (Grady et *al.*, 1999).

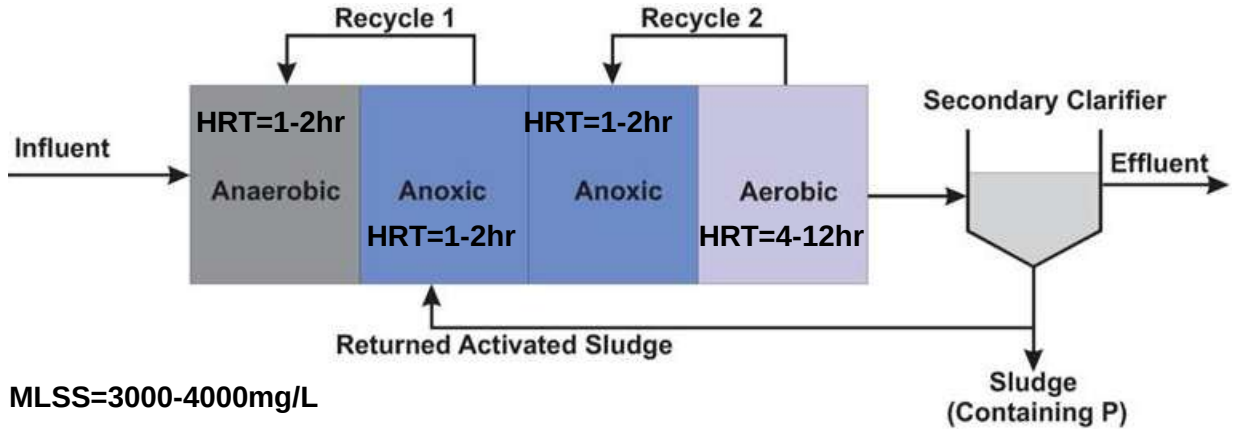


Figure 2-35 Modified UCT Process

2.5 Conclusions

This section has discussed pertinent research associated with simultaneous nitrification, denitrification and biological phosphorus removal (SNDP) process. It comprises three processes subject to the same environmental conditions. Microbial nitrification is a key process in the removal of NH_3-N (ammonia-nitrogen) from wastewater. The biological oxidation of NH_3-N to NO_3^- takes place through a two-step process in which AOBs convert NH_3-N to NO_2^- and NOB oxidize NO_2^- to NO_3^-. In a simultaneous nitrification-denitrification, NO_3^- and NO_2^- formed during nitrification is reduced to N_2 by heterotrophic denitrifiers. To promote the utilization of NO_3^-, an anoxic phase is required where NO_3^- is the only electron acceptor. Biological phosphorus removal (BPR) can be promoted along with nitrification and denitrification. BPR is carried out by specialized heterotrophic bacteria called phosphorus accumulating organisms (PAOs) when they are subject to strict anaerobic conditions followed by aerobic or anoxic stages. PAOs and denitrifying PAOs uptake VFAs and concurrently produce and store

intracellular PHAs. In the anoxic/aerobic phases, PAOs/DPAOs break down their internal PHAs and utilize the released energy to uptake phosphorus and accumulate poly-phosphates.

To develop SNDP processes, factors such as nitrogen and carbon loadings, biomass, hydraulic residence time, pH and temperature play major roles. Anaerobic and anoxic zones placed ahead of aerobic zones act as biological selectors that minimize the growth of filamentous microorganisms and improve the sludge settlability (van Haandel et *al.*, 2007). More details about SNDP process are discussed in later chapters.

Chapter 3 – Design, Operation and Performance Evaluation of a Vertical Bioreactor for Simultaneous Ammonia and Phosphorus Removal from Wastewater

3.1 Introduction

Among current environmental issues, nutrient pollution has become an overwhelming problem for many countries around the world. Excessive use of fertilizers in the agricultural sector and daily human activities are the main sources of nutrient pollution. This in turn causes extensive economic losses because of the degradation of fisheries, tourist facilities and residential properties. This global problem can be mitigated with more stringent environmental regulations, and advanced designs and processes. Three parameters are of paramount importance in the design of a wastewater treatment plant: 1) high standards of effluent quality, 2) minimal construction space (footprint) and 3) construction, installation and operation & maintenance costs of treatment technologies/plants. Successful technologies in wastewater treatment including nutrient removal, must meet the above design criteria. For example, upgrade/expansion of plants in urban areas are limited by construction space due to demographic pressures and high regulatory demands. Increasing chemical complexity in industrial and domestic wastewater, flowrates variability, mixing, and the removal of emerging contaminants impose further constraints in the development and control of wastewater treatment processes. The selection and adoption of a technology depends on its competitive costs as well. Selection of a technology in water/wastewater engineering are both site specific and time dependent, and include therefore a considerable degree of uncertainty. Some researchers have empirically estimated the construction and operating costs of wastewater treatment plants and concluded that the construction costs are the main item in total investment costs. This is especially true in high density urban areas. Among the important factors affecting construction cost, land and excavation costs as well as the cost of construction material rank very high as shown by Quasim et *al*. (1992). The considerable reduction in footprint and construction cost of vertical bioreactors

provide significant advantages. The cost evaluations of vertical bioreactors are beyond the scope of this thesis; however, their important characteristics and advantages against horizontal activated sludge basins are explained in details in chapter 3 and 5.

In this chapter, a novel vertical bioreactor with completely different operation and configuration is presented. Also, the bioreactor design and a nutrient removal process development are described in depth.

3.1.1 Research Objectives

In some wastewater treatment plants, ammonia removal processes such as nitrification and denitrification are accompanied by biological phosphorus removal (BPR). However, due to hypersensitivity of the phosphorus removing organisms many plants adopt chemical treatments for phosphorus removal. A list of the most common suspended- growth processes which carry out both biological nitrogen removal and biological phosphorus removal is shown below:

- 3 Stage Pho-Redox (A^2/O)
- 5 Stage Bardenpho
- Modified university of Cape Town (Modified UCT)
- Oxidation Ditch
- Sequencing Batch Reactor (SBR)

The most appropriate reactor configuration for a biological nutrient removal (BNR) process depends on factors such as the target effluent quality, influent quality, process control, biological process complexity, and available foot print. Many of the existing BNR processes take place in planar, horizontal basins with large footprint. As pointed out before, construction space limitations is one of the problems facing municipal wastewater treatment facilities. The

significance of the pilot scale vertical bioreactor designed and used to produce an effective BNR process is described below:

- Because of its vertical configuration, this bioreactor has lower land requirements and much lower excavation costs than comparable planar bioreactors;
- The cylindrical symmetry and vertical configuration of this bioreactor provides more construction flexibility which is suited for retrofitting of obsolete facilities in constrained or heavily populated areas;
- Construction materials of vertical bioreactors includes PVC or fiver glass which are highly durable, lighter and have lower costs compared to concrete structures that are widely used in wasyewater treatment plants;
- The circular cross section of this bioreactor provides a better mixing and mass transfer because it avoids the stagnancies which normally develop in the corners of rectangular bioreactors;
- The vertical configuration of this bioreactor provides well mixed, smooth flow of water from one stage to the other without additional need for pumps;
- Fewer number of pumps and mixers significantly reduces the energy consumptions and consequently the capital, operational and maintenance costs;
- The configuration of the bioreactor permitted the creation of the environmental conditions leading to the formation of ecosystems that favor the growth of new microbial species as suggested by Littleton et *al.* (2003);
- Simultaneous nitrification-denitrification-BPR developed in this vertical bioreactor is an ecologically sound process as it requires less organic carbon and produces less sludge than conventional nitrification-denitrification and BPR processes.

The main disadvantage of a vertical bioreactor is insufficient operational and process experience. This is not surprising since the horizontal concrete-type reactors in wastewater treatment have been investigated for decades and there are thousands of research projects and publications dedicated to them. In comparison, the vertical bioreactor designed, operated and evaluated herein is the first of its kind and requires future testing and further investigation.

3.2 Materials and Methods

3.2.1 Experimental Set-up

A vertical bioreactor with three consecutive stages, Anoxic 1, Anoxic 2 and Aerobic stages was built to cultivate a mixed culture of heterotrophic/autotrophic nitrifiers, denitrifiers, denitrifying PAOs (DPAOs). This bioreactor was aligned with an Anaerobic Lateral Unit (ALU) of 60 L capacity. The ALU provided a strict anaerobic condition to cultivate and promote the growth of PAOs. The pilot scale bioreactor of cylindrical cross-section was made of high density polyethylene (HDPE) and a working volume of 65 L. The rate of synthetic feed to the bioreactor was maintained at 10 L/hr (240L/day). The three stages of the bioreactor were separated from each other using rigid plastic boards bolted on top of each other. Wastewater flowed by gravity through external pipes from Anoxic stage 1 to Anoxic 2 and finally to the Aerobic stage. A recycle stream from the Aerobic stage to Anoxic 1 provided mixing and created a uniform composition of microorganisms and nutrients throughout the bioreactor. A 90 L cylindrical clarifier was used to separate the biomass from the treated effluent. The settled biomass was pumped continuously (by a metering pump) from the clarifier to the ALU at a rate of 15 L/hr. Finally, the treated effluent was tested for 1) nutrients concentrations, 2) evaluation of nutrient removal process and 3) reactor's performance. The experimental set-up was equipped with pH

meter, temperature, dissolved oxygen (DO) sensors and flowmeters. All the sensors and transmitters were connected to a data acquisition system which recorded and monitored the bioreactor continuously. The multistage vertical bioreactor was designed, constructed and tested in the Water Technologies Laboratory at Ryerson University in Toronto. The experimental unit was the result of a series of modification that began with the CUBEN bioreactor (Figure 3-1) and reached its maximum experimental flexibility (Figure 3-2).

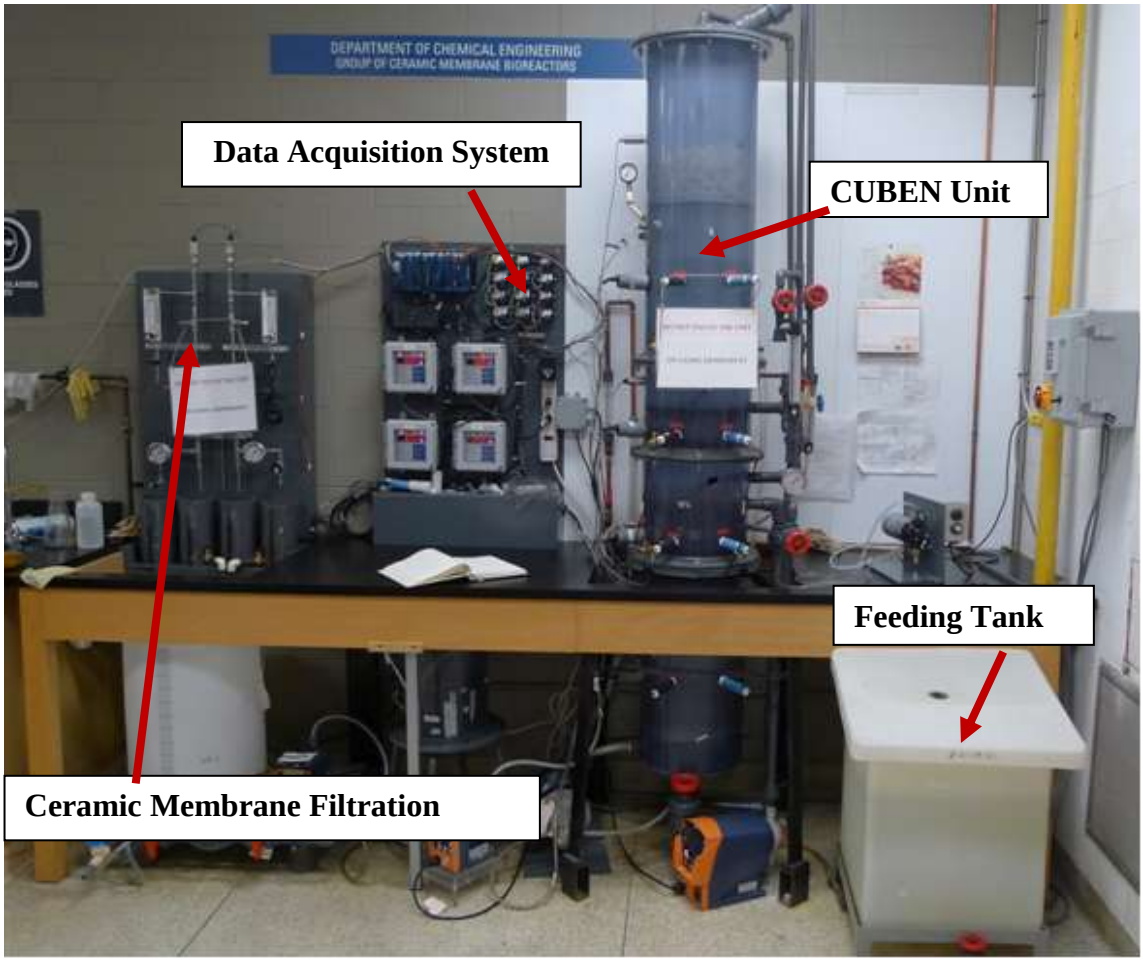


Figure 3–1 Compact Upright Bioreactor for the Elimination of Nutrient (CUBEN)

Figure 3-2 shows the experimental facility where this PhD experiment was carried out.

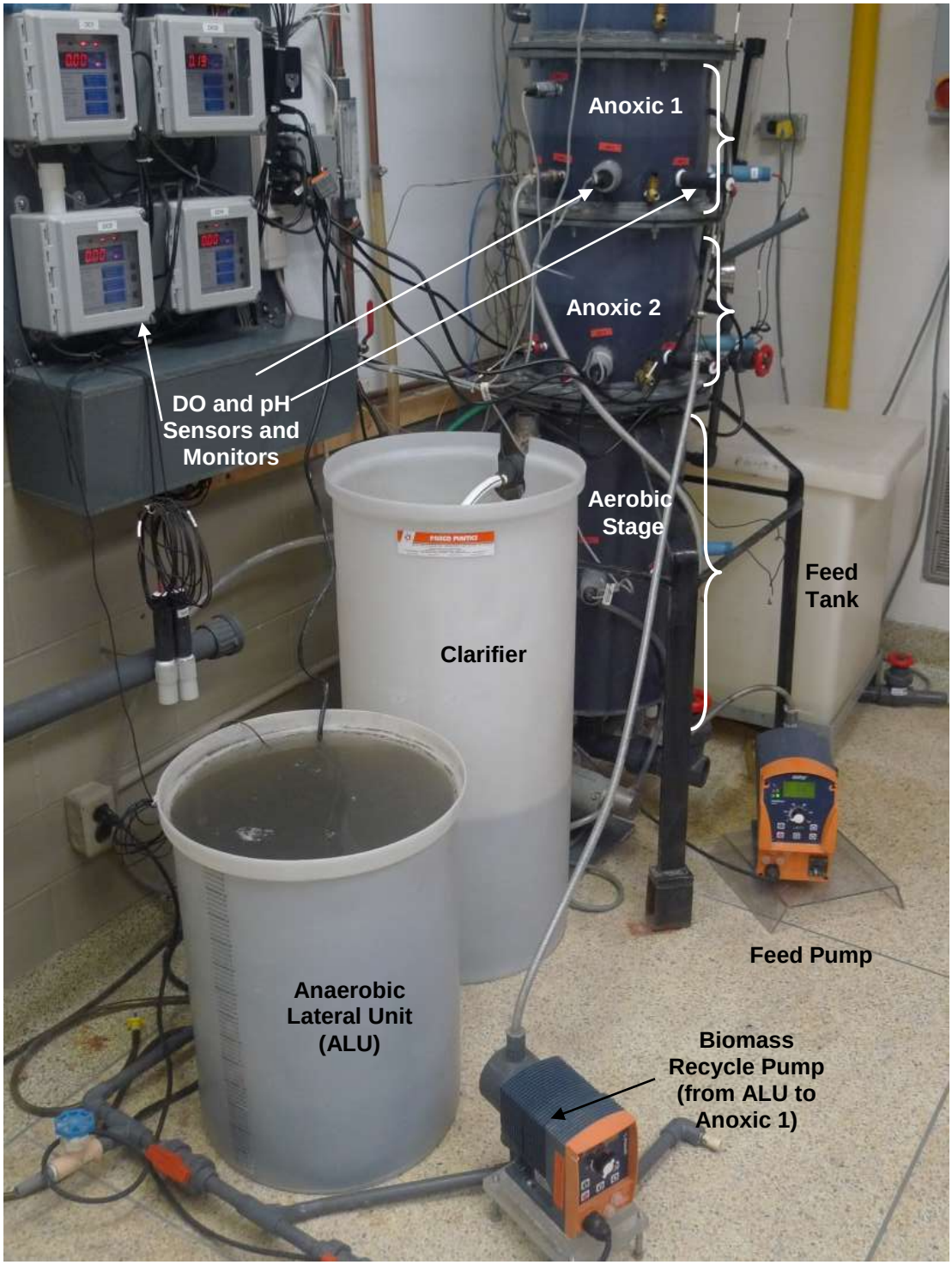


Figure 3–2 Photo of the Bioreactor and Experimental Facility (Modified CUBEN)

Figure 3-3 shows the schematic diagram of experimental set-up.

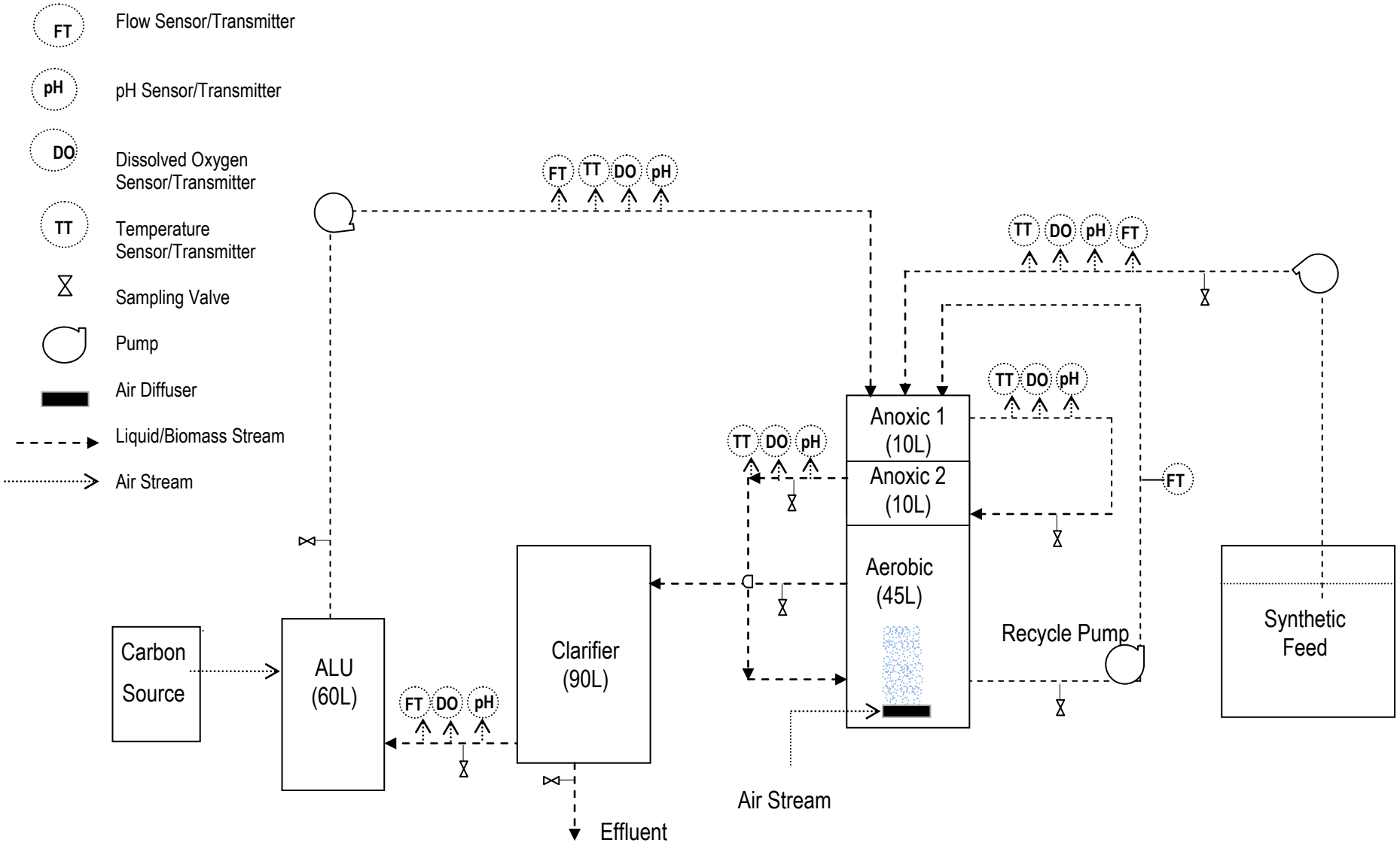


Figure 3–3 Block Diagram of the Vertical Bioreactor aligned with the Clarifier and Anaerobic Lateral Unit (ALU)

3.2.2 Process Development Methodology

To develop simultaneous nitrification-denitrification removal the following design parameters were adopted from studies by Jetten (1997) and Winkler (2011):

- Solids residence time (SRT): 50 days;
- Dissolved oxygen (DO) concentration in the Aerobic stage: 2.5-3.5 mg/L;
- DO concentration in Anoxic 1 and Anoxic 2: <0.1mg/L;
- pH: 7-7.5;
- DO in the Aerobic stage was varied to obtain optimum NO_2^- concentration and avoid NO_2^- accumulation in the Anoxic stages;
- Temprature: 15-25 ^oC.

Critical chemical and physical parameters in developing a successful BPR process include:

- A mixture of acetic acid, propionic acid and butyric acid was found to be the best carbon source to favor the growth of PAOs over glycogen accummulating organisms (GAOs); the PAOs competitors (Randall et *al.*, 1992 and Reddy et *al.*, 1998);
- COD to TP ratio in the influent was maintained at 30:1 (COD:TP) (Reza and Alvarez-Cuenca, 2013);
- Optimum DO concentration of 2.5-3.5 mg/L was used. DO has shown a significant effect on the PAO-GAO competition. High DO concentrations (i.e. 4.5-5.0 mg/L) reduce the BPR efficiency (Oehmen et *al.*, 2007);
- Low pH (below 6.5) can be detrimental for the BPR process since a low pH enviornment can promote the growth of GAOs. Calcium bicarbonate and sodium hyrdroxide were added to maintain the pH of the proccess within 7-7.5 range;

- The temperature was maintained within the rage of 20 to 25^{o}C. Researchers like Baetens (2001) reported that temperatures higher than 30^{o}C can negatively affect the BPR.

Design parameters and operating conditions mentioned above are summarized in Table 3-1.

Table 3-1 Operating conditions in the three stages of the bioreactor and the ALU

Parameters	**Three stages of the bioreactor**		**Anaerobic Lateral Unit (ALU)**
	Anoxic 1 & 2	**Aerobic**	
DO (mg/L)	<0.1	2.5-3.5	0
COD (mg/L)	500-300	250-100	1600
TP (mg/L)	30 (feed conc.) <TP <35	<1	50-60 (TP release by PAOs)
pH	7-7.5	7-7.5	7-7.5
Temperature	22-25^{o}C	22-25^{o}C	22-25^{o}C
HRT	2.5hours	4hours	4 hours
SRT	50 days	50days	50 days

3.2.3 Operating Procedure

The bioreactor's influent was a synthetic wastewater composed of the following compounds: NH_4Cl (11.25g), $KH_2\ PO_4$ (2.77g), Na_2HPO_4 (3.125g), $Na_2HPO_4H_2O$ (2.807g), Urea CH_4N_2O (5g), Calcium Carbonate (5g), $CaCl_2.H_2O$ (1.5g), $MgSO_4.7H_2O$ (1.5), Na_2SO_4 (1.5g), $FeCl_3$ (1.5g/L), $ZnCl_2$ (0.12g/L) and EDTA (7mg/L). These compounds were added and mixed in tap water in a 60L feed tank. A mixture of acetic acid (10ml), propionic acid (10ml), butyric acid (10ml) and sugar 20(g) was added to the ALU. All of these chemicals were supplied by VWR International. The feed composition is presented in Table 3-2.

Table 3-2 Synthetic Wastewater Composition

Nutrient	**Concentration (mg/L)**
NH_3-N	45 ± 1.8
NO_3-N	32 ± 0.9
*NO_2^-	33 ± 1.3
TP	32.6 ± 0.7
Organic Carbon Addition to the ALU	
**COD Concentrations	1400-1600 (mg/L)

* NO_2^- concentration in synthetic feed was due to the chemical reaction of urea in water. No nitrite containing compounds were used.
** COD concentration formed by adding a mixture of propionic, butyric and acetic acids as well as sugar.

The bioreactor was inoculated on November 10, 2012 with activated sludge from the North Toronto Wastewater Treatment Plant. During the start-up period, the biomass was maintained and internally recycled within the bioreactor for approximately three (3) months. During this period, samples were collected regularly from the bioreactor to detect any reduction in ammonia and phosphorus concentrations.

3.2.4 Analytical Methods

TKN was measured using the HACH 8075 Method. NH_3-N was determined by the HACH Method 8038, based on the Nessler method explained in the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). NO_3-N was determined by HACH Method 8171, based on the Cadmium reduction method. NO_2^- was measured following the Ferrous Sulfate Method **(**HACH 8153). TP and PO_4^{-3} concentrations were measured using the

Molybdovanadate Method with Acid Persulfate Digestion HACH Method 10127. COD was measured using the HACH Method 8000.

3.3 Results and Discussion

3.3.1 Nitrogen Compounds Removal

Simultaneous nitrification-denitrification was detected 3 months after the reactor start-up. It took approximately six (6) months to reach steady state. At steady state, NH_3-N, NO_2^- and NO_3-N concentrations in the three stages of the bioreactor were consistent as shown in Table 3-3.

Table 3-3 Average Concentrations of the Nitrogen Compounds in the Bioreactor after 200 of Continuous Operation

Stages	Nutrients	Influent Concentration (mg/l)	Effluent Concentration (mg/l)
Anoxic Stage 1	NH_3-N	50±5	20±5
	NO_2^-	~	60±5
	NO_3-N	30	10±5
Anoxic Stage 2	NH_3-N	20±5	10±5
	NO_2^-	60±5	40±5
	NO_3-N	10±5	~5
Aerobic Stage	NH_3-N	10±5	~5
	NO_2^-	40±5	10±5
	NO_3-N	~5	~5
Effluent (clarifier)	NH_3-N	~5	<1
	NO_2^-	10±5	<1
	NO_3-N	~5	<1

NH_3-N removal was observed 90 days after the start-up of the reactor. The NH_3-N removal during the period of 90-200 days was unsteady but explicable. It can be observed from

Figure 3-4 that NH_3-N was mainly converted in the first Anoxic stage where NO_2^- concentration was high (shown in Figure 3-5).

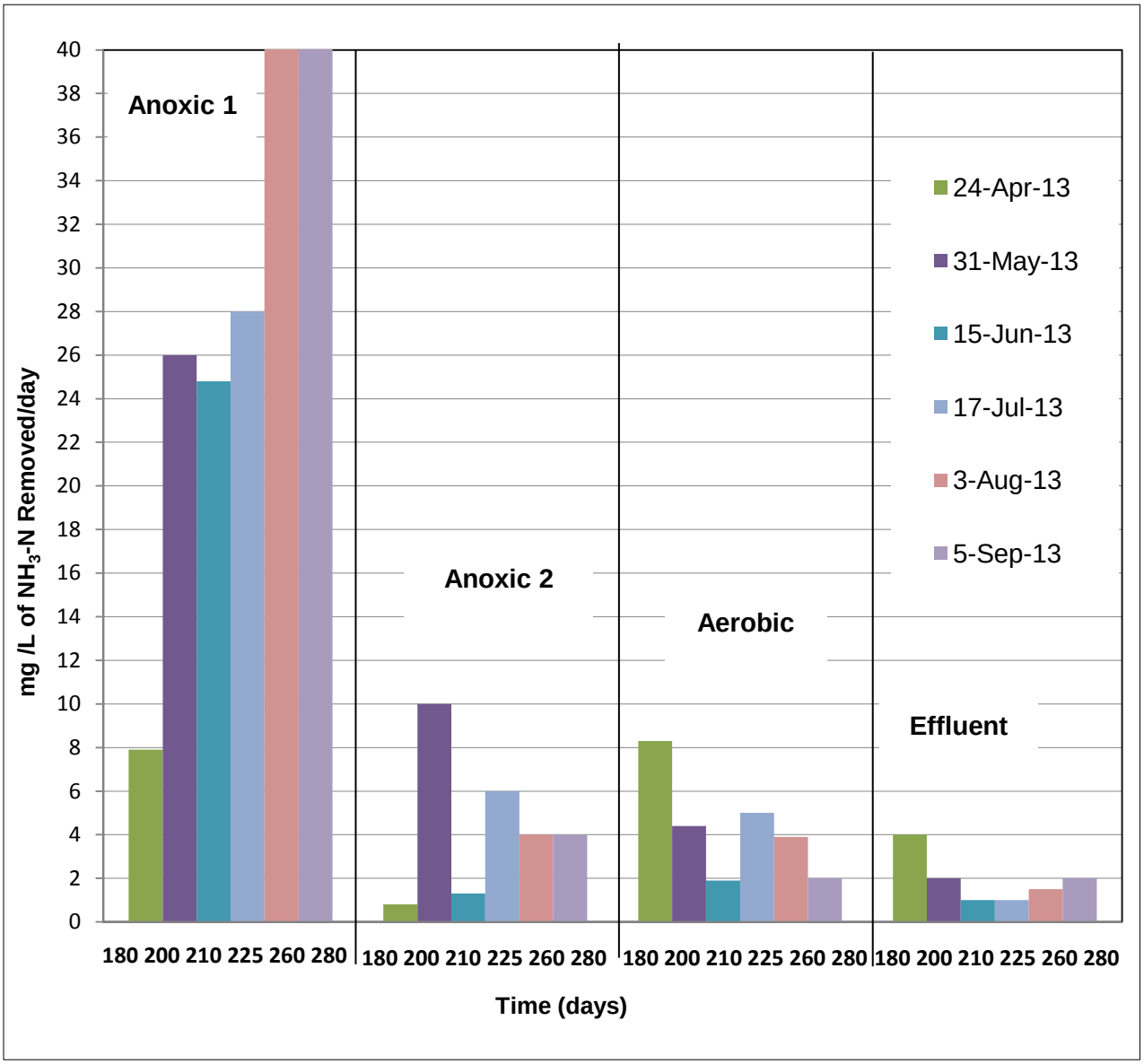


Figure 3–4 Daily NH_3-N removal in the three stages of the bioreactor and the effluent

As shown in Figure 3-4, while the NH_3-N removal rate increased in Anoxic 1, it decreased in the Aerobic stage. Substantial removal of NH_3-N occurred in the absence of DO and the presence of NO_2^- and NO_3-N in the Anoxic stage. The minimum removal rate in the Aerobic stage revealed the presence of both nitrification and denitrification.

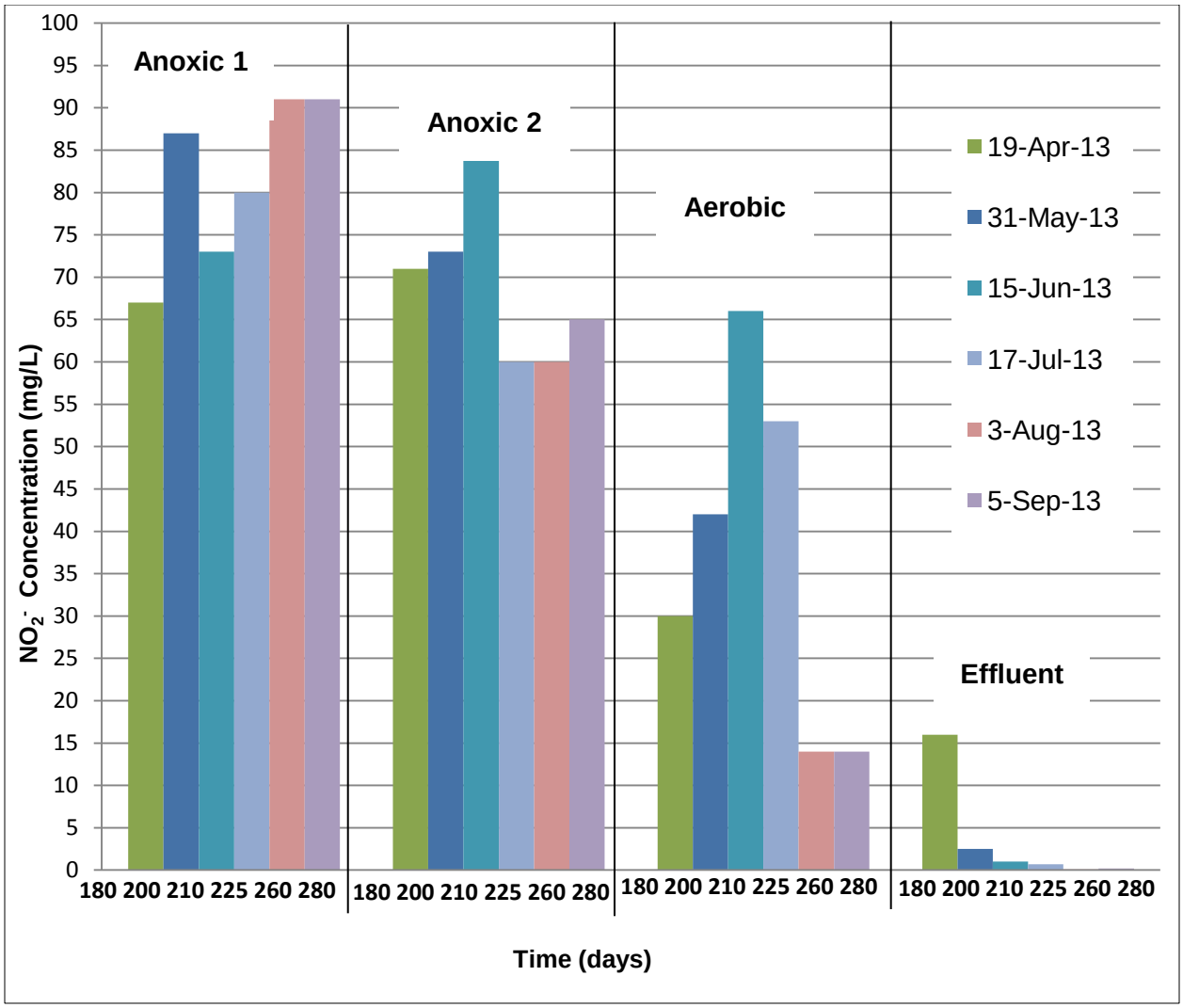


Figure 3–5 NO_2^- concentrations in the three stages of the bioreactor and the effluent

Figure 3-6 shows the concentration of NH_3-N in the influent and effluent streams between days 100 to 320. NH_3-N concentration in the effluent was found irregular during the first 200 days from the start-up date. Over time, NH_3-N in the effluent decreased to below 5 mg/L while influent concentration was kept constant at 50±5 (mg/L).

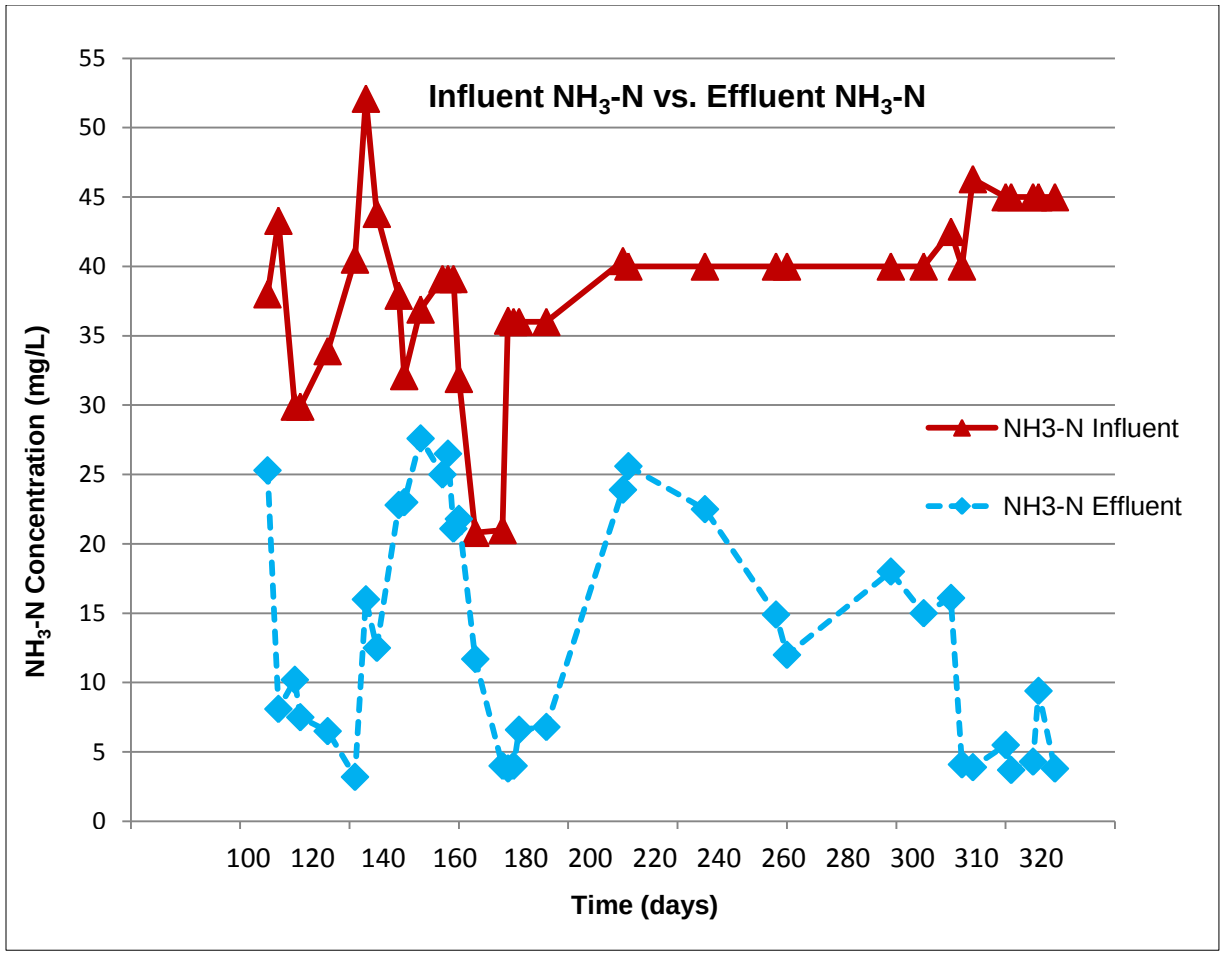


Figure 3–6 Influent and effluent concentrations of NH_3-N

NO_2^- along with NH_3-N and NO_3-N concentrations were measured regularly three times per week. Figure 3-6 illustrates a period of high NO_2^- fluctuations in all three stages of the reactor. NO_2^- concentration in the Aerobic stage was found the highest (200mg/L) between 180 and 200 days of reactor operation. After 200 days (shown on Figure 3-7 by an arrow), the NO_2^- profile showed a decreasing trend in the Aerobic stage with minimal variations in Anoxic 1 and 2. Stable NO_2^- concentration in the reactor together with consistent NH_3-N removal rate implied that steady state simultaneous nitrification-denitrification had been achieved.

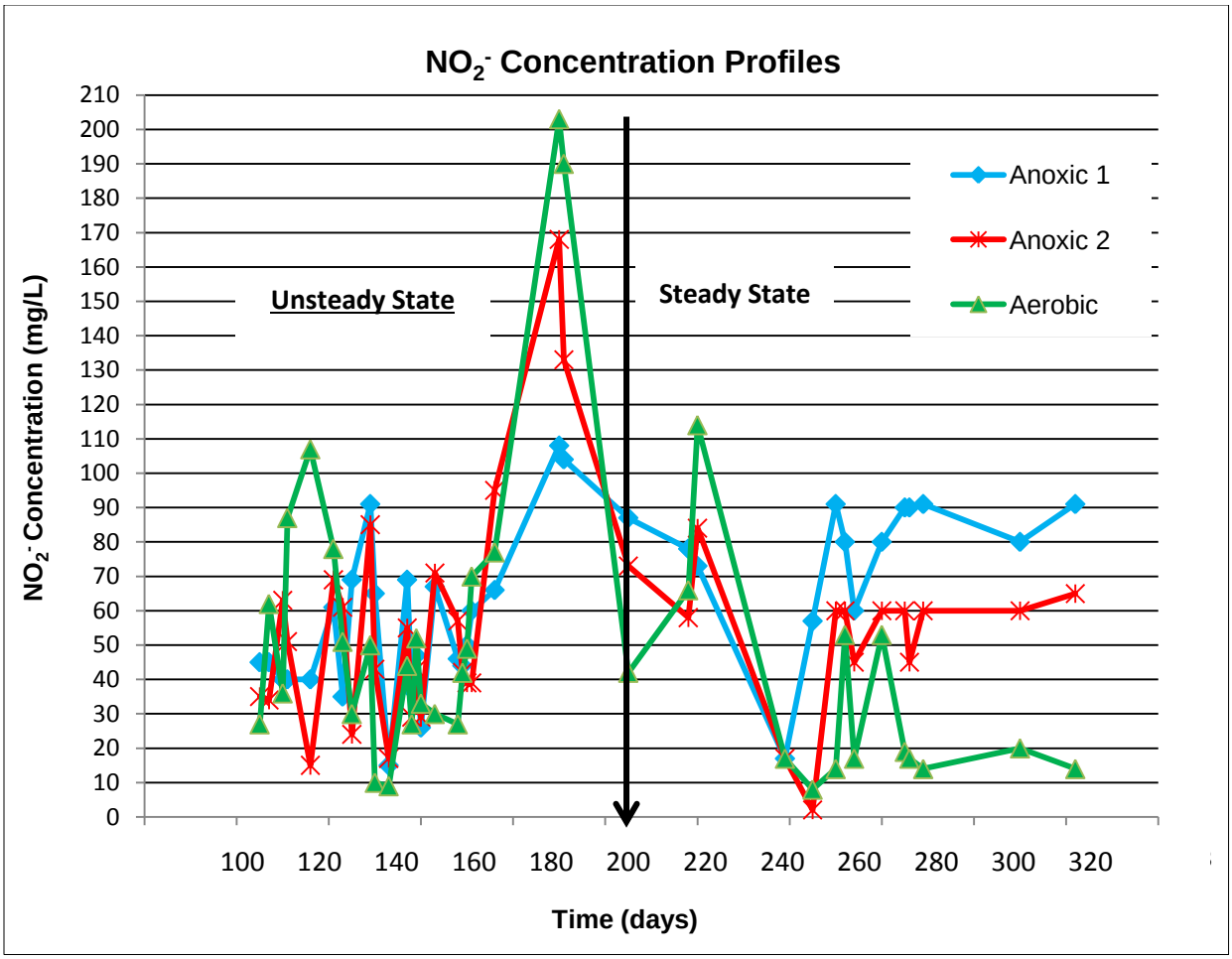


Figure 3–7 NO_2^- concentrations in the three stages of the bioreactor

After 200 days of operation, Anoxic 1 had higher NO_2^- concentration which can be related to high rate of simultaneous nitrification-denitrification in this stage than in Anoxic 2 and Aerobic stages. Figure 3-8, shows the concentrations of NO_3-N from day 100 to 320. After 200 days, NO_3-N concentration in the effluent decreased from approximately 18 mg/L to below 1 mg/L. The descending trend in NO_3-N implied that over time partial nitrification surpassed full nitrification, evidenced by high NO_2^- in Anoxic 1. Thus Figures 3-6, 3-7 and 3-8 confirm the stability, consistency and overall performance of the simultaneous nitrification-denitrification process from day 200 till day 320.

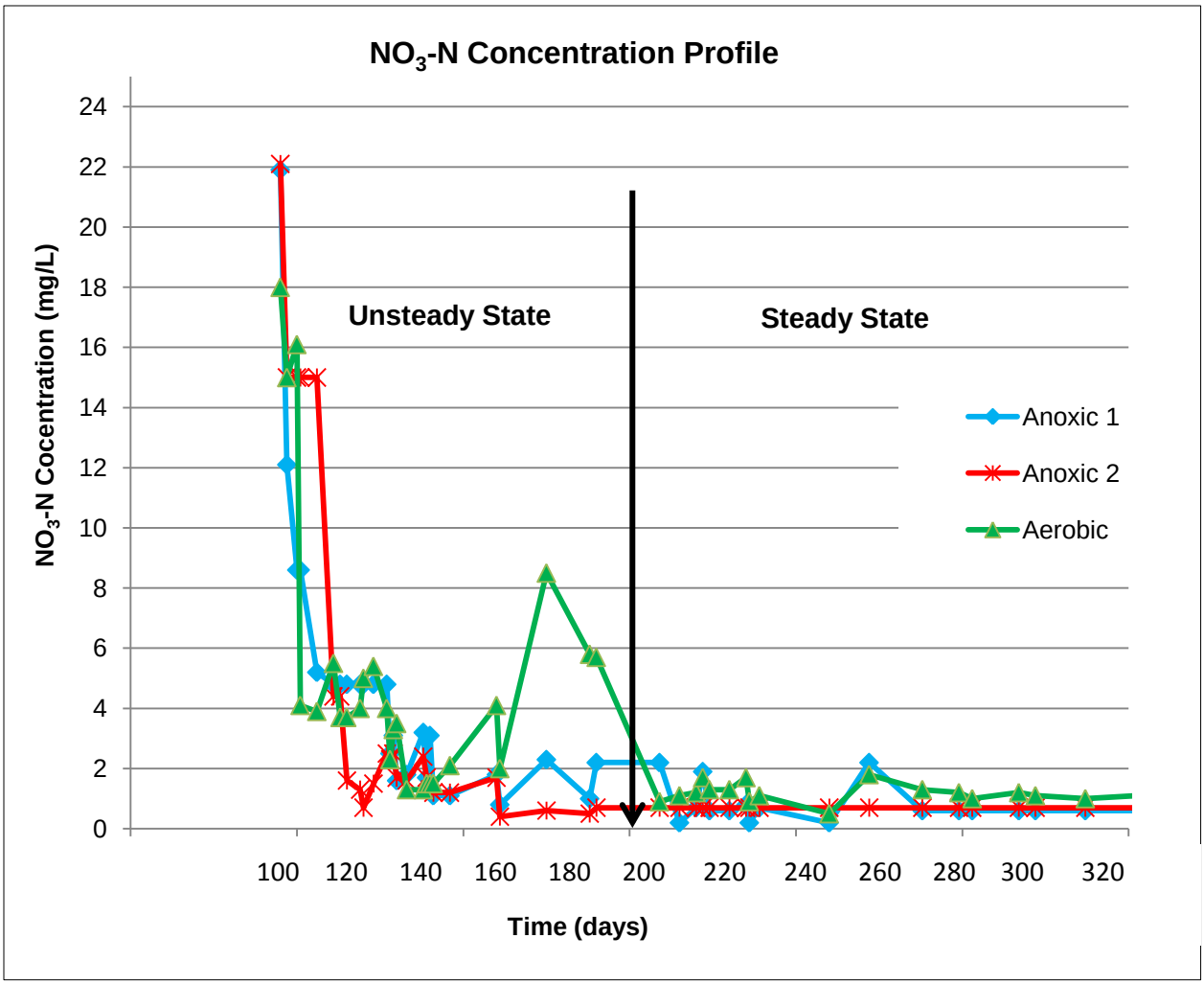


Figure 3–8 NO_3-N concentrations in the three stages of the bioreactor

3.3.2 Biological Phosphorus Removal

As shown in Figure 3-9, the biological phosphorus removal started much later than simultaneous nitrification-denitrification. It took almost 230 days (ca. 7 months) to detect BPR activity. The phosphorus removal efficiency increased from 21%, observed on July 4th, 2013, to 93% on December, 2013 (see Appendix 1). High NO_2^- concentration in the bioreactor, during the first seven (7) months, confirmed the inhibitory effect of NO_2^- on PAOs and DPAOs. This inhibitory effect was diminished once NO_2^- concentration in the reactor was below 100 mg/L due to NO_2^- utilization by denitrifiers. A number of researchers have confirmed the negative effects

of TP, DO and NO_2^- on simultaneous nitrification-denitrification. Saito (2004) reported that NO_2^- could have negative effects on the phosphorus uptake rates of PAOs/ DPAOs. In this study, when simultaneous nitrification-denitrification reached steady state condition, the NO_2^- fluctuations in the reactor were attenuated and PAOs/DPAOs started to uptake TP from the wastewater. TP concentration in the influent was kept constant at 32 mg/L whereas in the effluent the concentration of phosphorous gradually reduced to 2 mg/L. Figure 3-9 exhibits TP concentrations in the influent, the effluent and the ALU. The effluent TP concentration declined after 230 days, indicating that successful BPR was underway.

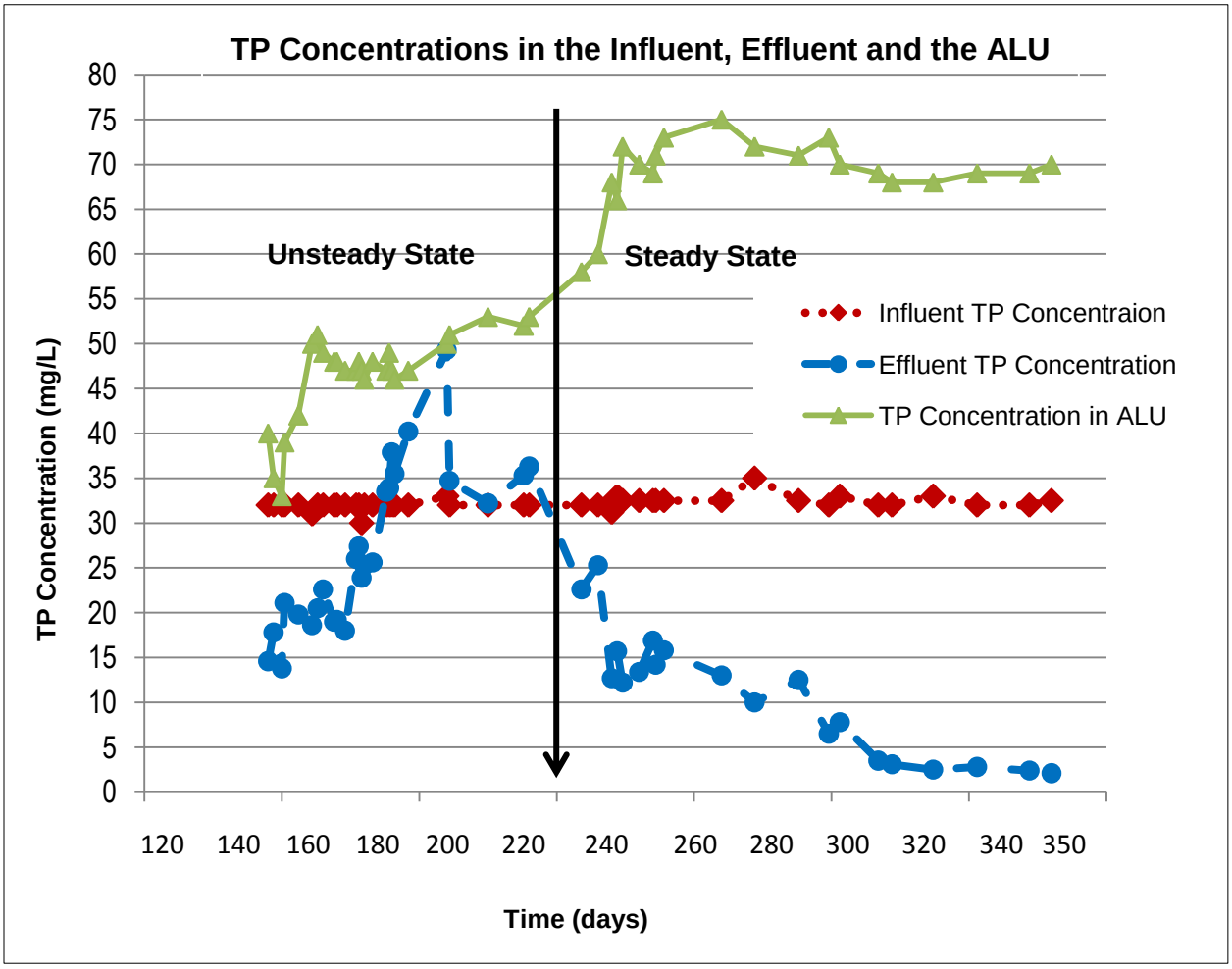


Figure 3–9 TP concentration profiles in the influent, effluent and ALU

There were no changes in the ammonia removal efficiency from the moment the BPR process became stable in the reactor. This was an indication that PAOs, DPAOs, and AOBs can coexist under common environmental conditions even though they have distinctive nutrient removal activities and start-up phases. The action of ALU with 4 hours of HRT and addition of volatile organic carbon (approximately 1200-1600 mg/L) was essential to develop the BPR process. TP concentration in ALU was 75 mg/L, which was more than double the TP amount in the synthetic feed (32 mg/L). High TP concentration in the ALU was due to the phosphorus release by PAOs/DPAOs. The experimental data confirmed that TP removal occurred in all three stages of the bioreactor. This BNR performance has not been reported in the scientific literature before.

3.3.3 Simultaneous Nitrification-Denitrification-BPR (SNDP) Process

The mixed liquor suspended solids (MLSS) concentration in the bioreactor varied over time and was as high as 5000 mg/L when the simultaneous nitrification-denitrification-BPR was at steady state. The MLSS concentration of 5000 mg/L was found in all three stages of the bioreactor due to complete mixing condition. The complete mixed operation was maintained by high internal recycle of 20 L/hr (recycling from Aerobic to Anoxic 1) and continuous operation of the bioreactor (24 hours per day, 7 days per week). Figures 3-10 to 3-14 present the results obtained from the three stages of the bioreactor and from the secondary clarifier. Figure 3-10 illustrates the nutrient concentrations in the feed.

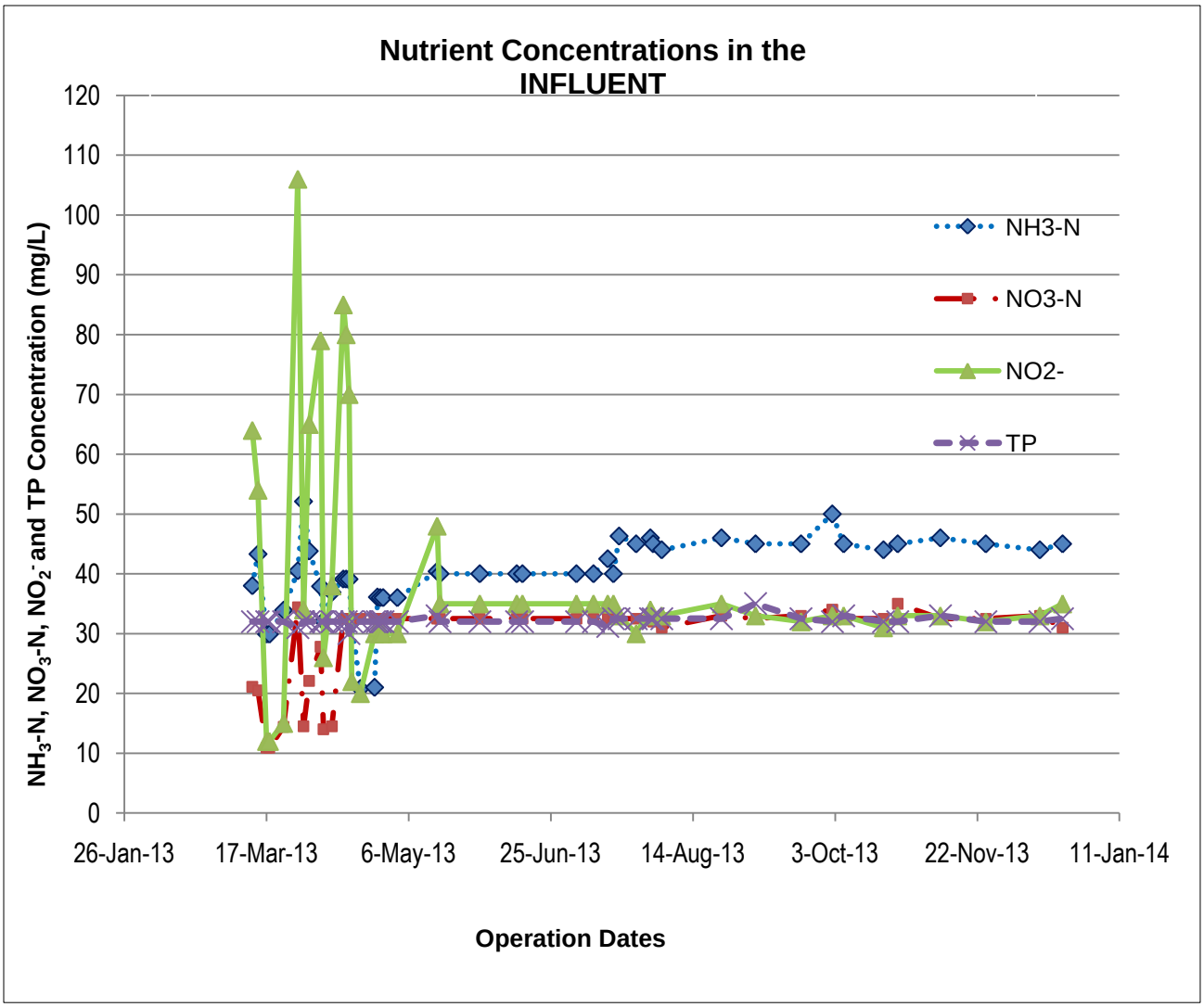


Figure 3–10 Nutrient Concentration in the Influent to the Bioreactor

During the start-up period, there were strong fluctuations in NO_2^- concentration (March-May 2013). This was due to the sodium nitrite addition in the synthetic feed. Early in May, the addition of sodium nitrite in the feed was discontinued.

Figure 3-11 illustrates the concentration profiles of NH_3-N, NO_2^-, NO_3-N and TP in the first Anoxic stage. As it was observed in Figure 3-4, the NH_3-N removal rate was very high in Anoxic 1. Figure 3-5 showed very high NO_2^- concentration in Anoxic 1 suggesting that NH_3-N

was predominantly removed by NO_2^-. Furthermore, NO_3-N concentration was very low (almost zero) in this stage which indicated that NO_3-N did not contribute to NH_3-N removal.

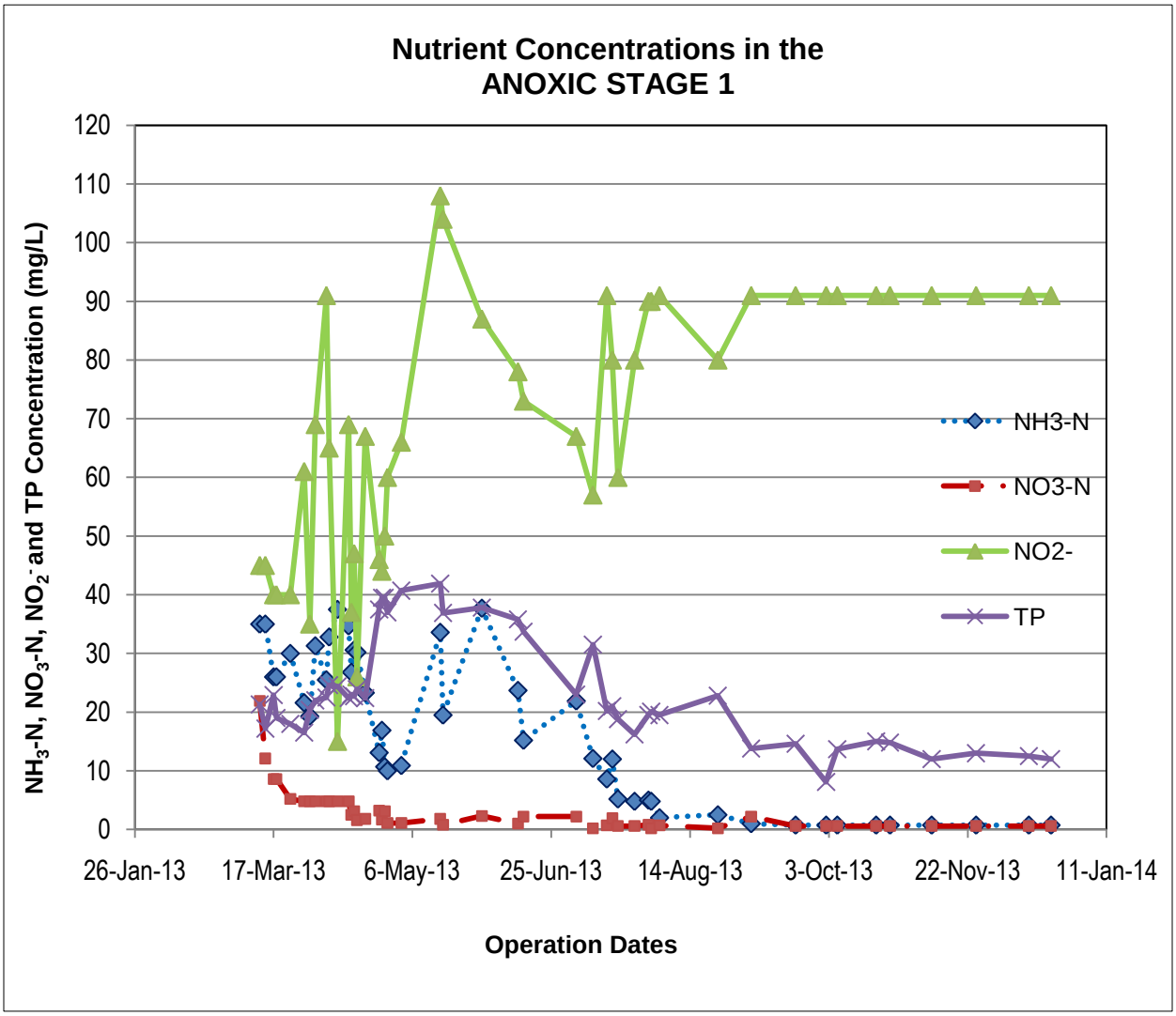


Figure 3–11 Nutrient Concentration in the Anoxic 1 Stage

Anoxic 2 and Aerobic stage results are presented in Figures 3-12 and 3-13. Nutrient concentrations, especially NO_2^-, were unstable from March to July 2013. In May 2013, NO_2^- concentration suddenly increased to above 110 mg/L and 170 mg/L in the Anoxic 1 and Anoxic 2 stages respectively. This was due to the NO_2^- accumulation in the bioreactor as the internal recycle was increased from 10 to 20 L/hr. Nutrient concentrations in Anoxic 1, Anoxic 2 and

Aerobic stage were consistent when the simultaneous nitrification-denitrification-BPR process reached steady state in August 2013, approximately 240 days after the start-up day.

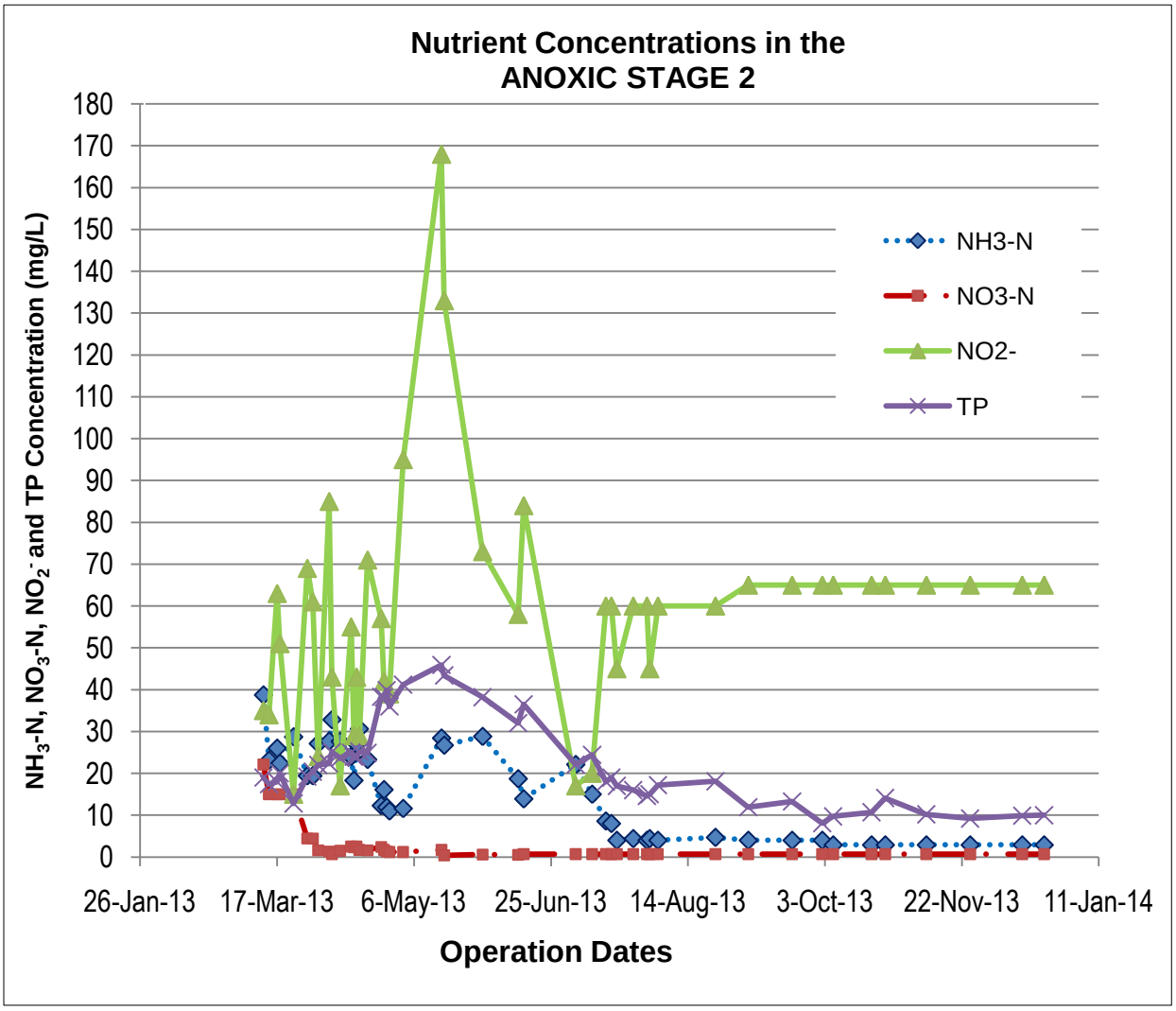


Figure 3–12 Nutrient Concentration in the Anoxic 2 Stage

As shown in Figure 3-13, the nutrient concentrations were unstable prior to July 2013. After that date simultaneous nitrification and denitrification stabilized and phosphorus uptake was achieved by the end of July.

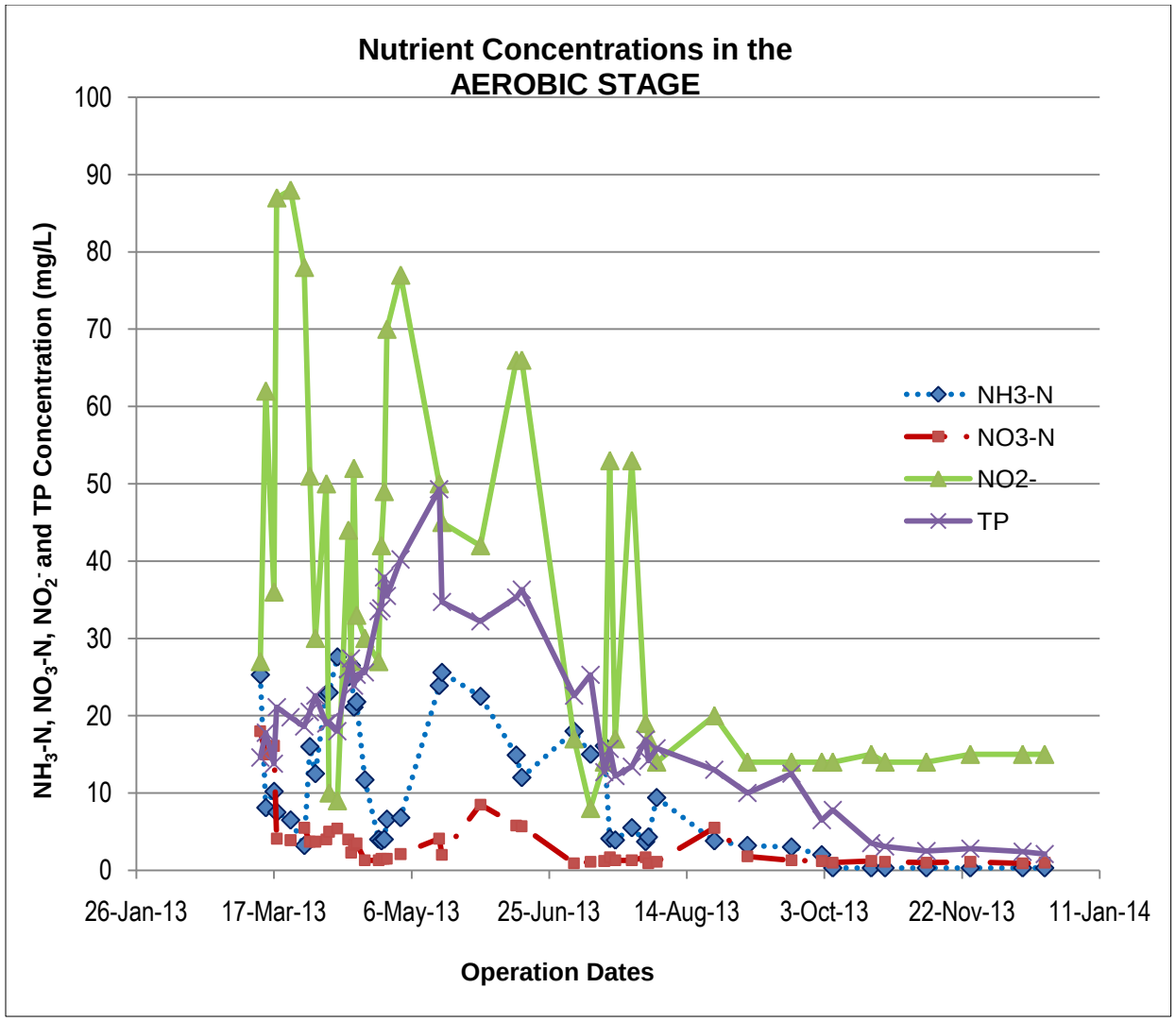


Figure 3–13 Nutrient Concentration in the Aerobic Stage

The secondary clarifier (90 L) was used to separate the biomass from the treated water. The physical characteristics of the biomass changed during the scope of this study. The settlability of the sludge in the effluent increased when the simultaneous nitrification-denitrification-BPR process was established. Figure 3-14 shows nutrient concentrations in the effluent leaving the secondary clarifier over time.

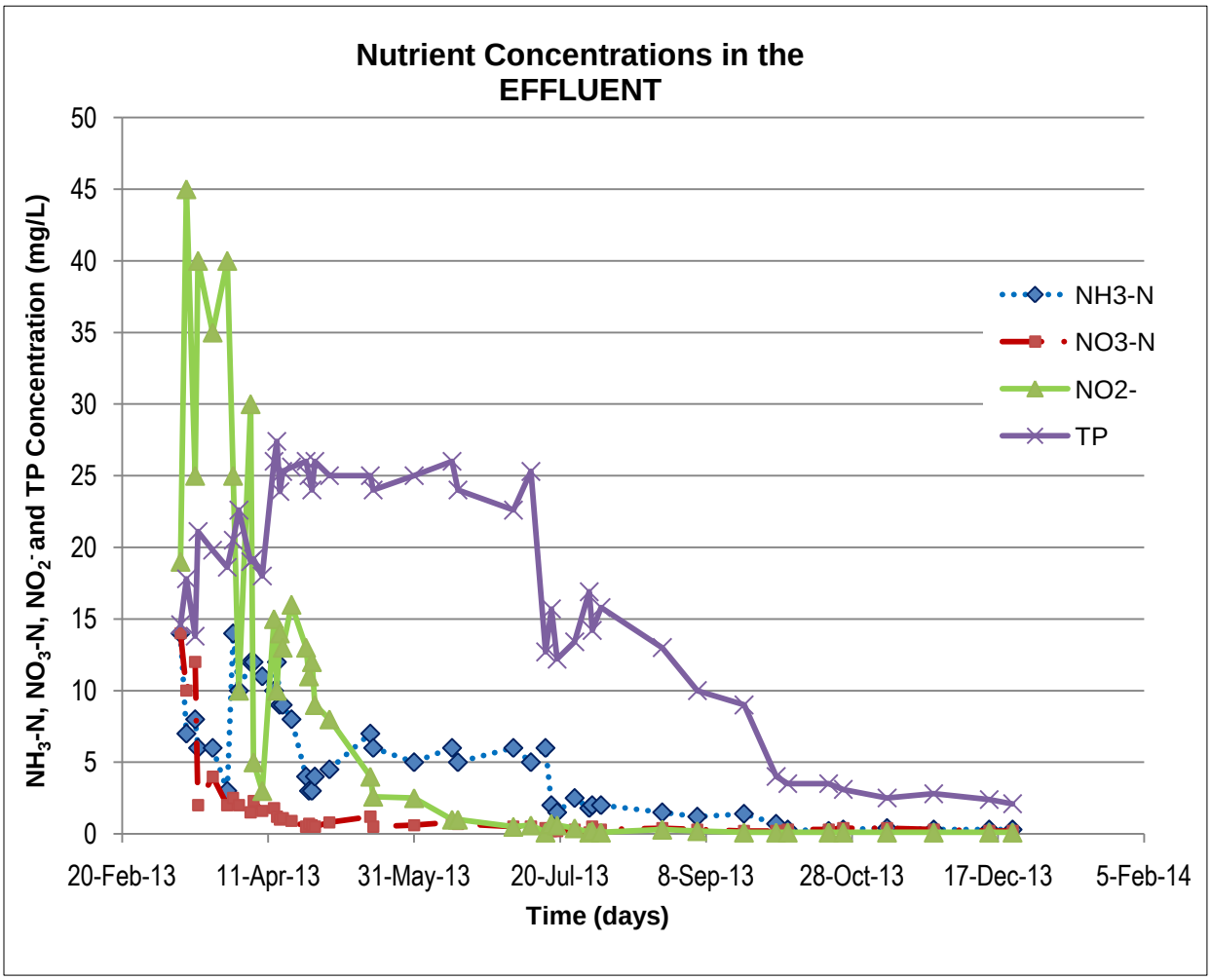


Figure 3–14 Nutrient concentrations in the effluent from the secondary clarifier

The concentrations of nitrogen compounds including NH_3-N, NO_3-N and NO_2^- overlapped and were virtually zero when simultaneous nitrification-denitrification was fully established. The TP concentration in the effluent leaving the secondary clarifier was gradually reduced from 25mg/L in July 2013 (240 days from the start-up) to less than 2 mg/L in December 2013 as shown in Figure 3-14.

Figure 3-15 summarizes ammonia and total phosphorus concentrations in the influent and effluent to the bioreactor from day 100 till 340.

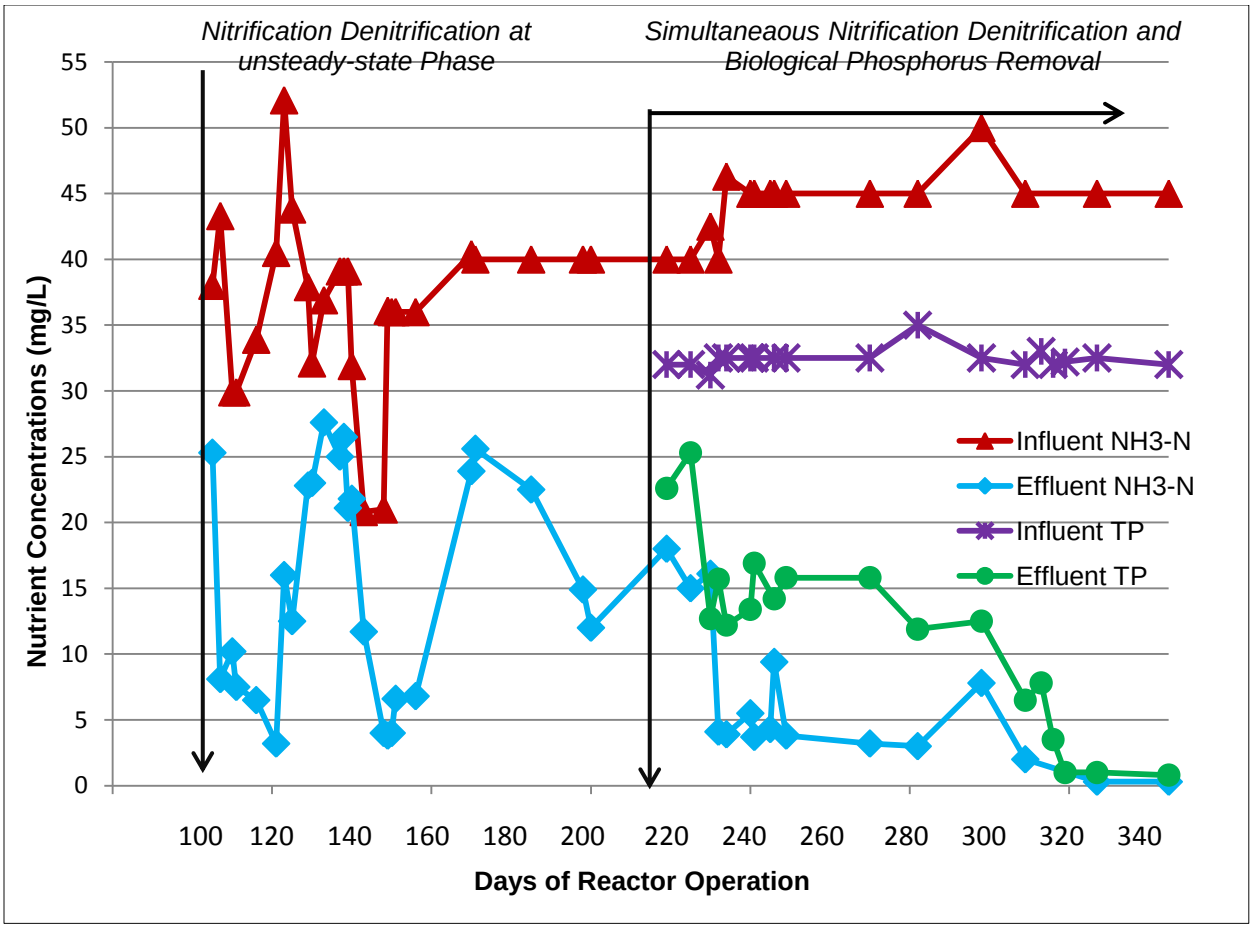


Figure 3–15 Nutrient concentration in the Influent and Effluent

The BNR performance in the last 6 months of the reactor operation is presented in Table 3-4.

Table 3-4 Reactor performance from day 110 to day 355

Parameter	Average Influent ± SD	Average Effluent ± SD	Nutrient removal Efficiency
TKN (mgN/L)	256 ± 41	23± 7	91%
NH_3-N(mgN/L)	45 ± 1.8	1.0 ±0.4	98%
NO_3-N (mgN/L)	32 ± 0.9	1.1± 0.1	97%
NO_2^- (mgN/L)	33 ± 1.3	0.8 ±0.5	98%
TP (mgP/L)	32 ± 0.64	1.02 ±0.33	97%
COD (mg/L)	526	97	82%

* SD: Standard Deviation

3.4 Conclusions

This chapter provided an overview of the vertical bioreactor design and development of a simultaneous nitrification-denitrification-BPR process in this bioreactor. The biological process developed was successful due to the bioreactor's innovative structure and performance. The most significant factors in developing simultaneous nitrification-denitrification-BPR process were:

- Sequential arrangement of Anoxic1, Anoxic 2 and Aerobic stages;
- Operation of the ALU in conjunction with the bioreactor and the biomass recycle from the ALU to the first Anoxic stage;
- Internal recycle stream from the Aerobic stage to Anoxic ;
- Maintaining neutral pH (7-7.5);
- Obtaining optimum NO_2^- concentration (below 100 mg/L).

The removal of nitrogen compounds including NH_3-N, NO_2^- and NO_3-N was over 95%. High NO_2^-concentration in the reactor was found to be a major inhibitor for the growth and microbial activities of PAOs and DPAOs. After 230 days, the BPR process was fully established and achieved over 95% TP removal. BPR occurred in all three stages of the bioreactor. As opposed to the high rate of NH_3-N removal in Anoxic 1, TP was equally removed in each stage. This indicated that PAOs and DPAOs performed equally under the two Anoxic stages and the Aerobic stage. This observation was in opposition to the view held by other researchers. There were no changes in the nitrogen removal efficiency from the moment the BPR process was detected in the bioreactor. This was an indication that PAOs, DPAOs, AOBs and NOBs coexisted under common environment.

Chapter 4 - Structure and Function of the Microbial Community Involved in the Simultaneous Nitrification, Denitrification and Biological Phosphorus Removal (SNDP)

4.1 Introduction

This chapter explores and discusses the microbial communities involved in the removal of ammonia and phosphorus in the experimental unit explained in chapter 3. The composition of the microbial community in the bioreactor was determined using phylogenetic analyses of the biomass samples and results were compared with the existing clone libraries. The results showed that the dominant bacterial groups were affiliated with the sub-class of β-*proteobacteria* (28.5%) from which 23% were from the genus *Zoogloea*. Another dominant group 67% were *Bacteriodites* from which 60.6% were a member of *Saprospiracae* genus. Against this author's initial prediction, there was no autotrophic anaerobic ammonium oxidizing bacteria commonly known as Anammox. Furthermore, *Acinetobater* and *candidatus* group that are widely known as PAOs were absent in the microbial structure. These results indicate that unique microbial structures with possibly new biochemical pathways were responsible for the removal of ammonia and phosphorus with such a high efficacy.

Objectives of the Molecular Biology Analysis: One of the main obstacles in the development of an industrial scale BNR process is the increasing complexity of its microbial communities. Another key issue is the insufficient knowledge of the microbial structure and ecophysiology of microbial populations in BNR processes. Most of the micro-organisms involved in BNR have been identified through culture-independent studies in well-controlled laboratory-scale reactors (Amann, 1995). Detection and quantification of these uncultured organisms can only be accomplished by molecular biology techniques using group or clone-specific oligo nucleotide probes. Previous research studies (Nielsen, et *al.*, 2010) have shown that there are six main functional groups in BNR processes including:

- Polyphosphate accumulating organisms (PAOs);

- Glycogen accumulating organisms (GAOs);
- Filamentous bacteria;
- Nitrifying organisms;
- Denitrifiers;
- Fermenting bacteria;
- Hydrolysing bacteria.

PAOs and GAOs: Based on extensive studies of the function of probe-defined species, PAOs are largely related to both *Rhodocyclus* (RPAO or *Accumulibacter*) (Crocetti et *al*., 2002; Hesselmann et *al.*, 1999) and certain *Actinobacteria* (Kong et *al.*, 2005). PAOs are able to uptake acetate under anaerobic conditions and form PHAs. As explained before, under aerobic/anoxic condition, PAOs can utilize the energy of their intracellular PHAs to uptake phosphorus from wastewater. GAOs are able to uptake acetate from wastewater under anaerobic conditions and form PHAs however they are unable to uptake phosphorus in the subsequent aerobic/anoxic stage. GAOs are generally known as the PAO's competitors and believed to be detrimental for the BPR process. GAOs mainly belong to the group of γ-*proteobacteria* (*Competibacter*) (Crocetti et *al.*, 2002; Kong et *al.*, 2004; Wong et *al.*, 2005).

Nitrifiers and Denitrifiers: Ammonia oxidizing bacteria (AOBs) involved in the first step of nitrification are primarily of β-*proteobacteria* and belong to the genus of *Nitrosomonas*, *Nitrosospira* (Mobarry et *al.*, 1996). The second step of nitrification is completed by a diverse group of nitrite oxidizing bacteria (NOBs) that are mainly α-*proteobacteria* including *Nitrospira* and *Nitrobacter*. NOBs, belonging to the phylum *Nitrospira*, are found to be more prevalent in BNR plants (Thomsen et *al.*, 2007). The identity and function of a significant fraction of the

biomass in BNR may still be unresolved, demanding scientists to conduct more advanced studies in full-scale treatment plants to identify other potentially important micro-organisms.

The motivation for the present research was to gain further insight into the microbial ecology of the biomass samples taken from the three stages of the vertical bioreactor. A better understanding of the microbial ecology can help to optimize the biological process and improve the design of the bioreactor. Comprehensive knowledge of the microbial community can support the optimization of the existing nutrient removal processes and enable the development of novel BNR processes.

The co-existence of microorganisms in BNR processes is highly influenced by three redox conditions, 1) anaerobic, 2) anoxic, and 3) aerobic. Microorganisms in BNR processes require adapting constantly to the variation of redox conditions which in turn are influenced by the bioreactor configuration, HRTs, SRTs and nutrient concentrations. These dynamic conditions promote mutation, niche partitioning, cellular versatility and variation in microbial activity in each redox zones (Nielsen et *al.*, 2010). Niche partitioning is due to the dynamic conditions where certain microorganisms are more suited for growth in one zone or another. The niche partitioning is further driven by metabolic specialization due to both the diversity of substrates in wastewater and the hydrolysis of macromolecules.

4.2 Materials and Methods

Microbial analysis of the samples from the Anoxic and Aerobic stages of the bioreactor was completed following the steps below:

i) DNA extractions using the Powersoil DNA Isolation Kit, and Polymerization change reaction (PCR) using bacteria-specific primers, and

ii) DNA sequencing using the MiSeq® system.

i) DNA Extraction and Polymerization Chain Reaction (PCR)

To analyze the microbial population, 2 and 4 µL biomass samples were taken from the two Anoxic and the Aerobic stages of the bioreactor. The biomass samples were taken in November 2013 when the process in the bioreactor was at steady state condition. The biomass from Anoxic 1 and 2 were mixed and analyzed as one biomass sample. The biomass sample from the Aerobic stage was collected and analyzed in a separate test tube. The 2 and 4 µL samples were centrifuged followed by DNA extraction with the Powersoil DNA Isolation Kit. The Powersoil kit contained solutions labelled as C1 through C6 whose specific chemical mixtures and exact composition were withheld as proprietary reagents. The protocol that follows involved a harsh cell lysis and rigorous purification.

The Powersoil DNA kit contained Power Bead Tubes to which 0.25 mg of each Anoxic and the Aerobic biomass samples were added. The tubes were then mixed by gentle vortexing. 60 µL of solution C1 were added and the tubes were vortexed for 5 seconds. After a centrifugation step at 10,000 x g for 30 seconds at room temperature, the supernatant was transferred to a clean 2 mL Collection Tube. Then, 250 µL of solution C2 were added and the tubes were vortexed for 5 seconds. The tubes were incubated at 4°C for 5 min and then centrifuged at room temperature for 1 min at 10,000 x g. Next, 600 µL of supernatant were transferred to a clean 2 mL collection tube avoiding the pellet. 200 µL of solution C3 were added and the tubes were briefly vortexed and incubated at 4°C for 5 min. The mixtures were centrifuged at room temperature for 1 min at 10,000 x g. Then, up to 750 µL of supernatant were transferred to a clean 2 mL Collection Tube. Next, 1200 µL of solution C4 were added to the supernatant and the mixtures vortexed for 5 seconds. Approximately 675 µL of the mixture were loaded onto a Spin Filter and centrifuged at 10,000 x g for 1 min at room temperature. The flow

through was discarded, and an additional 675 µL of supernatant was added to the Spin Filter and centrifuged at 10,000 x g for 1 min at room temperature. The same was done with the remaining volume of supernatant. Then 500 µL of solution C5 were added and centrifuged at room temperature for 30 seconds at 10,000 x g. After discarding the flow through, the samples were centrifuged again at room temperature for 1 min at 10,000 x g and the Spin Filters were placed in a clean 2 mL collection tube. Then 100 µL of solution C6 were added to the center of the white filter membrane. Centrifugation was performed at room temperature for 30 seconds at 10,000 x g and the Spin Filter was discarded. Finally, a large amount of DNA from all extracts was recovered and ready for Polymerization Change Reaction (PCR). Bacteria-specific primers were used for amplification of 10 ug of template per sample (30 reaction cycles) and expected a ~425-base PCR product (excluding primer sequences, which were removed for analysis).

ii) DNA Sequencing

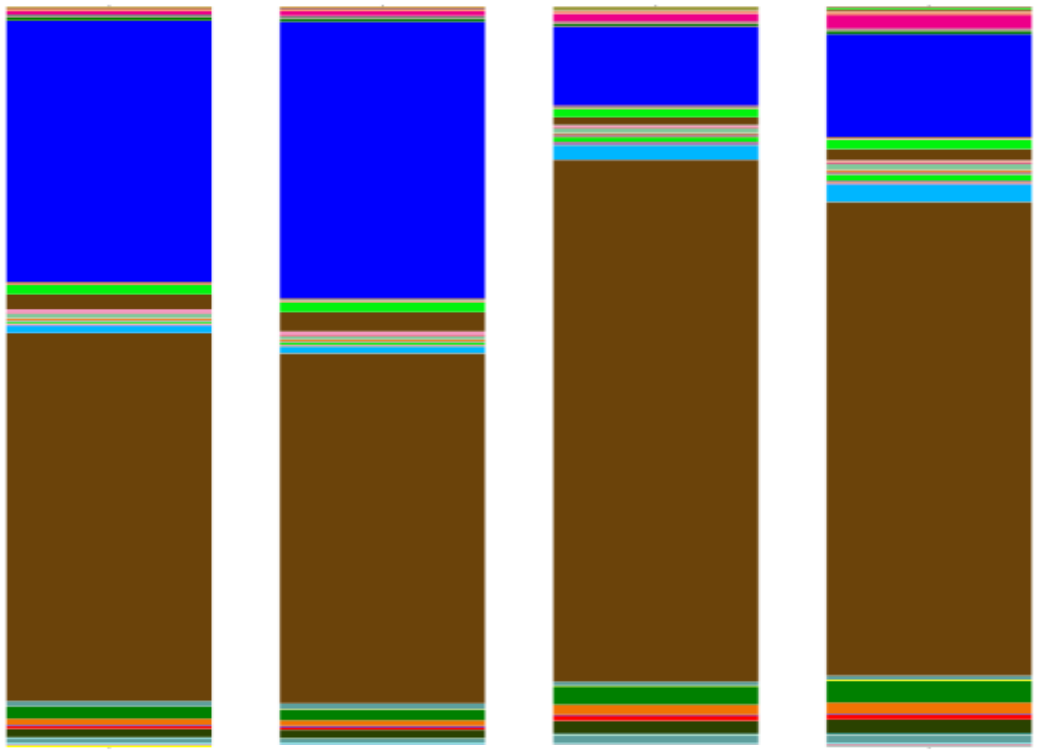
The PCR products were sequenced using a MiSeq® system. UPARSE was used to determine the structure of the microbial community. This software compressed all the data into unique taxa that were within 97% similarity to one another. The most abundant sequence within each 97% cluster was selected as the representative sequence for each operational taxonomic unit (OTU). Using the microbial sequencing, a total of 8,768,510 pairs were recovered. An OTU is typically defined as a cluster of reads with 97% similarity. For consistency, database matching is often done after clustering so that some OTUs are assigned to species and others are flagged as novel or unknown. Table 4-1 shows detailed breakdown of the bacteria in each sample.

Table 4-1 Total microbial population in the biomass samples

Bacteria/ Sample	**Anoxic (2µL)**	**Aerobic (2µL)**	**Anoxic (4µL)**	**Aerobic (4µL)**
Number of Bacteria	1,769,540	1,769,537	1,769,538	1,769,540
Total	3,539,077		3,539,078	

4.3 Results and Discussion

The results obtained from DNA sequencing showed that the samples from Anoxic and Aerobic stages of the bioreactor contained an abundance of *Saprospiraceae and Zoogloea.*



Legend:

k__Bacteria;p__Bacteroidetes;c__Saprospirae;o__Saprospirales;**f__Saprospiraceae;g__unknown**

k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o_Rhodocyclales;f_**_Rhodocyclaceae;g_Zoogloea**

k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Comamonadaceae; Other

Figure 4–1 Schematics representation of the bacterial composition and distribution in the biomass taken from the Anoxic and Aerobic stages of the bioreactor

As it can be seen from the legend in Figure 4-1, an unknown genus of *Saprospiraceae* and genus of *Zoogloea* a member of the class of *Betaproteobacteria* dominated the biomass samples. *Saprospiraceae* belongs to a group of protein-hydrolyzing epiphytic rods which have been observed to grow attached to filaments such as *Chloroflexi*, *Proterobacteria*, and candidate phylum TM7 (Xia et *al.*, 2008).

4.3.1 Taxonomic Microbial Diversity

The taxonomic microbial diversity analysis showed a significant presence of heterotrophic bacteria in all the stages of the bioreactor. Denitrifiers belonging to the family of *Zoogloea and Thauera* were found to be among the most abundant species in the samples. In addition, *Rhodocyclales*, *Actinobacteria* and *Bacteroidetes* were identified. In previous studies by Hesselsoe et *al.*, (2009), *Rhodocyclales* were found in activated sludge samples with nitrifying–denitrifying and phosphorus removing activities. The most important result of the microbial analysis was the abundance of *Saprospiraceae* in all biomass samples. *Saprospiraceae* which belong to the phylum of *Bacteroidetes* found in this study, were only 96% identical to the nearest Genbank sequence meaning that this dominant bacterium was likely a distinct species with unknown metabolism. The only indication of *Saprospiraceae* in the literature was their presence in SBR samples taken from a BNR plants (Ginige et. *al.*, 2004). Further studies would be required to study the microbial activities of *Saprospiraceae* under the aerobic and anoxic conditions. Figure 4-2 shows the taxonomic hierarchy of the microbial community in the vertical bioreactor.

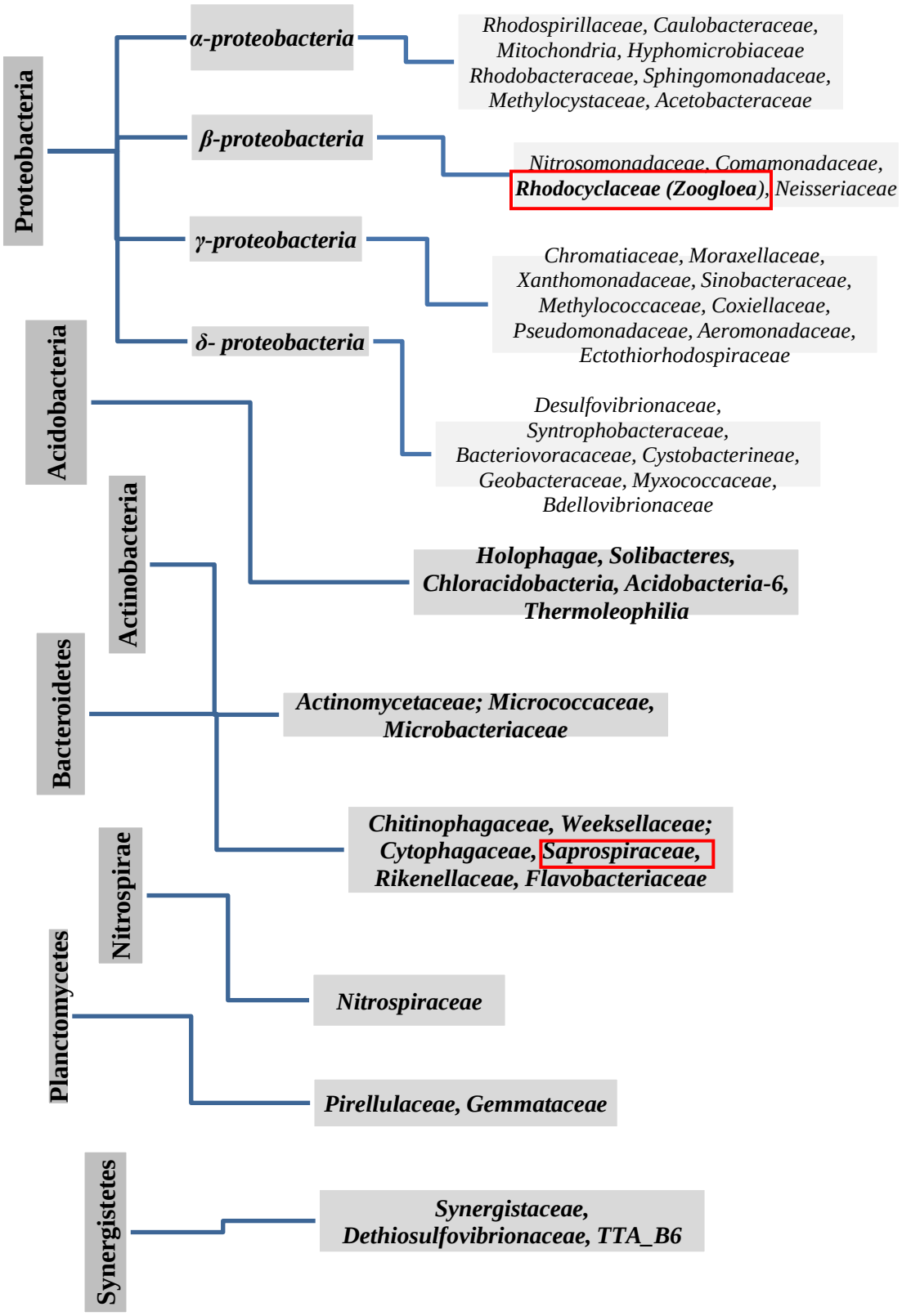


Figure 4–2 Taxonomic hierarchy of the microbial population in the bioreactor

The OTU results of the biomass samples are presented in Table 4-2.

Table 4-2 Taxonomic hierarchy of the microbial population in the bioreactor

Phylum	Class	Family	Fraction in Total Population
Proteobacteria	α-proteobacteria	Rhodospirillaceae, Caulobacteraceae, mitochondria, H yphomicrobiaceae, Rhodobacteraceae, Sphingomonadaceae, Methylocystaceae, Acetobacteraceae	1.15%
	β-proteobacteria	Nitrosomonadaceae, (0.03%) Comamonadaceae, (1.3%) Rhodocyclaceae (Zoogloea), (23%) Neisseriaceae	28.5%
	γ-proteobacteria	Chromatiaceae, Moraxellaceae, Xanthomonadaceae, Sinobacteraceae, Methylococcaceae, Coxiellaceae, Pseudomonadaceae, Aeromonadaceae,	0.55%
	δ- proteobacteria	Desulfovibrionaceae, (0.23%) Syntrophobacteraceae, Bacteriovoracaceae, Cystobacterineae, Geobacteraceae, Myxococcaceae, Bdellovibrionaceae	1.097%
Acidobacteria	Holophagae; Solibacteres; Chloracidobacteria; Acidobacteria-6; Thermoleophilia		0.15%
Actinobacteria	Actinomycetaceae; Micrococcaceae; Microbacteriaceae		0.00441%
Bacteroidetes	Chitinophagaceae; Weeksellaceae; Cytophagaceae; Rikenellaceae; Flavobacteriaceae; *Saprospiraceae*,61%;		67%
Nitrospirae	Nitrospiraceae		1.27 E-05%
Planctomycetes	Pirellulaceae; Gemmataceae		0.0361%
Synergistetes	Synergistaceae; Dethiosulfovibrionaceae; TTA_B6		6.78 E-06
Firmicutes	Clostridia		0.542%
Chloroflexi	Thermomicrobia; Anaerolineae		0.087%

4.3.2 Symbiotic relationship among *Zoogloea* sp. and *Saprospiraceae*

This analysis did show a strong presence of *Zoogloea sp*. of the *β-Proteobacteria* class and an unknown genus of the S*aprospiraceae* family, a member of *Bacteroidetes* phylum. *Saprospiraceae* formed 67.5% of bacteria in both the Anoxic 1 and 2 stages and 48.5% of bacteria in the Aerobic stage of the bioreactor. *Zoogloea* formed 11.5% and 36% of total bacteria in the Anoxic and the Aerobic stages respectively. These microbial distributions in anoxic and aerobic environments have not been found in any laboratory scale nor large scale BNR plants.

Zoogloea

Zoogloea sp. belongs to the β-*Proteobacteria* class and has long been identified as a denitrifier. *Zoogloea* has also been involved in the oxidation of ammonia to nitrite which is carried out by nitrifiers. According to Bano and Hollibaugh (2000), the spatial distribution and diversity of ammonia oxidizing bacteria of the beta subdivision of the class *Proteobacteria* have been found in nature specifically in the Arctic. Furthermore, a research study conducted by Roinestad and Yall (1970) confirmed the presence of polyphosphate granules in *Zoogloea* cells. Polyphosphate granules are mostly detected in the PAOs or DPAOs cells. These findings suggest that some species of *Zoogloea* have phosphorus uptake and denitrification capabilities.

Laboratory FISH analysis has confirmed two dominant groups of phosphate accumulating organisms: *candidatus Accumulibacter Phosphatis* and uncultured *Rhodocylus* a member of the class β-*proteobacteria*. As it can be observed in Figure 4-2, *Zoogloea* belongs to *Rhodocylus* order. Data obtained from several molecular studies done by Crocetti et *al*., (2000), Hesselmann et *al*., (1999) and Liu et *al*., (2001) concluded that BPR activities have been found in bacteria closely related to members of the genus *Rhodocyclus* (class: β-*Proteobacteria*). Culture-independent methods have identified the β-Proteobacterial *Rhodocyclus*-related

Candidatus ‘Accumulibacter phosphatis’ and the actinobacterial genus *Tetrasphera* to be important PAOs (Hesselmann et *al.*, 1999; Zilles et *al.*, 2002; Kong et *al.*, 2004; Kong et *al.*, 2005). They also have been found to be capable of denitrification (Kong et *al.*, 2004; Flowers et *al.*, 2008; Kristiansen et *al.*, 2012). The basic difference between these two PAO groups is that they use different carbon sources. *Accumulibacter* mainly utilizes volatile fatty acids (VFA) (e.g. acetate, propionate) (Kong et *al.*, 2004, Kong et *al.*, 2005; Flowers et *al.*, 2008), while the carbon source for *Tetrasphaera* consist of amino acids. Importantly, *Tetrasphaera* can also ferment glucose and hydrolyse starch, which is very different from *Accumulibacter* (Kong et *al.*, 2005, Kong et *al.*, 2007). The *Accumulibacter* and *Tetrasphera* were not found in this bioreactor. These scientific findings have cast doubts on the significance of the role of the previously known PAOs (i.e. *acinetobacters*) in BPR process. *Rhodocyclus* are now attracting more interest as possible phosphorus-accumulating bacteria. According to Grady (2011), microbial populations of *Zoogloea*, *Thauera* and *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* readily flocculate in activated sludge processes. As shown in Figure 4-2 and Table 4-2, these floc-forming organisms were abundant in the bioreactor and were capable of flocculation which played a very important role in biomass settling in the secondary clarifier.

Saprospiraceae

As discussed before, an unknown genus of S*aprospiraceae*, member of Bacteroidetes phylum formed 67.5% of the biomass composition in the Anoxic sample and 48.5% of in the Aerobic sample. *Saprospiraceae* is mainly known as protein hydrolyser as indicated by Xia (2007). They have been widely found in BNR plants; however, their functions, metabolism and contribution to the removal of nutrients are undetermined. Inexplicably, a group of protein-hydrolyzing bacteria affiliated with the family *Saprospiraceae* have been observed to grow

closely attached to filaments belonging to the phyla *Chloroflexi*, *Proterobacteria*, and TM7 (Xia et *al.*, 2008). This is called epiphytic growth which has been confirmed by Miura et *al.*, (2007) and Albertsen et *al*., (2013). They found that many non-filamentous bacteria involved in the hydrolysis of polysaccharides and proteins in BPR ecosystems are commonly affiliated with the phyla *Bacteroidetes* (such as *Saprospiraceae*), *Chloroflexi*, and candidate division TM7. The advantage of epiphytic growth is currently unknown, however, it is hypothesized that such interactions may be symbiotic, where attachment protects epifloral bacteria from being washout and, in return, the epiphytic rods provide amino acid substrates to their filamentous hosts (Xia et *al.*, 2008). It appears that a comparable ecosystem in this bioreactor might have created such a microbial structure.

Comamonadaceae

In the samples taken from the bioreactor, *Comamonadaceae* (β-*proteobacteria*) formed 1.32% and 1.63% of the biomass in the Aerobic and Anoxic stages. FISH analysis combined with DAPI staining by Ge, et *al.*, (2015) confirmed that the bacterial cells of *Comamonadaceae* contained polyphosphate, identifying them as the key polyphosphate accumulating organisms

Filamentous and Hydrolyzing Bacteria

Hydrolysis carried out by microorganisms is the first step in the degradation of most organic matter. The abundant macromolecules entering wastewater treatment plants are typically lipids, polysaccharides, and proteins. Hydrolysis is a slow process and is usually the rate-controlling step in a BNR process involved in phosphorus and ammonia removal. The hydrolysates such as amino acids and their fermentation products are important sources of carbon and nitrogen for microorganisms. Under anaerobic conditions, hydrolysate can be further fermented to short-chain fatty acids (SCFAs), which are important carbon and energy sources for

PAOs and denitrifiers (Kong et *al.*, 2004, 2005 and Thomsen et *al.*, 2007). The fermentation process takes place under anaerobic condition and during the process mainly carbohydrates and amino acids are transformed into acids such as lactic acid and acetic acid.

Microbial hydrolysis in activated sludge is carried out by extracellular enzymes (exoenzymes) excreted by microorganisms. These exoenzymes are primarily located on the cell surfaces, where hydrolysis and release of partly degraded macromolecules are repeated until hydrolytic fragments are small enough to be assimilated by the microorganisms (Confer and Logan, 1998; Goel et *al.*, 2005). Surface-associated activity of phosphatases, lipases and some other exoenzymes in activated sludge can be detected using epifluorescence microscopy.

Xia et *al.*, (2007) and Kong et *al.*, (2008), found strong connection among filamentous *Streptococcus, Microthrix* and *Chloroflexi* and fermenting bacteria such as *Tetrasphaera*. Xia et *al.*, (2008) found strong correlations between the filamentous bacteria such as *Chloroflexi* and protein hydrolysing bacteria *Saprospiraceae*. This relation is meaningful, since it has shown that *Saprospiraceae* has an epiphytic growth on the filamentous bacteria. Furthermore, Kong et *al.*, (2008) found that fermenting bacteria namely *Tetrasphaera*, and filamentous *Streptococcus* and hydrolyzing bacteria of *Saprospiraceae* constitute a small group in Danish BPR plants. While excess filamentous bacteria result in serious operational problems such as sludge settling and foaming issues in wastewater treatment plants (Seviour and Nielsen, 2010), they are essential for hydrolysis of macromolecules.

Both hydrolysing and fermenting bacteria are poorly studied and there are no other studies investigating their presence in full-scale BNR plants. The concentrations of *Chloroflexi* in the Anoxic and Aerobic stages of this bioreactor were 0.06% and 0.09% respectively. Due to the disproportionate presence of these two microbial groups (*Chloroflexi* and *Saprospiraceae*) in

this research compared to that of Xia (2008), such a strong correlation seems unlikely. The sequencing results of the biomass samples shown in Figure 4-2 indicate that other filamentous bacteria such as *Streptococcus* and *Microthrix* did not exist in this study.

Figure 4-3 illustrates the microbial interactions and process bio-products in the three main environmental phases: Anaerobic (ALU), and the bioreactor′s Anoxic and Aerobic stages. The three coloured rectangular boxes represent biomass flocs or granules in the three different stages.

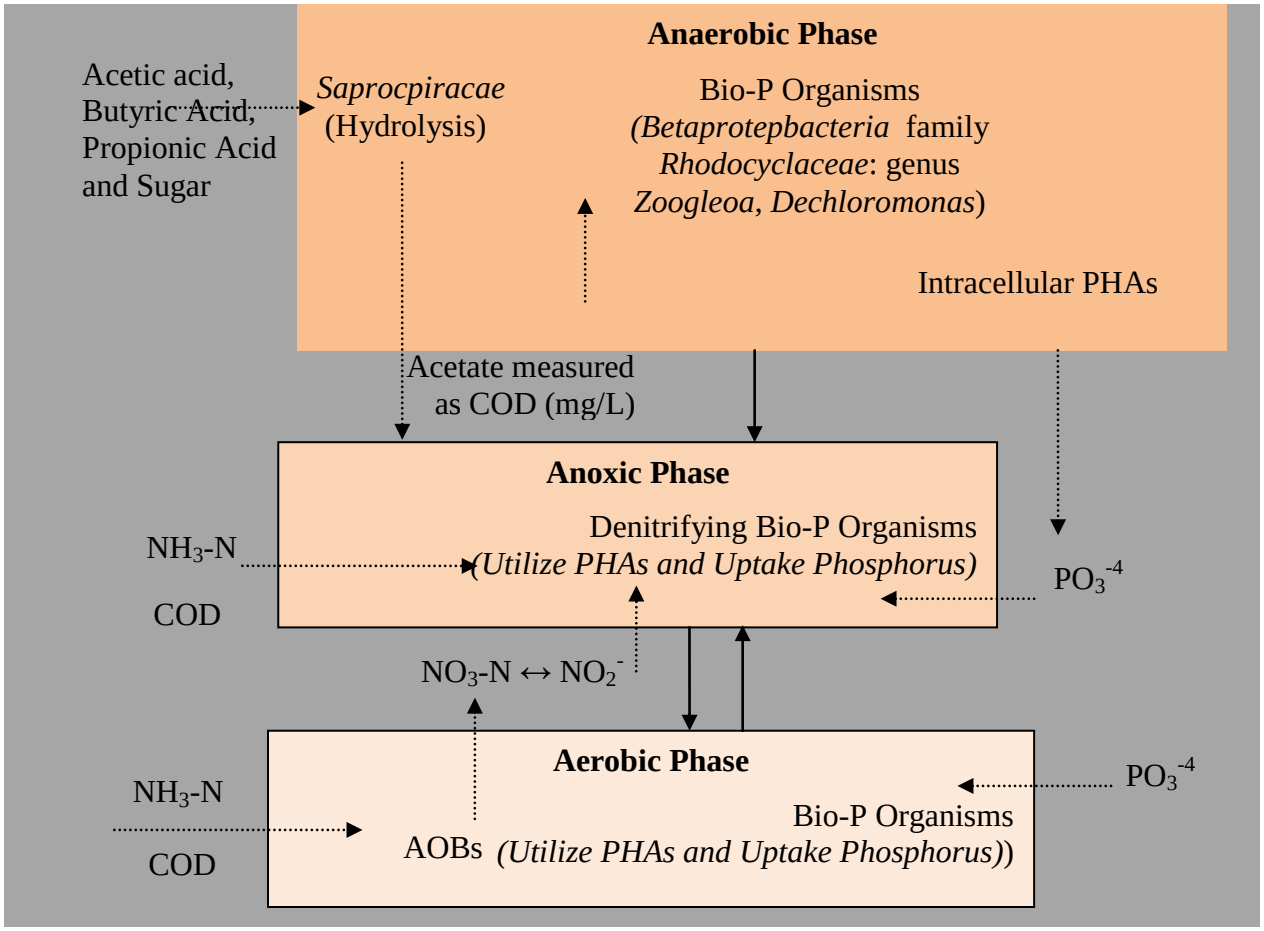


Figure 4–3 Proposed microbial interactions in the Anaerobic (ALU), Anoxic and Aerobic stages of the bioreactor

Table 4-3 shows a comparative table between the various microbial groups in this study vs. finding by other researchers.

Table 4-3 Comparison between microbial groups in the present work and studies by other researchers

Functional Group	**Phylum (p), Class (c), Order (o), Family (f), Genus (g), Species (s)**	**This Study Reza (2016) (% in the samples)**	**Past Studies (% in the samples)**	
AOBs	c_ *γ-proteobacteria* c_*β-proteobacteria*	32 (Unknown Genus)	1.2-8.2	Mobarry et *al.*, (1996)
NOBs	c_ *α-proteobacteria*; g_*Nitrospira*	none	0.2-5.7	Thomsen et *al.*, (2004)
Denitrifiers	c_ *β-proteobacteria*; f_*Rhodocyclaceae*; g_ *Zoogloea*	12-36	0.2-5.2	Rossello-Mora et *al.*, (1995)
Denitrifying PAO	Most *Accumulibacter*	None	1-10.8	Crocetti et *al.*, (2000)
PAOs	c_ *Actinobacteria*;	0.00441	0.01-5	Hesselman et *al.*, (1999) and Beer (2006)
	c_ *γ-proteobacteria*;	0.55	2-7	
	c_ *β-proteobacteria*; f_*Rhodocyclaceae*;	28.5	34-89	
GAOs	Defluviicoccus; *Thioalkanivibrio*;	none	0-0.2	Wong et *al.*, (2005) Kong et *al.*, (2007)
Protein Hydrolyzers	*Saprospiraceae;* *Chloroflexi;*	49-67	1.2-12.2	Kong et. *al.*, (2007)

Biomass Flocs

Floc formation is an important feature which drives community assembly in a BPR ecosystem. The ecological factors contributing to floc formation are complex and poorly understood (van der Gast et *al.*, 2008; Ayarza et *al*., 2011). Extracellular polymeric substances (EPS) typically excreted by microorganisms contribute to floc formation in wastewater. EPS contains exoenzymes which fragment macromolecules into smaller molecules (Confer and Logan, 1998; Wingender et *al*., 1999). Flocs are typically 50-100 μm in diameter and contain a complex mixture of different micro-colonies, filamentous bacteria, and EPS (Nielsen et *al.*, 2012). According to Grady et *al.* (2011) and Forde and Fitzgerald (2003), microbial floc assemblage is important for the following reasons:

1) flocs avoid biomass wash out and produce longer biomass retention in the reactor.

2) flocs create cellular resistance against chemical and enzymatic breakdown.

3) flocs protect microorganisms from predators.

In this study, the abundance of *Saprospiraceae* in the biomass samples most likely contributed to floc formation and subsequently facilitated the separation of the biomass in the secondary clarifier. The floc formation by *Saprospiraceae* needs to be studied in more details and their impact on the overall efficiency of BNR processes needs further investigation.

4.4 Conclusions

In this chapter, the genomic sequencing of the biomass is presented. While the genomic studies greatly contribute to the clarification of every single bacterium in the samples, they do not provide any information about the functional capabilities of these microorganisms. These are the most salient conclusion herein presented:

- A detailed literature review of the functional groups found in the samples provided some understating on the possible functionality and metabolism of *Saprospiraceae*, the most abundant and dominant microorganism in the biomass.
- The second most abundant group of the bacteria in the samples was found to belong to the genus of *Zoogloea* (member of β-*proteobacteria* class).
- Past studies have shown that *Zoogloea* can function as both nitrifiers and denitrifiers with intracellular poly-phosphates accumulation capabilities. The multi-functionality of *Zoogloea* depends on the redox environments and substrate availabilities.
- The microbial analysis in this study suggested that there were likely epiphytic growth and symbiotic relationship between the two groups of *Saprospiraceae* and *Zoogloea*.

Chapter 5 - Comparative Study of the Simultaneous Nitrification-Nitrification-BPR Process (SNDP) in a Vertical Continuous Flow Reactor

5.1 Introduction

Complete biological nutrient removal includes three processes. These are: nitrification, denitrification and biological phosphorus removal. The present work has integrated these processes in a single sludge, continuous flow vertical bioreactor. Chapter 5 presents the results of the study on the simultaneous nitrification-denitrification-biological phosphorus removal (SNDP) process with the following objectives:

- To show the feasibility of a simultaneous nitrification and denitrifying phosphorus removal in a single sludge, continuous flow vertical bioreactor.
- To demonstrate that the BNR perfromance in the present work is comparable or superior to existing, planar conventional bioreactors, and
- To compare its TN/COD and TP/COD ratios with those of other reactors, sepecifically SBRs and activated sluge.

5.2 Materials and Methods

5.2.1Analytical Procedures

The analytical equipment used consisted of a Hach test kits with the following instruments:

- A spectrophotometer (DR2700) used to analyze concentrations of NH_3-N, NO_2^-, NO_3-N, TP and COD.
- An anaerobic digester (DRB200) used for COD analysis.
- Filtering system with 0.45µm filter paper used prior to the analytical measurements.

Samples were taken continuously for over 350 days from all three stages of the bioreactor, the feed and the effluent leaving the clarifier. The experimental facility used for this study was previously discussed in chapter 3.

5.2.2 Bioreactor Operational Parameters

Among the various operational parameters that influence the SNDP process, the most important ones are the temperature, pH, flowrate stability, and nutrients and organic compounds. The bioreactor was inoculated with activated sludge from a wastewater treatment plant in Toronto, ON. The biomass was maintained and internally recycled within the bioreactor for approximately three (3) months to cultivate a mixed culture of heterotrophic/autotrophic nitrifiers, denitrifiers and PAOs

The overall working volume of the bioreactor was 65 L (Figure 5-1). The synthetic feed was pumped at a rate of 10 L/hr to the first Anoxic stage, then flowed by gravity through external pipelines from Anoxic 1 to Anoxic 2 and finally to the Aerobic stage. A recycle stream from the Aerobic stage to Anoxic 1 stage provided mixing and created a uniform composition of microorganisms and nutrients throughout the bioreactor. This bioreactor was aligned with a secondary clarifier and an anaerobic lateral unit (ALU) of 60-L capacity. Effluent from the reactor passed through a secondary clarifier where biomass settled at the bottom of the clarifier and was transferred to the ALU. The ALU provided strict anaerobic conditions to cultivate, and promote the growth of PAOs as well as to maintain the biomass within the system. The supernatant taken from the top of the clarifier, was filtered (0.45µ filter paper) and tested for nutrient concentration. Figure 5-1 is a process flow diagram showing the three stages of the vertical bioreactor, side-stream ALU and other ancillary units.

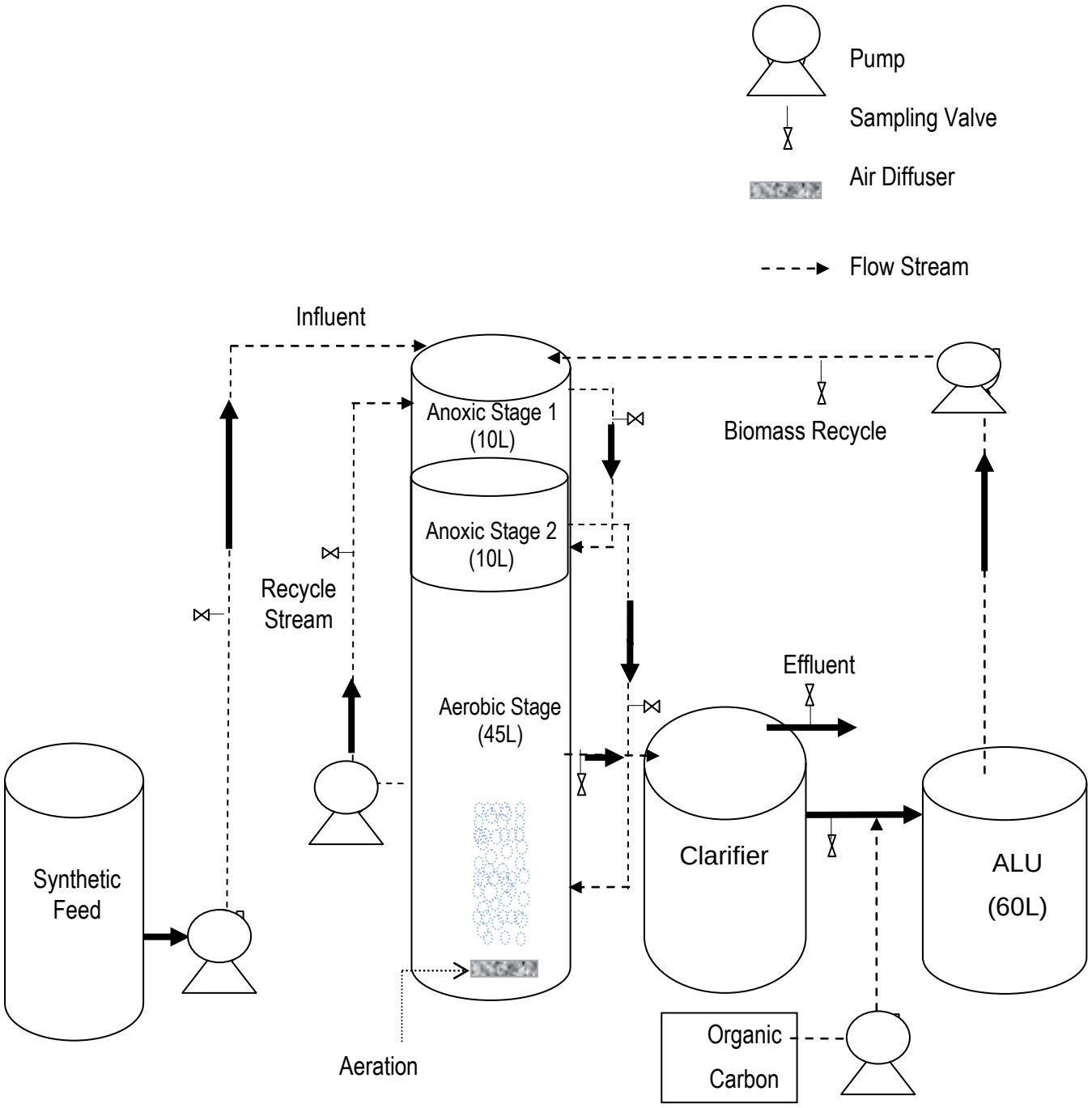


Figure 5–1 Block diagram of the vertical bioreactor with the side stream ALU

5.2.3 Composition of the Feed

The bioreactor's influent was a synthetic wastewater that could represent well effluent from the secondary treatment and having the following composition shown in Table 5-1.

Table 5-1 Composition of the Synthetic Wastewater

Nutrient	**Concentration/Mass**
NH_3-N	45 ± 1.8 (mg/L)
NO_3-N	32 ± 0.9 (mg/L)
*NO_2^-	33 ± 1.3(mg/L)
Total Phosphorus (TP)	32.6±0.7 (mg/L)
Urea CH_4N_2O	5 (g) / 65L of water
Calcium Carbonate	5 (g) /65L of water
$MgSO_4.7H_2O$ (Magnesium Sulphate-heptahydrate)	1.5 (g) /65L of water
$CaCl_2.H_2O$ (calcium chloride Dihydrate)	1.5 (g)/ 65 L of water
$Na_2\ SO_4$ (Sodium Sulphate)	1.5 (g) /65 L of water
$FeCl_3$ (Iron III chloride or Ferric chloride)	1.5 (g /L)
$ZnCl_2$ (Zinc chloride)	0.12 (g/L)
EDTA	7 (mg/l)

* NO_2^- concentration in synthetic feed was due to the chemical reaction of urea in water.

A mixture of acetic acid (10ml), propionic acid (10ml), butyric acid (10ml) and sugar 20(g) was added to the ALU. The organic mixture provided a COD of approximately 1600 mg/L. Specified parameters presented in Table 5-2 were used to create an environment for nitrifiers, denitrifiers and PAOs/DPAOs to inhabit and grow. These key parameters were used to size each stage of the bioreactor, to size the ALU and to determine the optimum nutrient concentrations and environmental factors (i.e. temperature and pH).

Table 5-2 Operating conditions in the ALU and the three stages of the bioreactor

Parameters	ALU	Bioreactor Stages		References
		Anoxic 1 & 2	Aerobic	
DO (mg/L)	0	<0.5	2.5-3.5	(Jetten et *al.*, 1997)
COD (mg/L)	1600	500-300	250-100	(Alvarez-Cuenca and Reza, 2013)
COD:TP	30:1	-	-	(Reza and Alvarez-Cuenca, 2013) (Randall, et *al.*, 1992)
pH	7-7.5	7-7.5	7-7.5	(Alvarez-Cuenca and Reza, 2013)
Temperature	15-25^{o}C	15-25^{o}C	15-25^{o}C	(Baetens, 2001)
HRT	4 hours	2.5hours	4hours	(Reza and Alvarez-Cuenca, 2013)
SRT	50 days	50 days	50days	-

5.3 Results and Discussion

5.3.1 Bioreactor Performance

This section focuses on the evaluation of the performance of the bioreactor developed in this PhD research compared with other existing processes. Analytical results were recorded and analyzed to study the microbial processes. Results showed that ammonia removal began 140 days after start-up. NH_3-N, NO_2^- and NO_3-N concentrations in the effluent were unsteady for approximately 240 days after the start-up of the reactor's operation. The ammonia removal process reached steady state as concentrations were stable and consistent from day 240 until the end of the experiments. Over time, NH_3-N trends in the effluent decreased to below 1 mg/L while influent concentrations were kept constant at 45 ± 1.8 (mg/L) as shown in Figure 5-2.

Table 5-3 summarizes the average nutrient concentrations and associated material balances from various sampling locations.

Table 5-3 Summary of the results for phosphorus, nitrogen and carbon compounds (mg/L) in the present bioreactor

Parameters (Mean ± SD)	**NH_3-N**	**NO_2^-**	**NO_3-N**	**TP**	**COD**	**TN**	**DO**	**Total Carbon**
Feed	45 ± 1.8	33 ± 1.3	32 ± 0.9	32.6±0.7	526	272±7.5	1.25	-
Anoxic 1	2.6 ± 0.56	87± 3.9	0.74± 0.14	14.8±4	217	96.8± 4.8	<0.1	-
Anoxic 2	2.87± 1.2	61± 6.3	0.7± 0.1	12 ±3.3	153	79.4± 4.4	<0.1	-
Aerobic	1.0± 0.4	14.4±0.5	1.1± 0.1	7.4±0.7	97	23.2±8.1	2.2±0.2	-
Effluent	0.7± 0.5	0.8± 0.5	0.3± 0.1	2.7±0.4	80	4.3± 1.2	<0.1	-
ALU	-	-	-	55±5	1600	-	-	-
Biomass	-	-	-	AX:175 AE:113 Average:144	-	240± 5	-	230±2

*AX means Anoxic Stage; AE means Aerobic Stage; Biomass samples were taken from Anoxic 1 and 2 (mixed) and Aerobic stage of the bioreactor. Their TP, TN and TC contents were measured. Their average concentrations are presented in the above table.

The mixed liquor suspended solids (MLSS) concentration at steady state was approximately 5000 mg/L. To maintain the MLSS concentration of 5000 mg/L and SRT of 50 days, 0.14g of biomass per day was removed and discarded from the three stages of the bioreactor. Total phosphorus (TP), total organic nitrogen (TN), total carbon (TC) and COD concentrations of the biomass in the two anoxic stages and Aerobic stage were analyzed after the process was stabilized after 300 days. Chemical Analysis showed that the average TP concentrations in the biomass were 175 and 113 mg/L in the two Anoxic stages and the Aerobic

stage respectively. Total organic nitrogen was 165 mg/L and total carbon was 247 mg/L in the biomass. The microbial analysis showed a high amount of nitrifiers and denitrifiers in the biomass and there was no a trace or indication of Anammox bacteria in the samples. Therefore, simultaneous nitrification-denitrification seemed to be the only pathway for conversion of NH_3-N to N_2. Figure 5-2 presents NH_3-N, NO_3-N and NO_2^- concentrations in the influent and effluent. Figure 5-2 shows a high variability in nitrogen concentrations during the early stages of the experiment (first 200 days). From day 200 until the end of the experimental work (day 360), concentrations in the effluent showed a stable and consistent trend.

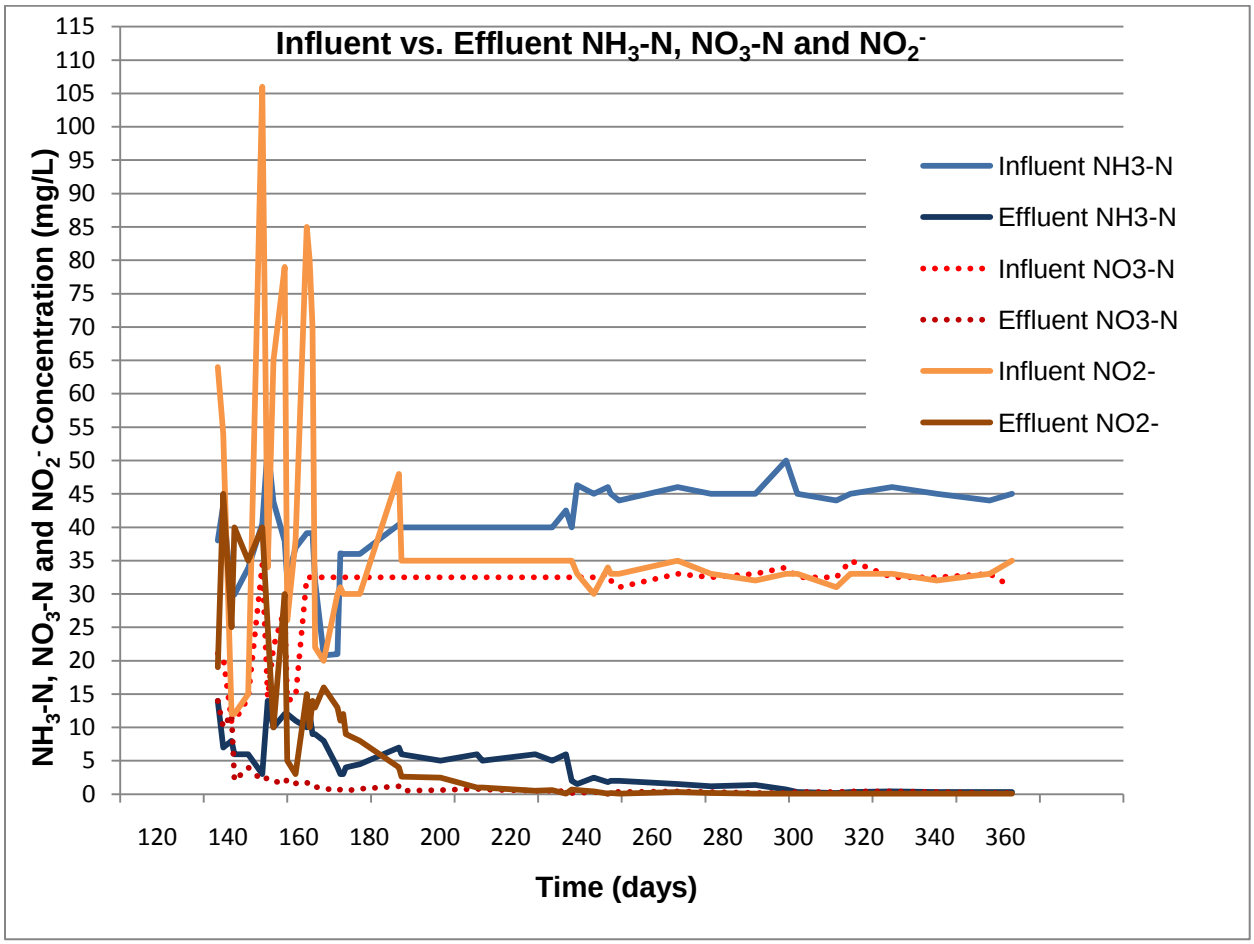


Figure 5–2 Average concentrations of the nitrogen compounds in the influent and effluent throughout the experimental period

Biological phosphorus removal started much later than nitrogen removal. It took almost 230 days (ca. 7 months) from the reactor start-up date to detect BPR. The phosphorus removal efficiency in the effluent increased from 21%, observed on July 4th, 2013, to over 90% on December, 2013. High NO_2^- concentration in the bioreactor, during the first seven (7) months was found as the main inhibitor of PAOs. This inhibitory effect diminished once NO_2^- concentration in the reactor was less than 100 mg/L due to utilization by nitrifiers and denitrifiers. This result is consistent with the findings by Saito (2004) who reported that NO_2^- could inhibit the phosphorus uptake by the PAOs. A number of researchers have confirmed the negative effect of NO_2^-, free nitrous acid (HNO_2) and other the protonated species of nitrite on aerobic phosphorus uptake of PAOs (Pochana and Keller, 1999; Zeng et *al.*, 2014; Zhou et *al.*, 2007). In the present study, when nitrification-denitrification reached steady state, the NO_2^- fluctuation in the reactor decreased. As a result, PAOs and denitrifying PAOs (DPAOs) started to uptake TP from wastewater. TP concentration in the influent was kept constant at 32.6±0.7 mg/L while it gradually reduced to 2.7±0.4mg/L in the effluent leaving the clarifier.

As shown in Table 5-2 and 5-3, the action of ALU with 4 hours of HRT and addition of volatile organic carbon (approximately 1600 mg/L) were essential in developing the BPR process. TP concentration in ALU was 55±5 mg/L. High TP concentration in the ALU was due to the phosphorus release by PAOs and denitrifying PAOs (DPAOs). The experimental data confirmed that TP removal occurred in all three stages of the vertical bioreactor whereas NH_3-N removal mainly took place in the first Anoxic stage. TP was equally removed in each stage as shown in Figure 5-3. This shows that PAOs and DPAOs performed equally in the two Anoxic stages and the Aerobic stage. This observation differed from studies by other researchers who

claimed that PAOs had higher growth yield and phosphorus uptake rate than DPAOs (Hu et *al.*, 2002; Johwan et *al.*, 2002).

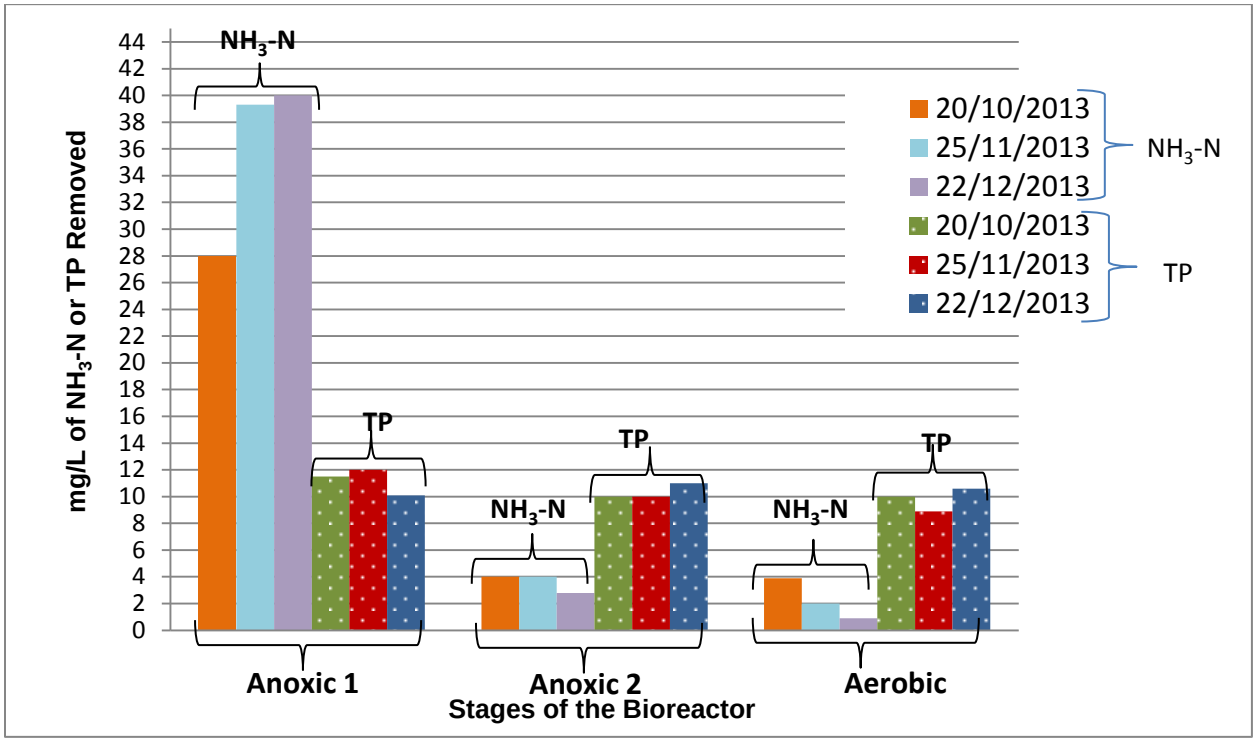


Figure 5–3 NH_3-N and TP removed in the three stages of the bioreactor during the last 3 months of the experiment

Figure 5-3 shows how ammonia concentration decreased from 28 mg/L in the Anoxic 1 to below 4 mg/L in the Aerobic stage's effluent during October 2013. NH_3-N reduced even further during November and December 2013 from approximately 40 mg/L to below 2 mg/L. This reduction of NH_3-N in the absence of DO and presence of NO_2^- and NO_3-N in Anoxic 1 and Anoxic 2 confirmed that simultaneous nitrification denitrification was successfully achieved. The physical characteristics of the biomass changed during the scope of this study. The settleability of the sludge in the clarifier increased when simultaneous nitrification-denitrification-BPR process reached steady state. According to Grady (2011), microbial

populations of *Zoogloea*, *Thauera*, *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* readily flocculate in activated sludge processes. As shown in chapter 4, these floc-forming organisms were abundant in the present bioreactor and were capable of flocculation which played a very important role in biomass settling in the secondary clarifier. There were no changes in the nitrogen removal efficiency from the moment the BPR process was established in the reactor. This indicated the above bacteria coexist under the same environmental conditions even though they have distinctive microbial activities and growth rates. Figure 5-4 provides an expanded view of the COD concentration in the bioreactor with increase in the rate of removal of TN and TP in 390 minutes (6.5 hours of HRT). The average TP removal rate was 0.13 (mg/L.min) and the TN removal rate was found to be 1.6 (mg/L.min).

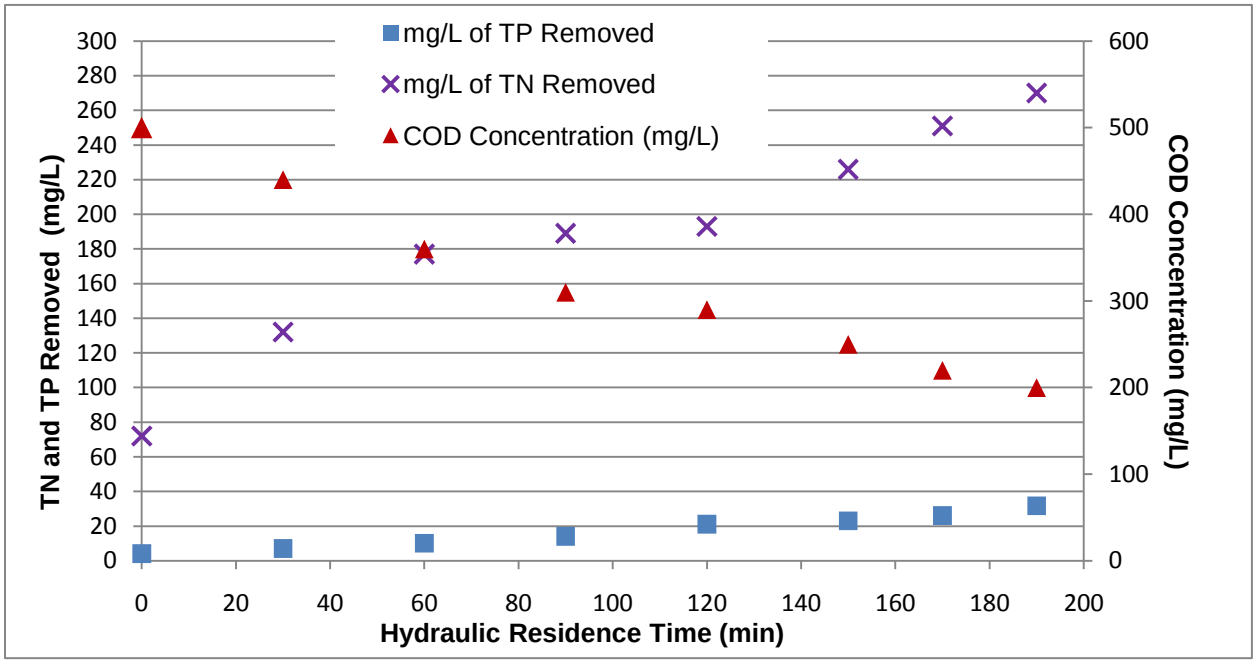


Figure 5–4 TP and TN removal rate relative to COD concentration over time

Figure 5-4 illustrates that the decrease in COD concentration, in the three stages of the bioreactor, was mainly caused by the simultaneous nitrification-denitrification. Both nitrification and denitrification require the presence of readily biodegradable COD. Whereas, the energy

required for the BPR comes from the intracellular PHAs. The analytical results showed that the SNDP process requires a COD to TN ratio of 1.9:1. This is much lower than C:N ratio found by Yang et *al.*, (2005), Hanki et *al.*, (1990), Pochana and Keller (1999). Low C:N ratio indicated that SNDP in this vertical bioreactor required much lower organic carbon than conventional bioreactors.

5.3.2 Single Sludge Continuous Flow Bioreactor

Biological nitrogen removal was observed 140 days after the start-up date and reached steady state after 240 days as shown in Figure 5-2. NH_3-N concentration in the influent was 45 ± 1.8 mg/L and below 1 mg/L in the effluent resulting in a 95% NH_3-N removal efficiency. The NO_2^- concentrations in Anoxic 1, 2 and Aerobic stages were 87 ± 3.9, 61 ± 6.3 and 14.4 ± 0.5 mg/L respectively. These values were much higher than NO_3-N concentrations of 0.74 ± 0.14, 0.7 ± 0.1 and 1.1 ± 0.1 mg/L in the three stages of the bioreactor. Figure 5-5, 5-6, 5-7, 5-8 and 5-9 show the concentrations of NH_3-N, NO_3-N, NO_2^- and COD during the last six (6) months of the reactor operation when the SNDP process reached steady state.

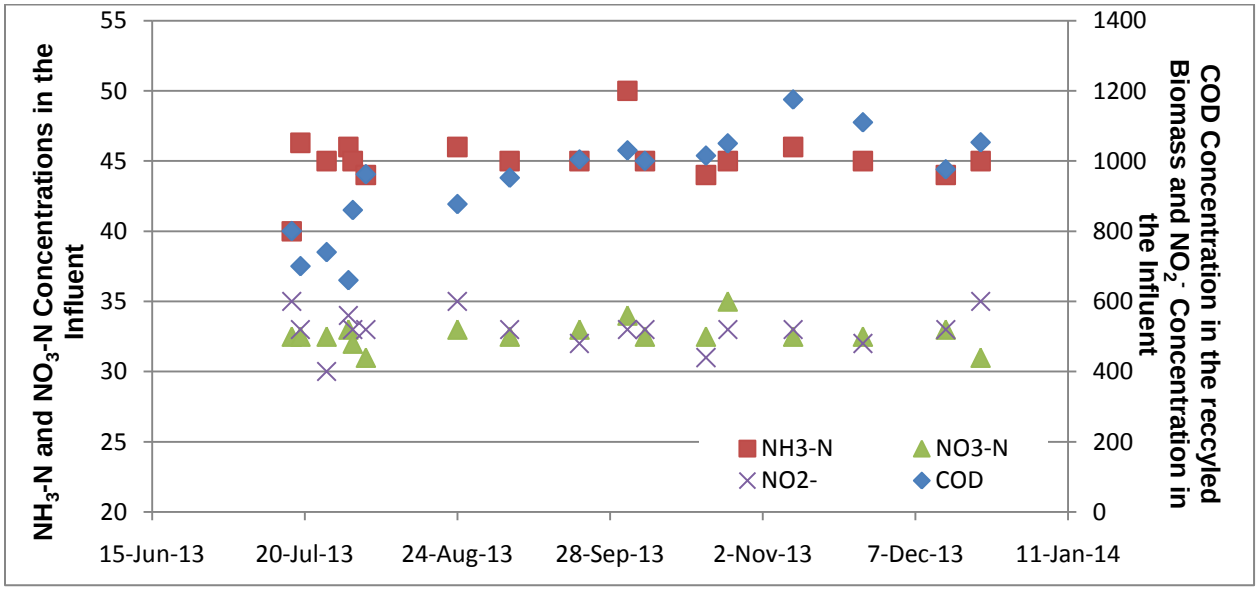


Figure 5–5 NH_3-N, NO_3-N, NO_2^- Concentrations in the FEED and COD Concentrations in the Recycled Biomass

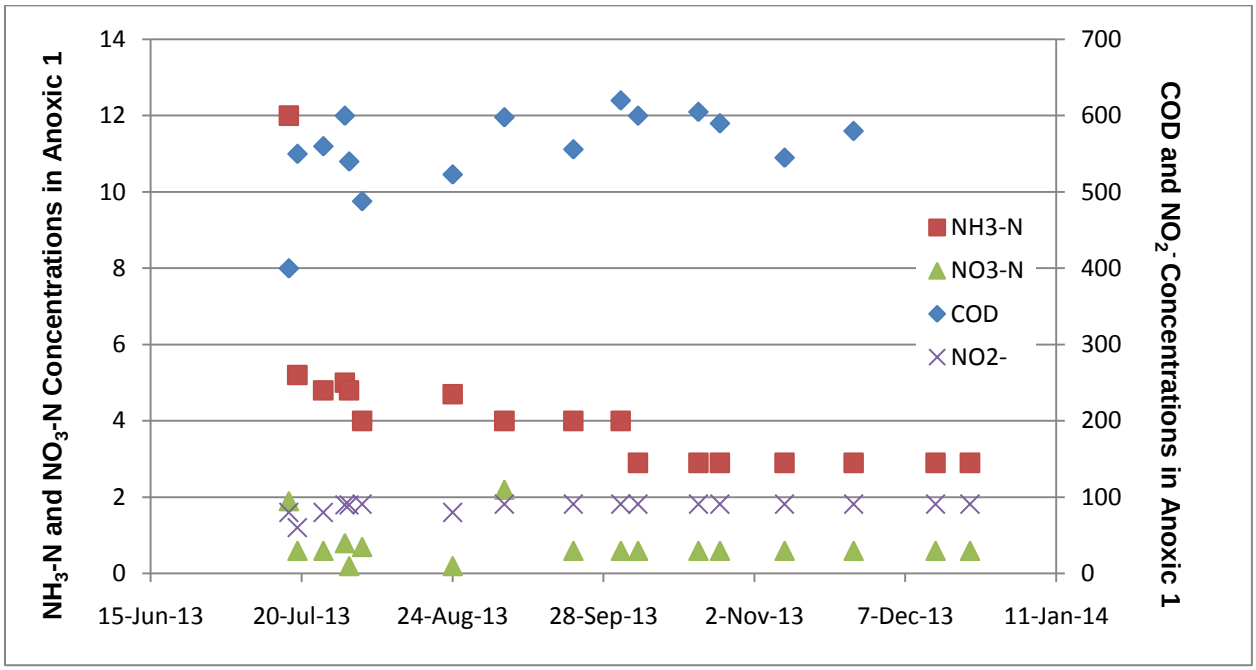


Figure 5–6 NH_3-N, NO_3-N, NO_2^- and COD Concentrations in the ANOXIC 1 Sample

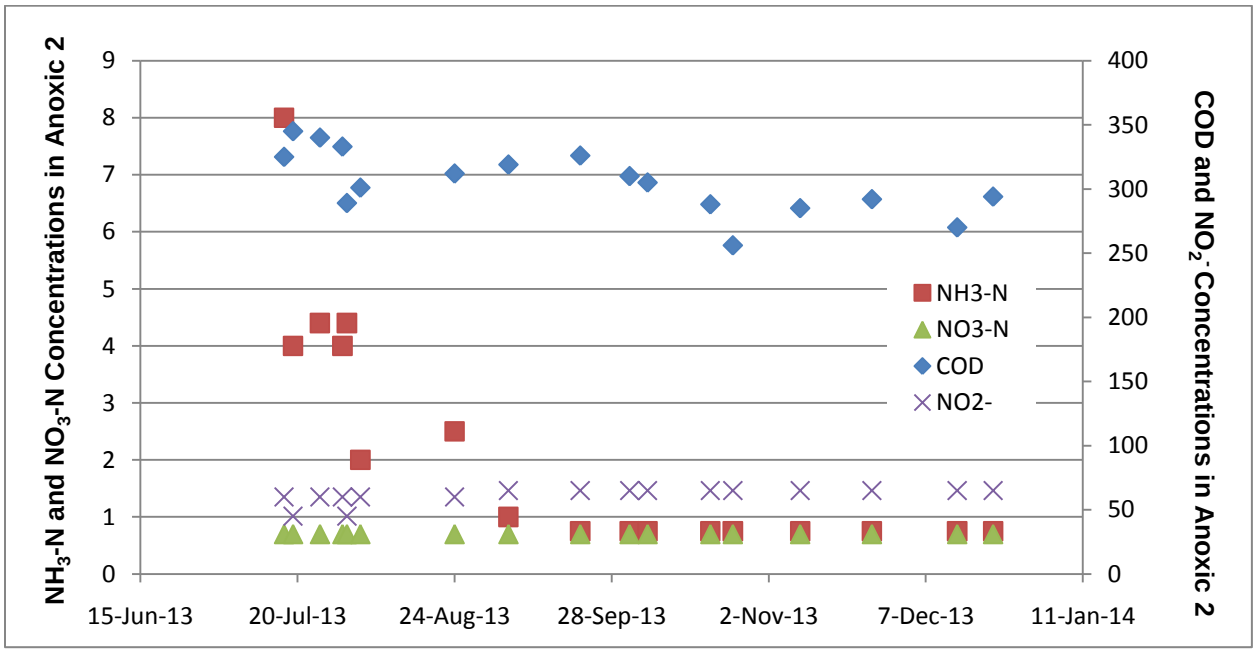


Figure 5–7 NH_3-N, NO_3-N, NO_2^- and COD Concentrations in the ANOXIC 2 Sample

Figure 5-8 shows that COD concentration in the Aerobic stage decreased over time. The reduction in COD availability did not have negative impact on the SNDP process efficiency.

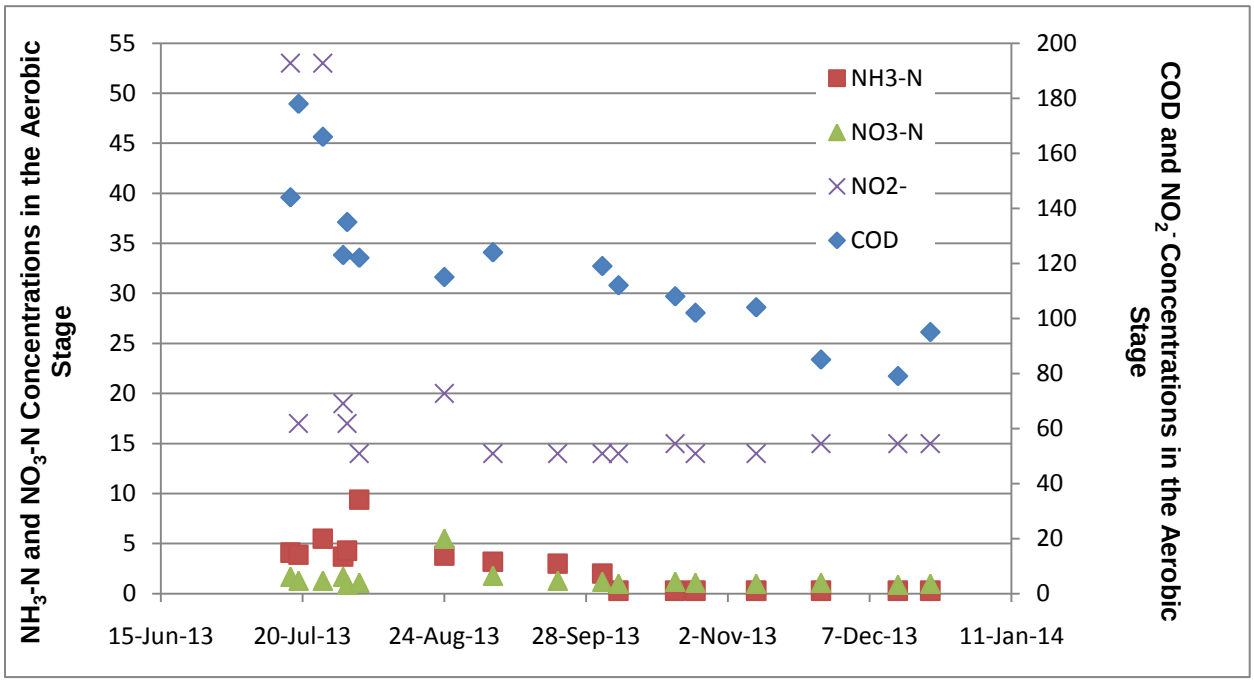


Figure 5–8 NH_3-N, NO_3-N, NO_2^- and COD Concentrations in the AEROBIC Sample

The phosphorus uptake inhibition at NO_2^- concentration ranging from 122 mg/L to 814 mg/L has been widely reported by and Keller (1999), Zhou et *al*. (2007) and Zeng et *al*. (2014). Phosphorus removal was observed 230 days after the start-up however the rate of phosphorus uptake was minimal due to the high concentration of NO_2^- in the bioreactor which was above 120 mg/L. Present experimental data confirmed that biological phosphorus removal reached steady state after 320 days. Phosphorus removal was carried out in the Anoxic and Aerobic stages of the bioreactor with removal efficiency above 90%. High phosphorus uptake rate throughout the bioreactor indicated that PAOs and DPAOs performed comparably under anoxic and aerobic conditions. TP in the influent was constant at 32.6 ± 0.7 and was reduced to 2.7 ± 0.4 mg/L in the effluent shown in Figure 5-9. The average COD concentration in the recycled biomass leaving the ALU and entering Anoxic 1 was 526 mg/L.

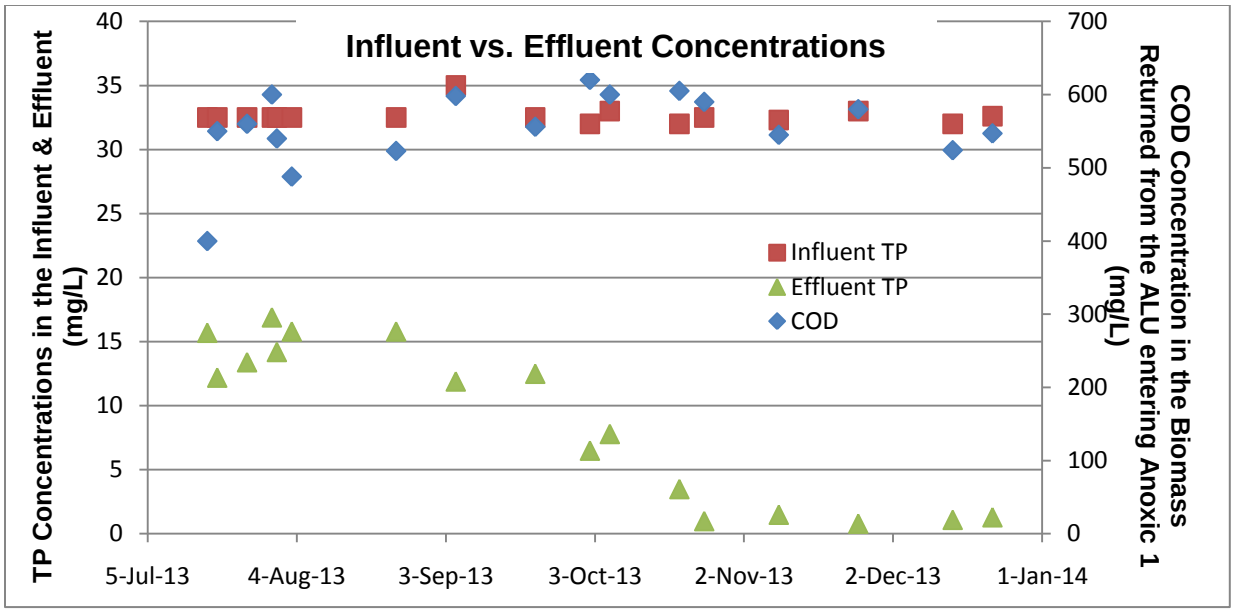


Figure 5–9 Influent and Effluent TP Concentrations at various COD Concentrations Recycled from the ALU.

Figure 5-10 provides a detailed view of nutrient concentrations in various streams.

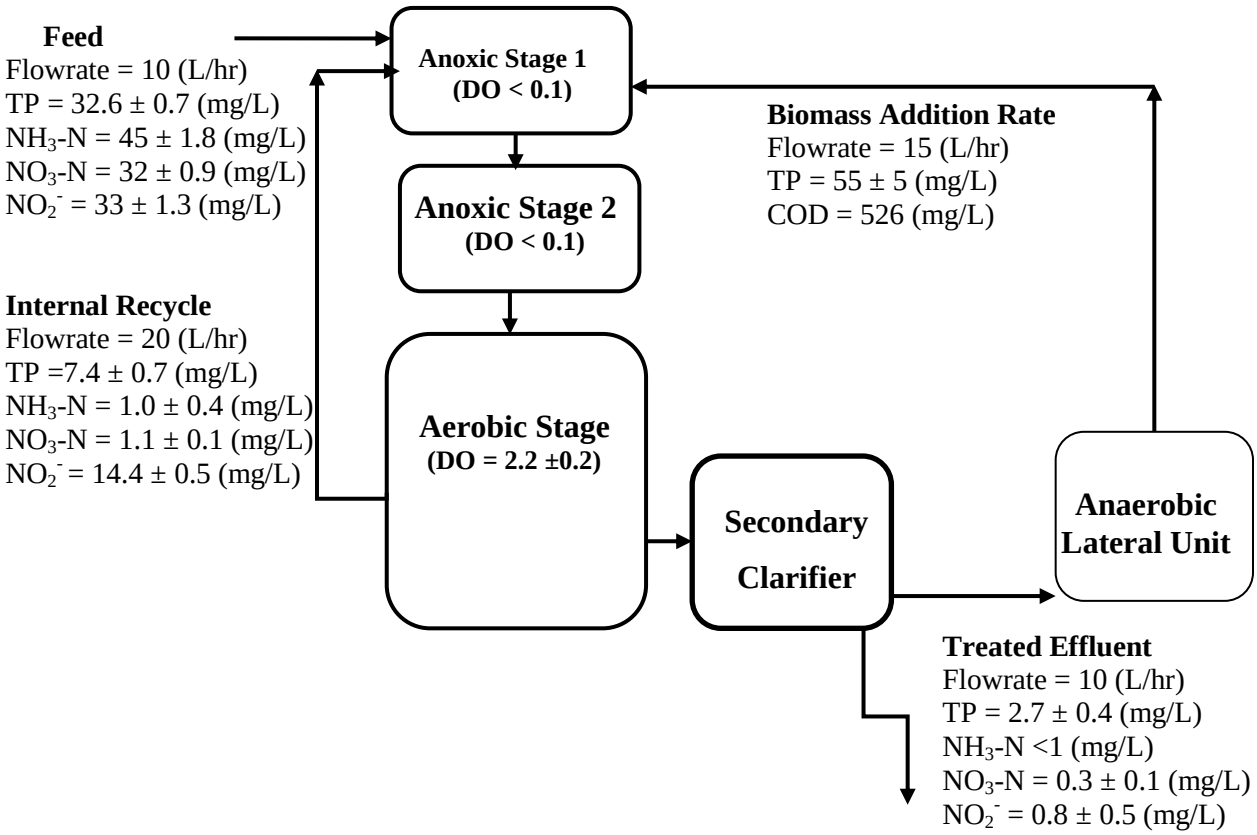


Figure 5–10 Flow characteristics throughout the experimental set up

5.3.3 Feasibility of a Single Sludge Continuous Vertical Bioreactor

Both nitrification and denitrification require the presence of organic matter such as readily biodegradable COD. Whereas, the energy required for phosphorus uptake comes from utilization of intracellular polyhydroxyalkanoates (PHAs). The present results show that to achieve a 95% TN removal in a nitrification-denitrification process, the TN/COD ratio was 0.6 gTN/ gCOD. This ratio is much higher than the results presented by other researchers (Table 5-4) using SBRs or activated sludge processes. High TN:COD and TP:COD ratios indicated that simultaneous nitrification-denitrification-BPR in this reactor required much lower organic carbon than conventional bioreactors. Table 5-4 compares the N/C and P/C ratios of conventional nutrient removal systems with the ratios used in this work.

Table 5-4 Comparative evaluation of the process performance using N/C and P/C ratios

N/C (gTN/ gCOD)	P/C (gTP /gCOD)	Reactor/Process Type	References
0.6	0.034	Present bioreactor	**This study** **Reza (2016)**
0.263	0.037	Two sludge planar system	Kuba et *al.* (1996)
0.1	0.02	SBR	Helness (2007)
0.05-0.3	N/A	SBR	Yang et *al.* (2005)
0.5-0.7	N/A	SBR	Gustavsson et *al.* (2011)
0.14	N/A	Activated Sludge	Collivignarelli and Bertanza (1999)

According to Yang et *al.* (2005) experimental results, microbial activity of heterotrophs decreased when N/C ratio increased from 0.05 to 0.3 while the nitrification rate increased

significantly. In the present research, simultaneous nitrification-denitrification rate reached its maximum at 0.6 (gTN/gCOD).

The experimental unit including both the reactor configuration and the role of ALU enabled the removal of 32 mgTP/L and 267 mgTN/L at the expense of 526 mgCOD /L. Substantial reduction of NH_3-N in the absence of DO and presence of NO_2^- and NO_3-N in the first and second Anoxic stages showed that simultaneous nitrification-denitrification was highly successful in this vertical bioreactor.

As shown in Figure 5-8, NH_3-N, NO_3-N and NO_2^- concentrations in the Aerobic stage were extremely low from July until December 2013. This indicated that simultaneous nitrification-denitrification was successfully achieved after July. In contrast, TP concentrations in the effluent (Figure 5-9) were inconsistent suggesting that biological phosphorus removal process was not fully established in July 2013.

From July till September 2013, the TP concentration in the effluent was reduced gradually implying that denitrifying phosphorus removal rate increased over time. As shown in Table 5-4, the single sludge vertical reactor showed clearly higher TN and TP removal efficiencies than SBR and activated sludge reactors. The integration of biological phosphorus removal with simultaneous nitrification-denitrification is not common due to the inhibitory impact of NO_2^-. Therefore, chemicals are mainly used to remove phosphorus from wastewater when simultaneous nitrification-denitrification process is carried out. One of the major finding of this study was to achieve a successful biological phosphorus removal along with simultaneous nitrification-denitrification. The NO_2^- fluctuation in the reactor decreased significantly when nitrification-denitrification reached steady state. As a result, PAOs and DPAOs started to uptake TP from wastewater.

Slow growth rate of NOBs compared to AOBs has been found to be the main contributor to NO_2^- accumulation. The present continuous flow reactor with staging sequences of Anoxic and Aerobic environments provided an acclimatized condition for DPAOs. The vertical configuration of the reactor and high rate of internal recycle provided steady flow conditions and homogenous mixing which may have enhanced the simultaneous nitrification and denitrifying phosphorus removal. That is, not only nitrogen and phosphorus removal were improved, but also organic carbon requirements were reduced. According to Abeling and Seyfried (1992) and Katsogiannis et *al.* (2003), simultaneous nitrification-denitrification presents significant advantages, as it reduces both oxygen requirement by approximately 25% for nitrification, and organic carbon requirement by 40% for denitrification. Furthermore, denitrifying phosphorus removal produces even greater oxygen and carbon savings since denitrification and phosphorus uptake were accomplished simultaneously by DPAOs.

5.4 Conclusions

The results presented herein show the successful performance of a multistage vertical bioreactor in which a new microbial process for nutrient removal has been developed. This process satisfies the design criteria of foot print minimization and high simultaneous removal of both nitrogen and phosphorous. In this study, a simultaneous nitrification-denitrification-BPR process was developed in a single sludge continuous flow vertical bioreactor. The nutrient removal process was successful due to the bioreactor's staging sequence and smooth mixing flow by gravity from the Anoxic to the Aerobic stage. The participation of various nutrient removal species and their efficient performances were the result of a receptive ecosystem

provided by the both bioreactor and the ALU. From the results obtained in this study, it can be concluded that:

- Simultaneous nitratification-denitratification and biological phosphorus removal can be achieved in a vertical bioreactor by successive Anoxic-Aerobic stages aligned with an anaerobic unit (ALU).
- Due to this configuration, mixing was smooth therefore flow did not create intense turbulent and cell breakage.
- Ammonia removal efficiency of over 95% and biologial phosphorus removal of over 93% were realized with a hydraulic residence time of 2.5 hours in the Anoxic stages followed by 4 hours in the Aerobic stage, along with high internal recycle rate.
- High TN:COD and TP:COD ratios indicated that simultaneous nitrification-denitrification-BPR in this reactor required much lower organic carbon than conventional bioreactors. This a significant economic improvement since this process requires much lower organic carbon to remove both TN and TP.

Chapter 6 – Proposed Unstructured Models for Nitrification, Denitrification and Biological Phosphorus Removal

6.1 Introduction

A mathematical model is a quantitative representation of a phenomenon. This phenomenon is often very complex because of both the nature of the variables involved and the interaction among the variables. The purpose of a mathematical model is to make more understandable the phenomenon. This includes the prediction of the phenomenon's behaviour avoiding the development of unnecessary and unpredictable complexity. After all, a model that because of its complexity is seldom applied is a failed model. As indicated before the present work involves both the creation of a novel bioreactor and the study of the microbial ecosystems including new biological processes. Thus, the modelling of new BNR processes is indeed complex. This chapter presents three proposed models for the processes investigated namely nitrification, denitrification and BPR.

6.1.1 Structured and Unstructured Models

Kinetic analysis is a widely accepted route for describing the performance of biological processes and for predicting their performance. These kinetic models have been applied to various biological wastewater processes to determine the kinetic parameters of substrate removal. The validity of these models has been verified by comparing the experimental and predicted data at different substrate concentrations and variables.

Structured models take metabolic pathways into consideration whereas in unstructured kinetics models microorganisms are usually considered to be a component or the reactants in the system. It is important to note that most kinetic models and their integrated forms are nonlinear which makes exact solutions quite difficult. Saturation kinetics suggests that at low food/microorganism (F/M) ratio, the rates of removal of substrates are approximately

proportional to substrate concentration (first order in substrate concentration). On the other hand, at high F/M, the kinetic rates are independent of substrates concentration (Debik and Coskun, 2009). The unstructured kinetics models are frequently employed in modeling complex microbial systems because they are simple, but are accepted to represent experimental data (Hu et *al.* 2002). Examples of unstructured model used in wastewater treatment are:

i) Monod Equations

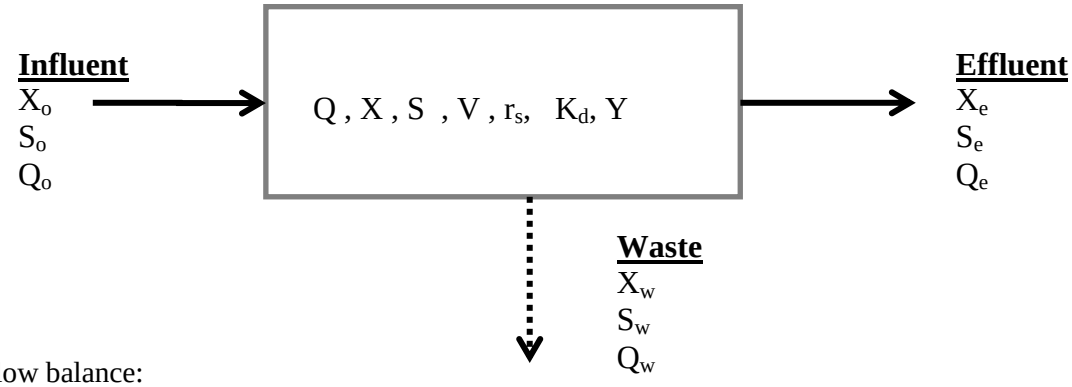
ii) Grau Second Order

iii) First Order Kinetic Models

iv) Modified Stover-Kincannon

The general material balance on the entire reactor must take into account both the removal/generation of biomass (X) and the substrate (S). The system under study consists of the entire volume enclosed in the control volume.

$$\frac{dX}{dt}V = Q_0 X_o - Q_e X_e - Q_w X_w + VYr_s + Vk_d X \qquad \text{[Eq. 6-1]}$$



Flow balance:

$Q_o = Q = Q_e + Q_w \rightarrow \boxed{Q_e = Q - Q_w}$ [Eq. 6-2]

Biomass Balance:

$X_o = X_e + X_w$ [Eq. 6-3]

By considering the masses of solids and substrates accumulated in the system each day, and the amounts entering and leaving the control volume during the same period, the amount of solids produced each day can be computed from the tree material balance equations (Eq. 6-1 to 6-3). That is,

$$\frac{dX}{dt}V = QX_o - [(Q - Q_w)X_e + Q_w X_W)] - VYr_s - Vk_d X \quad \text{[Eq. 6-4]}$$

Where:

X_0 is the biomass concentrations in the feed, (gVSS/L);

X is the biomass concentrations in the reactor, (gVSS/L);

X_e is the biomass concentrations in the reactor effluent, (gVSS/L);

X_w is the biomass concentrations in the wasted sludge (leaving clarifiers), (gVSS/L);

Q is flowrate (L/d);

Q_w waste flow rate (L/d);

V is the reactor volume (L);

K_d is the biomass decay rate constant (1/d);

Y is the yield coefficient (g VSS/gCOD).

r_s is rate of substrate uptake (mg/L.d)

In the case of substrates that contribute to the growth of the organisms, rates of substrate conversion $\left(\frac{dS}{dt}\right)$ are linked to the rates of growth $\left(\frac{dX}{dt}\right)$ and the biomass concentration increases as substrate is removed. The mathematical analysis of such growth-linked systems is more complex than that where microbial growth can be ignored. There are a number of situations where it may not be possible to quantify the concentration of substrate-degrading organisms in a heterogeneous microbial community (Yetilmezsoy and Sakar, 2008). However, the rate of substrate depletion can be measured. There are also situations in which the organism's

concentration remains essentially constant even as the substrate is degraded (i.e. no growth situation). Given these various features of biodegradation kinetics, different models including first-order, zero-order, logistic, Monod (with and without growth) and logarithmic models can be used to describe the rate of biological nutrient removal. At steady state, assuming no biomass formation in the reactor ($\frac{dX}{dt}$ = 0), and assuming no biomass concentration in the feed (X_o = 0) Eq. 6-4 can be rearranged and simplified as shown in Eq. 6-5.

$$\frac{(Q - Q_w)X_e + Q_w X_W}{VX} = -Y \frac{r_s}{X} - k_d \qquad \text{[Eq. 6-5]}$$

The inverse of the term on the left-hand side of Eq. 6-5 is defined as the solids residence time, SRT, (days). Thus, Eq.6-5 can be rewritten as:

$$\frac{1}{SRT} = -Y \frac{r_s}{X} - k_d \qquad \text{[Eq. 6-6]}$$

6.2 Materials and Methods

The experiments in this study were carried out in three (3) phases. Phase one (1) was performed in a 2.5 L batch reactor (R1) (Figure 6-1). The Batch reactor was used for modeling nitrification using the experimental data obtained in phase one (1), under various substrate concentrations and variable SRTs. The denitrification model was completed in Phase two (2). The experimental data were obtained from the operation of only Anoxic 1 and Anoxic 2 stages of the continuous flow vertical bioreactor. The Anoxic stages are labelled as (R2) in Figure 6-2. Biological phosphorus removal (or bio-P) process modeling was conducted in Phase three (3) utilizing the 65 L vertical continuous flow bioreactor with different substrate concentrations and

variable HRTs. The reactor used for bio-P modeling is labelled R3 as shown in Figure 6-3. Detailed operational methods and flow streams have been described in chapter 3.

6.2.1 Experimental Set-Up

Experimental Set-Up for Nitrification Modeling (Phase 1)

The laboratory-scale batch reactor (R1) with 2.5 L working volume was operated at 25±2°C to perform Kinetics tests. The biomass used in the batch reactor was drawn from the continuous flow vertical bioreactor during July 2013 to December 2013. The biomass was taken from the three stages of the vertical reactor to give a mixed microbial population that best represents the Kinetics of the microbial processes involved in the biological nutrient removal. In total, 6 runs were completed in the batch reactor with SRTs varying from 35-50 days. Approximately, 1L of biomass was used in all 6 runs. Prior to each run, the biomass was left to settle for 3 hours. The supernatant was discarded and the settled biomass was used for the batch experiments. The running cycle of R1 was 6 hours consisting of 30 minutes sampling intervals.

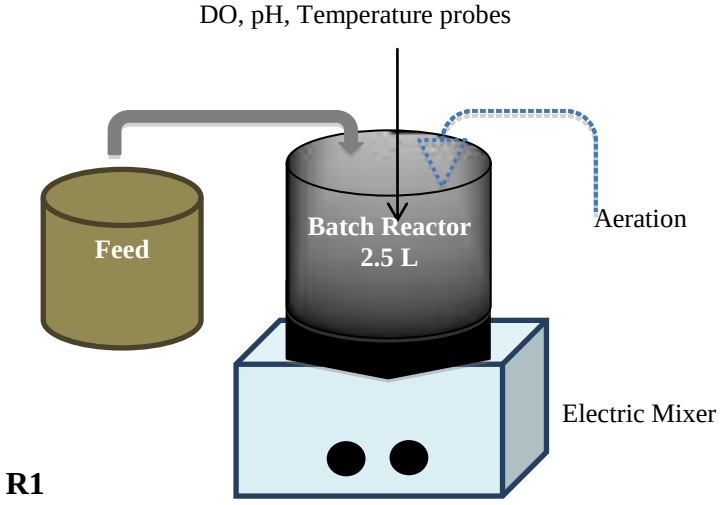


Figure 6–1 Schematic representation of R1 (Batch Reactor) used for modeling nitrification

Experimental Set-Up for Denitrification Modeling (Phase 2)

Anoxic1 and Anoxic 2 stages of the vertical bioreactor, discussed in Chapter 3, were used to collect data for denitrification modeling (boundaries shown in red line, Figure 6-2). The HRT, internal recycle and biomass stream from the Anaerobic Lateral Unit (ALU) were 10 L/hr, 20 L/hr and 15 L/hr respectively. Recycle stream was essential to transfer nitrite and nitrate (produced from the oxidation of ammonia) from the Aerobic stage to the Anoxic 1.

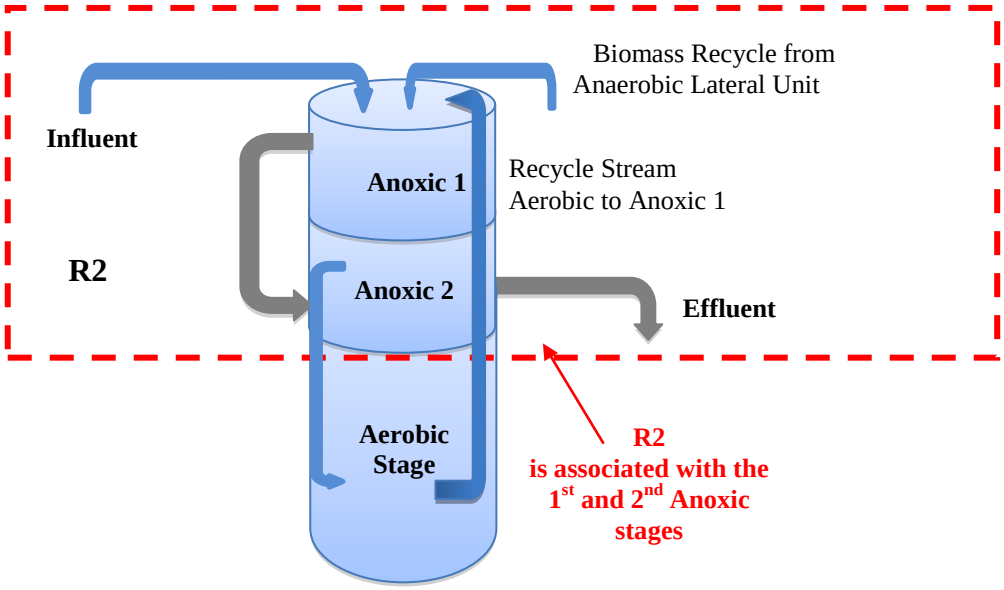


Figure 6–2 Schematic representation of the anoxic stages in the continuous flow vertical bioreactor (R2) used for modeling denitrification

Experimental Set-Up for BPR Modeling (Phase 3)

The vertical bioreactor discussed in chapter 3 was used to collect data for the development of the biological phosphorus removal model. The influent, internal recycle and biomass addition rate were maintained the same as described in R2.

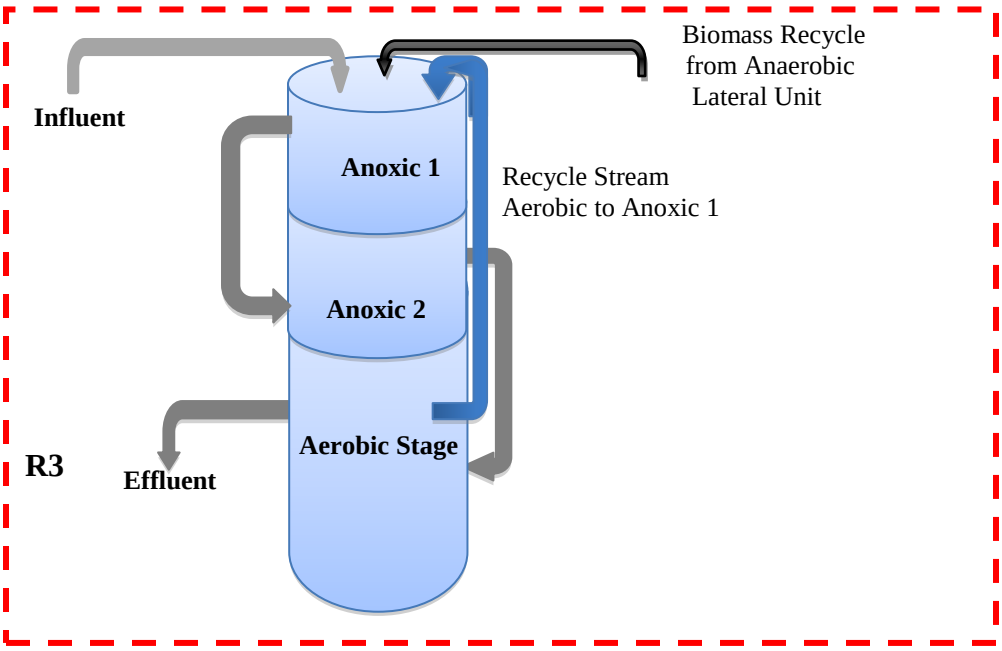


Figure 6–3 Schematic representation of the continuous flow vertical bioreactor (R3) used for modeling biological phosphorus removal

6.2.2 Operating and Sampling Methods

65 L of water were used to create a synthetic feed using the following chemicals: 7.5g-11.25 g NH4Cl, 5g Urea CH_4N_2O, 4.1g -12g KNO_3, 4.5g-11.5g $NaNO_2$, 3.125 g KH_2PO_4, 2.807g $Na_2HPO_4H_2O$, 1.512g/L $CaCl_2.H_2O$. Prior to chemical analysis, samples were pre-filtered with a 1.2 µm pore size glass fiber filter (Whatman GF/C) followed by a 0.45 µm pore size membrane filter (Gelman GN-6). All the samples were analyzed for COD, ammonia, nitrite, nitrate, phosphate, MLSS and MLVSS according to Standard Methods for the Examination of Water and Wastewater (APHA, 2012). All analyses were in duplicate. Temperature, pH and dissolved oxygen were measured using a bench top analyzer with specific probes. The composition of the synthetic feed was maintained the same for all experimental runs.

To obtain the best-fit for the proposed models, numerous samples were taken in the form of input variables to obtain the highest correlation between the experimental data and the values

predicted by the model. In the selection of input variables, the aim was also to investigate the effects of them on target values. Hence, the F test and the corresponding p values were determined to better evaluate the significance of the model. The statistical results are presented in section 6.3.4. Moreover, descriptive statistics of the residual errors were provided to better evaluate the adequacy of the models.

6.3 Results and Discussion

6.3.1 Modeling NH_3-N Removal Rate

For modeling the nitrification, the biomass was aerated for 2 hours to assure that all bulk ammonium was completely converted to nitrite/nitrate. One (1) L of biomass was mixed with one (1) L of feed in the batch reactor (R1). The results of the 6 runs in the batch experiments are summarized in Appendix 2-a.

The basic hypothesis of the nitrification kinetics is that substrate (NH_3-N) and O_2 are consumed via enzymatic reactions carried out only by the organisms with the specific enzymes. Therefore, rates of substrate removal are generally proportional to the concentration of organisms able to degrade the substrate and are dependent on (NH_3-N and O_2) the concentration characteristic of saturation kinetics. The relationship between the specific growth rate (μ), the rate limiting substrate concentration (S) and SRT can be expressed by the Monod empirical model as follows:

$$\mu = \frac{\mu_{max}.S}{K_s + S} \qquad \text{[Eq. 6-7]}$$

$$\mu = \frac{1}{\text{SRT}} + K_d \qquad \text{[Eq. 6-8]}$$

By replacing Eq. 6-7 in Eq. 6-8, the following equation is obtained:

$$\frac{\mu_{max}.\,S}{Ks\ +\ S} = \frac{1}{SRT} + K_d \qquad \text{[Eq. 6-9]}$$

Rearranging Eq. 6-9:

$$\frac{SRT}{1 + K_d SRT} = \frac{K_s}{\mu_{max}}\frac{1}{S} + \frac{1}{\mu_{max}} \qquad \text{[Eq. 6-10]}$$

The values for μ_{max} and K_S are determined from Figure 6-4, by plotting $\frac{SRT}{1+K_d SRT}$ vs. $\frac{1}{S}$ shown in Eq. 6-10.

Where substrate S is NH_3-N, μ_{max} and K_S represents maximum specific growth rate and half-saturation constant for nitrifying organisms involve in the removal of NH_3-N using electron donors (i.e. oxygen or possibly nitrite).

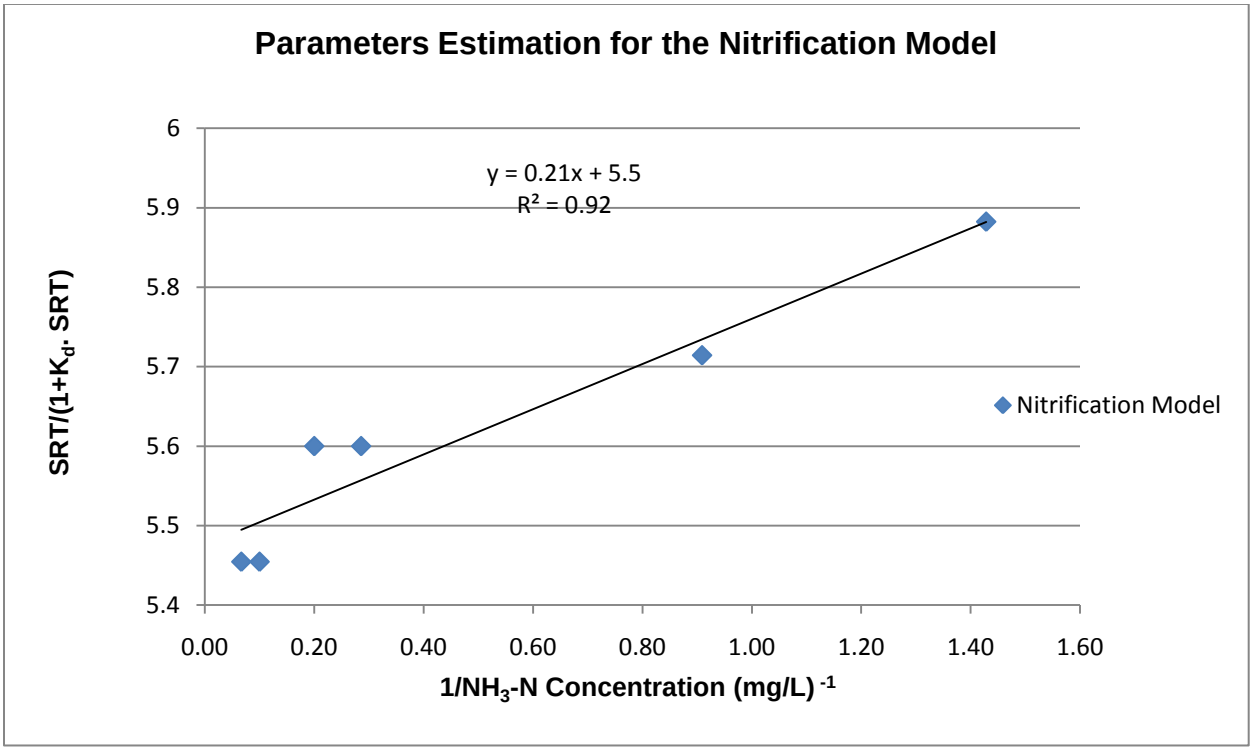


Figure 6–4 A plot of SRT/(1+K_d. SRT) vs. 1/NH_3-N for the determination of kinetic constants in nitrificaion

The yield (Y) can be found using the following relation:

$$Y = \frac{\mu_{max}}{K_s} \quad \text{[Eq. 6-11]}$$

Table 6-1 Nitrification Model Parameters @25^{o}C

μ_{AOB} (day^{-1})	K_{NH3-N}	Y	SRT (days)	K_d (day^{-1})
0.18	0.86	0.21	35-50	0.15 (adopted from ASM2d)*

*** Activated Sludge Model 2d**

$$\frac{0.18 \times S}{0.86 + S} = \frac{1}{SRT} + 0.15 \quad \text{[Eq. 6-12]}$$

Eq. 6-12 represents the first step of nitrification, i.e. the rate of oxidation of ammonium to nitrite. This author did not model the second step of nitrification (conversion of nitrite to nitrate). As mentioned in chapter 2, the second step of nitrification inolves oxidation of nitrite to nitrate by NOBs. The microbial analyses presented in chapter 4, concluded that there were no NOBs in the biomass samples. Therefore, the ammonia removal model takes into account only the first step of nitrification by AOBs since the second step did not occur.

The nitrification rate depends very much on the temperature of the bulk liquid. Therefore the parameters expressed in table 6-2 are temeperature dependant. To compare the specific growth rate found in this study with values obtained by other models, the correlation by Marais and Ekama (l976) was used to adjust all the specific growth rate to a standard value at 20°C, using: $\mu_{nT} = \mu_{n20} \cdot 1.123^{(T-20)}$;

Table 6-2 Compararison of μ_{max} @ 20^{o}C in different studies

μ_{max} (day^{-1})	k_d (day^{-1})	K_{NH3-N} (mg/L)	Reference
0.33	0	0.1	Downing et *al.* (1964)
0.33-0.65	0.04	1.0	Marais et *al.* (1976)
0.33	0.12	1.0	Lijklema (1973)
0.32	**0.15**	**0.86**	**This Study Reza (2016)**

6.3.2 Modeling Denitrification

For modeling the denitrification process, the 1st and 2nd Anoxic stages of the bioreactor (R2 in Figure 6-2) were used. Feed with specified doses of nitrites and nitrates were used to estimate the rate of denitrification relating to the rate of removal of a combined nitrite and nitrate. The denitrification rate evaluated at different HRTs and the experimental data were used to estimate the kinetic constants. Combined nitrite and nitrate balance on the differential volume of Anoxic 1 and 2 yields the following equation:

$$QdS = R_{DN}.\,dV = -\frac{R_m\,S}{K_{DN}+S}\,dV \qquad \text{[Eq. 6-13]}$$

Where $R_m = \mu_s X$ which is the maximum denitrification rate (mg/L.h);

K_{DN} is the saturation constant for denitrification (mg/L);

S_0 and S are the combined nitrate and nitrite concentrations in the influent and effluent (mg/L);

HRT is the hydraulic residence time (h).

Rearranging Eq. 6-13, equation 6-14 was obtained:

$$QdS = -\frac{R_m\,S}{K_{DN}+S}\,dV$$

$$\frac{Q}{dV} = -\frac{R_m\,S}{K_{DN}+S}\,\frac{1}{dS}$$

$$\frac{dV}{Q} = -\frac{1}{R_m}\left(\frac{K_{DN}+S}{S}\right)dS = -\frac{1}{R_m}\left(\frac{K_{DN}}{S}+1\right)dS \qquad \text{[Eq. 6-14]}$$

Integrating Eq. 6-14

$$\int\frac{dV}{Q} = -\int\frac{1}{R_m}\left(\frac{K_{DN}+S}{S}\right)dS = -\int\frac{1}{R_m}\left(\frac{K_{DN}}{S}+1\right)dS$$

Using the following boundary conditions, Eq. 6-14 can be integrated as follows:

$V_{t=o} = 0$; $S_{t=o} = 0$

$V_t = V$; $S_t = S$

$$\frac{\Delta V}{Q} = HRT = -\frac{K_{DN}}{R_m}\left(ln\frac{S_o}{S}\right) + \frac{1}{R_m}(S_o - S)$$

By rearranging the above integration, Eq. 6-15 is obtained:

$$\frac{R_m}{K_{DN}} - \frac{1}{K_{DN}}\left(\frac{So - S}{HRT}\right) = \frac{1}{HRT} ln\left(\frac{So}{S}\right) \quad \text{[Eq. 6-15]}$$

Plotting $\frac{1}{HRT} ln\left(\frac{S_o}{S}\right)$ vs. $\left(\frac{S_o - S}{HRT}\right)$ yields a straight line with slope $-\frac{1}{K_{DN}}$ and an intercept $\frac{R_m}{K_{DN}}$

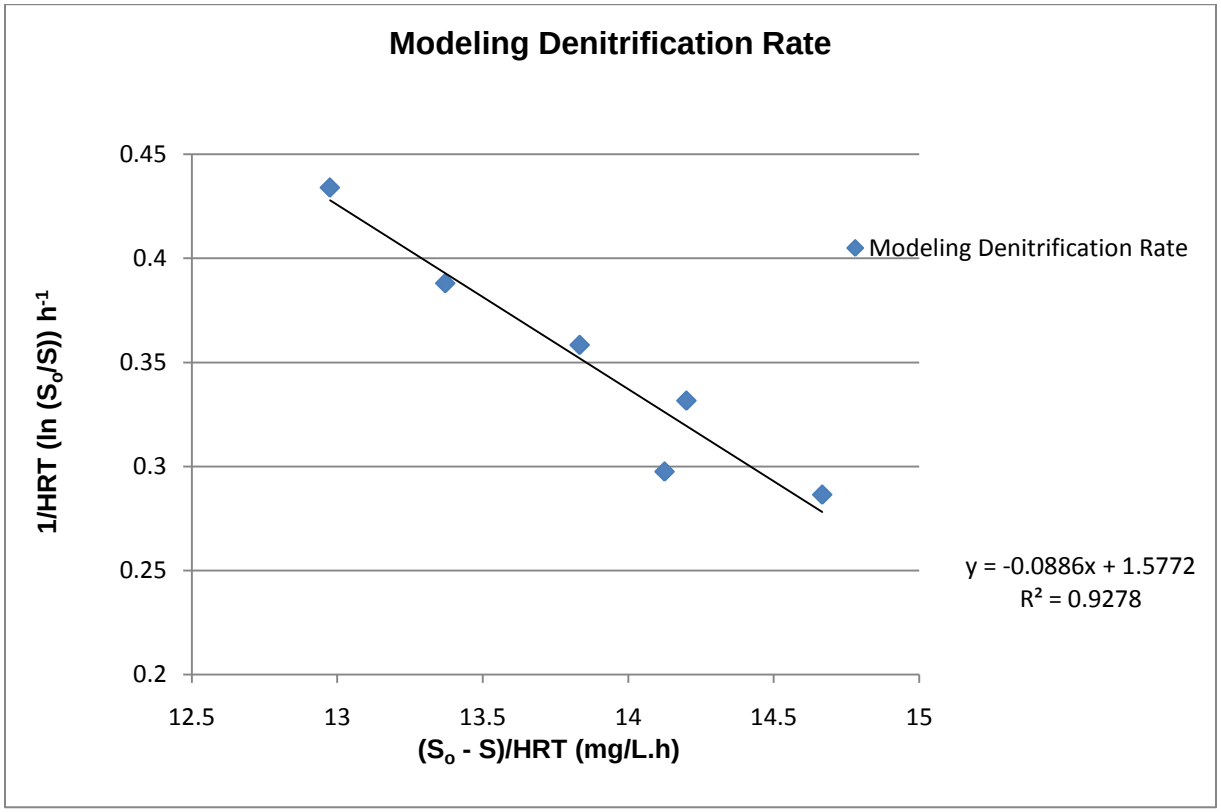


Figure 6–5 1/HRT(ln(S_o/S)) vs. (S_o-S)/HRT for the determination of kinetic constants in denitrificaion

$$-\frac{1}{K_{DN}} = -0.0886 \; and \; \frac{R_m}{K_{DN}} = 1.5772$$

So K_{DN} = 11.2 mg/L and R_m = 17.7mg/L.h;

Average X (or MLVSS) concentration was found1480 mg/L;

Therefore, $R_m = \mu_s X$, maximum specific growth rate (μ_s) was found 0.012 h^{-1} or 0.29 day^{-1}.

$$\frac{0.29X}{11.2} = -\frac{1}{11.2}\left(\frac{S_o - S}{HRT}\right) = \frac{1}{HRT} ln\left(\frac{S_o}{S}\right) \quad \text{[Eq. 6-16]}$$

Experimental data used to develop the Figure 6-5 is presented in Appendix 2-b.

Table 6-3 Compararison of kinetic parameters of denitrification in different studies

μ_{max} (day^{-1})	K_{DN} (mg/L)	Maximum Denitrifcation Rate (R_m) mg/L.h	X (mg/L)	Reference
0.23	0.27	11.7	1200	Dincer and Kargi (2000)
0.29	-	34	1800	Foglar (2003)
1.8	9.1	10.3	-	Stensel et *al.* (1973)
0.29	**11.2**	**17.7**	**1480**	**This study** **Reza (2016)**

The maximum specific growth rate (μ_s) of denitrifiers found in this study was comparable with other studies referenced in Table 6-3. The saturation constant for denitrification (K_{DN}) was greater than the values found in the referenced publications. The difference may have been due to the combined concentrations of nitrite and nitrate used to develop the model in this study. Furthermore, the denitrification model developed by Foglar (2003) was based on experimental data obtained from a batch reactor. However, in the present study, the two Anoxic stages of this continuous flow reactor were used to develop the denitrification model (Eq. 6-16).

6.3.3 Modeling Bio-P Removal

In the presence of stored PHAs, the accepted interpretation of an oxygen uptake (OU) profile to model aerobic bio-P removal is inaccurate because it is necessary to distinguish the OU required for COD oxidation from the break-down of the intracellular PHAs. The substrate

removal kinetics formulated by Grau (1976) as shown in Eq. 6-17 is a better indicator to describe in a simplified way the complex substrate removal in the bio-P process. It is assumed that the concentration of individual substrates in a complex mixture decreases linearly (i.e., zero-order kinetics and n=0) until the substrates are exhausted. Thus, the bio-P removal rate becomes a function of the ratio of the remaining substrates in the mixture to the substrates initially present. The exponent *n* corresponds to the order of reaction. Decrease in substrate concentration can be approximated by the ratio of concentrations at any time divided by initial substrate concentration shown as (S/S_0) (Grau et *al.*, 1975).

$$-\frac{dS}{dt} = K_{pX} + \left(\frac{S}{S_o}\right)^n \quad \text{[Eq. 6-17]}$$

Where, K_p is the order of the reaction in terms of the substrate removal rate $\left(\frac{mg\ S}{mgX\ day}\right)$;

For the initial removal period during which all substrates are still present in the mixture, the overall removal pattern resembles Monod kinetics. The integration of this model for n=1 yields a first order equations and for n= 2 a second-order kinetic equation is obtained.

First Order Model:

$$\frac{S}{S_o} = exp\left(-\frac{K_{1p}\ X_o\ HRT}{S_o}\right) \quad \text{[Eq. 6-18]}$$

Second Order Model:

$$\frac{S}{S_o} = \left(-\frac{1}{1 + \frac{K_{2p}\ X_o\ HRT}{S_o}}\right) \quad \text{[Eq. 6-19]}$$

Where ,

K_{1p} is the first order is substrate removal rate $\left(\frac{mg\ S}{mgX\ day}\right)$

K_{2p} is the second order removal rate $\left(\frac{L^2}{mgS\ mgX\ day}\right)$

Linearizing Eq. 6-18 by taking Ln of both sides, we can obtain Eq. 6-20.

$$-log\frac{S}{S_o} = \left(-\frac{K_{1p}\ X_o\ HRT}{S_o}\right) \quad \text{[Eq. 6-20]}$$

K_{1p} represents a kinetic parameter for 1st order Grau model:

Rearranging Eq. 6-19, yields the following equation:

$$\frac{S_o\ HRT}{S_o\text{-}S} = HRT - \left(\frac{S_o}{K_p\ X}\right) \quad \text{[Eq. 6-21]}$$

Simplifying Eq. 6-21,

$$\left(\frac{S_o}{K_p\ X}\right) = a$$

Therefore, second order Grau model becomes a linear equation shown below:

$$\frac{S_o\ HRT}{S_o\text{-}S} = a + b\ HRT \quad \text{[Eq. 6-22]}$$

And define,

$$\frac{S_o\text{-}S}{S_o} = Removal\ Effciency$$

Therefore,

$$\frac{HRT}{Removal\ Effciency} = a + b\ HRT \quad \text{[Eq. 6-23]}$$

It was found that the second order Grau model (Eq. 6-23) fitted the experimental data obtained from bio-P removal process much better that first order kinetic model (Eq. 6-18). The Bio-P process follows a multi-component substrate removal model where phosphorus and PHAs are substrates to be included in the model. Since, Eq. 6-23 was a more realistic model, it was used to represent the phosphorus removal process.

The efficiency of phosphorus removal was found at various HRTs.

Plotting $\frac{S_o\ HRT}{S_o\text{-}S}$ vs. HRT, gives the following plot with slope **b** and intercept **a**.

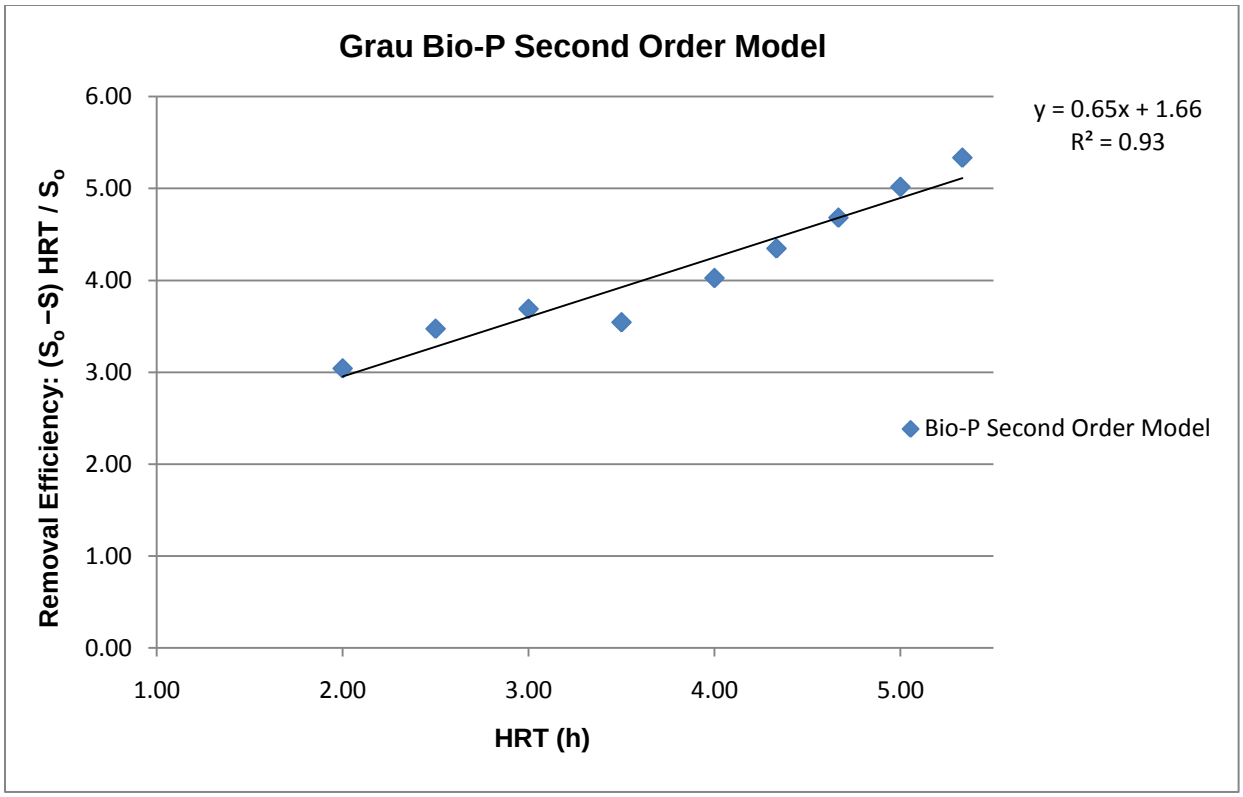


Figure 6–6 Plot of $(S_o - S)$ HRT / S_o vs. HRT for the determination of kinetic constants in biological phosporus removal

$$\frac{\text{HRT}}{\textit{Removal Effciency}} = 1.66 + 0.65\,\textit{HRT} \qquad \text{[Eq. 6-24]}$$

$$\left(\frac{32}{1.66 \text{ x } 1450}\right) = K_p$$

The raw data used to plot Figure 6-6 are presented in Appendix 2-c.

$K_p = 0.013$ $\left(\frac{\text{mg P}}{(\text{mgMLSS}) \text{ h}}\right)$ or $K_p = 0.32$ day^{-1} (maximum specific phosphorus uptake rate)

According to Filipe and Daigger (1999), the specific growth rate of PAOs and DPAOs (μ_{PAOs} and μ_{DPAOs}) can be estimated from the following relations:

$$\text{Aerobic Growth of PAOs on } X_{PHAs} = \mu_{PAOs} \frac{S_{O2}}{K_P + S_{O2}} \frac{X_{PHA}}{X_{PAO}} X_{PAO} \qquad \text{[Eq. 6-25]}$$

$$\text{Anoxic Growth of DPAOs on } X_{PHAs} = \mu_{PAOs}^{Anoxic} \frac{K_P}{K_P + S_{O2}} \frac{S_{NO3}}{K_{NO3} + S_{NO3}} \frac{X_{PHA}}{X_{PAO}} X_{DPAO} \quad \text{[Eq. 6-26]}$$

Where, K_{NO3} is found from the denitrification model (Eq. 6-14).

Using Eq. 6-25 and 6-26, μ_{PAOs} and μ_{DPAOs} were estimated. The results are shown in Table 6-4 and compared with kinetic parameters from past studies.

Table 6-4 Compararison of kinetic parameters of Bio-P in different studies

μ_{max} (day^{-1})	K_p (mgP . (mgMLSS day)$^{-1}$	Y_P (mgP/mgMLSS)	Reference
$\mu_{max\_Aerobic:}$ 1.12 μ_{max_Anoxic} : 1.36	0.32	0.69	**This study** **Reza (2016)**
$\mu_{max\_Aerobic}$: 2 μ_{max_Anoxic} : 1.2	0.103	0.63	(Henze et *al.* 2000)
$\mu_{max\_Aerobic}$: 0.9-1.1	2.6	0.48-0.55	Wentzel et *al.* (1989)
$\mu_{max\_Aerobic}$: 3.36 μ_{max_Anoxic} : 1.2	2.07	0.73	Filipe and Daigger (1999)

6.3.4 Statistical Analysis of the Models

The models were evaluated using the chi-square distribution with one degree of freedom (DF). The P-value approach was used to determine the significance of the variances between the experimental data and predicted values. Thus, we will be estimating the fitness of the NH_3-N oxidation (nitrification), NO_3-N removal (denitrification) and phosphorus removal (bio-P).

1) Nitrification Model

Table 6-5 Analysis of Variance (ANOVA) for Nitrification Model

Source of Variation	Sum of Squares	df*	Mean Square Error (MSE)	F_o**	$F_{Critical\ @\ CI(1-0.1)\%}$	*P-value*
Model (S***, SRT)	0.4	1	0.41	8.3	4.54	<0.1
Residual	13.5	4	3.37	0.82	-	-
Total	13.9	5	2.78	-	-	-

* df: Degrees of freedom; ** F of observed; *** NH_3-N concentration

The *P* value calculated for nitrification was <0.1 indicating the significance of the model. Below is the graphical representation of the experimental data vs. the predicted values obtained from the nitrification model (Eq. 6-12).

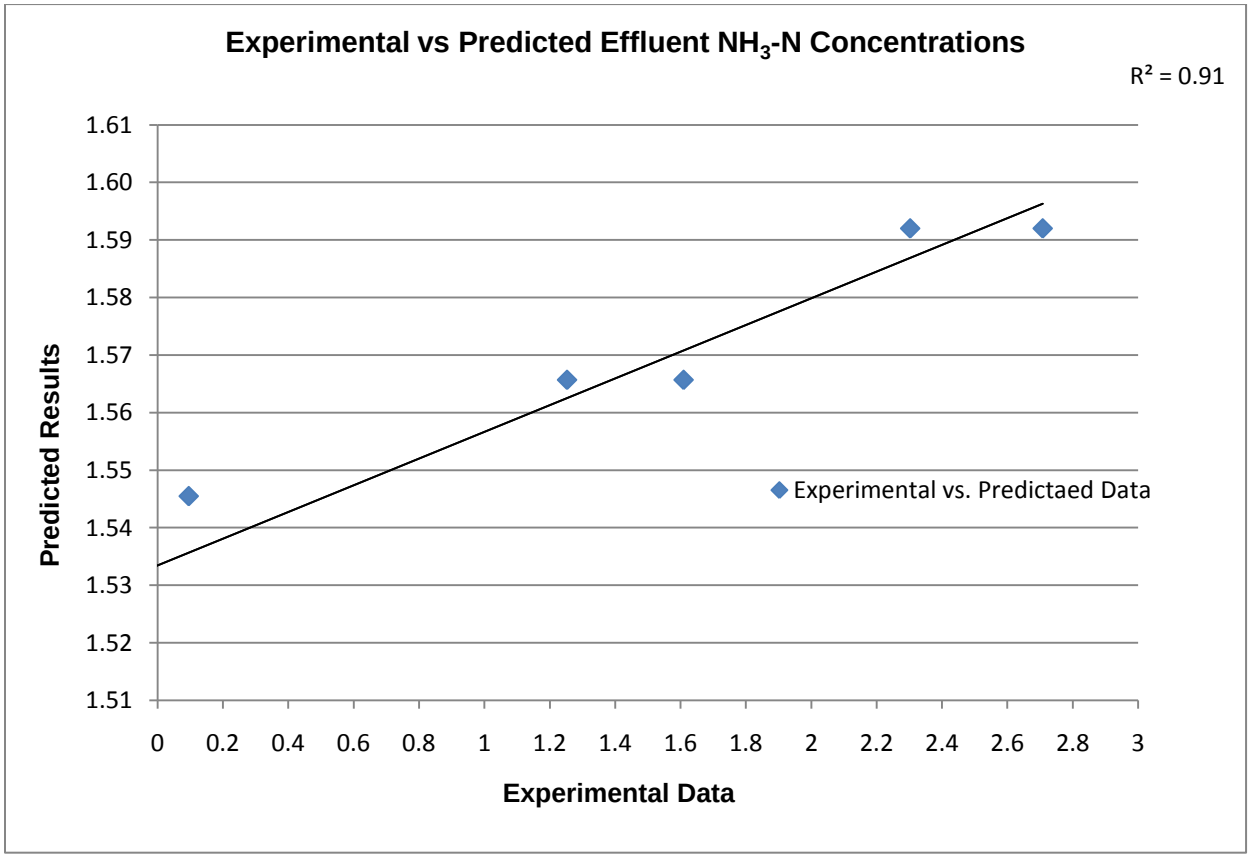


Figure 6–7 Predicted values vs. experimental data in the nitrification model

2) Denitrification Model

Table 6-6 Analysis of Variance (ANOVA) for the Denitrification Model

Source of Variation	Sum of Squares	df	MSE	F_o	$F_{Critical\ @\ CI(1-0.05)\%}$	*P*-Value
Model (S*, HRT)	7.16	1	7.16	9.9	7.71	<0.05
Residual	284	4	71.1	-	-	-
Total	291	5	58.3	-	-	-

* Combined NO_3-N and NO_2^-

From the ANOVA Table 6-6 for the denitrification model, the fitness of the model (Eq. 6-16) can be evaluated. To measure the adequacy of the denitrification model, experimental data vs. predicted values from the model were plotted in Figure 6-8.

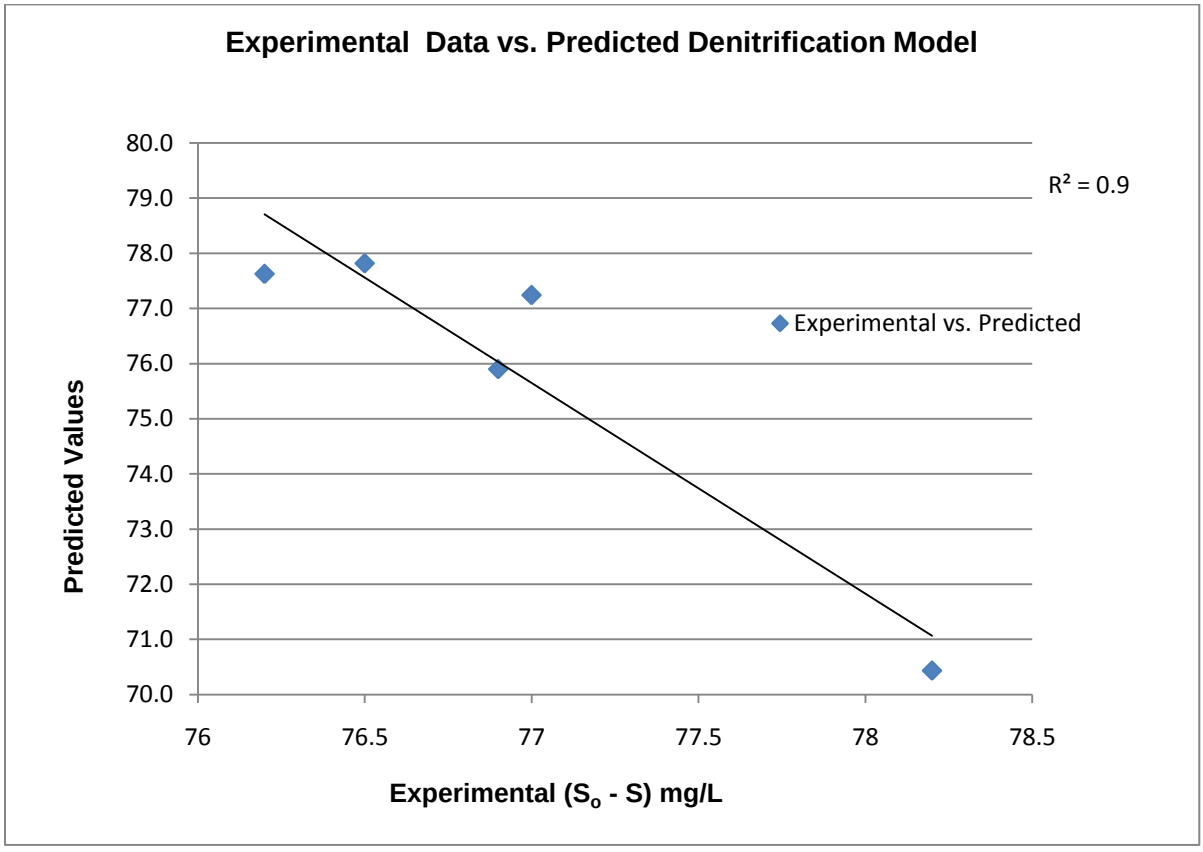


Figure 6–8 Predicted values vs. experimental data in the denitrification model

3) Bio-P Model

Table 6-7 Analysis of Variance (ANOVA) for Bio-P Model

Source of Variation	Sum of Squares	df	MSE	F_o	$F_{Critical\ @\ CI(1-0.1)\%}$	*P-value*
Model (E*, HRT)	147.8	1	147.8	4.27	4.06	<0.1
Residual	3156.7	5	631.3	-	-	-
Total	3304.5	6	550.7	-	-	-

* Phosphorus Removal Efficiency

From Table 6-7, the p-value <0.1 indicates that the model is indeed significant. The goodness of fit for the bio-P model is presented in Figure 6-9, by plotting experimental bio-P efficiency vs. predicted efficiencies found from the model.

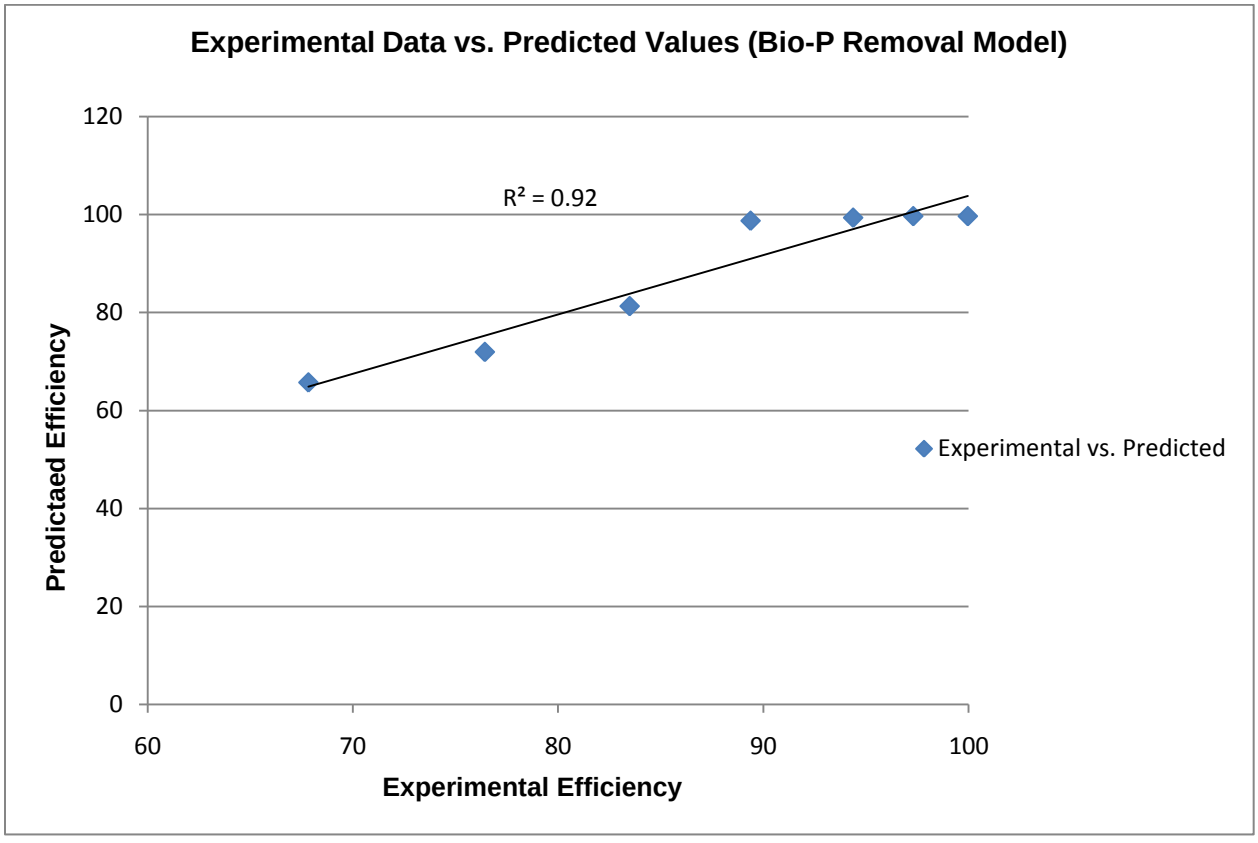


Figure 6–9 Predicted values vs. experimental data in the bio-P model

Figure 6-9 illustrates that the bio-P model is statistically satisfactory and the model predictions for phosphorus concentrations were in good agreement with the measured data as shown by the value of the correlation ($R^2 = 0.92$).

6.4 Conclusions

The focus of this chapter was to develop three mathematical models which could best describe the simultaneous nitrification-denitrification-BPR (SNDP) removal. Unique microbial structure of the process in the vertical continuous flow bioreactor was extremely complex. The experimental data were collected using three different experimental phases. These are the main conclusions pertaining to the chapter:

- The kinetic parameters were determined by a material balance where the substrate consumption was found with the modified Monod Equation.
- To develop the nitrification model, a 2.5 L batch reactor was used with the biomass taken from the continuous flow vertical bioreactor. Ammonia uptake was measured at different SRTs.
- The continuous flow reactor was used to develop mathematical models for denitrification and BPR processes. The rate of removal of combined nitrite and nitrate at different HRTs were studied and the data were used in developing an unstructured model for denitrification.
- The phosphorus uptake rates at various HRTs were measured and the raw data were used to form a Bio-P model.
- The three unstructured models could adequately predict the behaviour of each biological process.

The models presented in this chapter can be used for predictive analyses of the nutrient concentration variations in the bioreactor with an underlying assumption that the main reactions involved were fully known.

Chapter 7 –Study of the Operating Parameters of Simultaneous Nitrification, Denitrification and Biological Phosphorus Removal (SNDP) Process

7.1 Introduction

Simultaneous nitrification, denitrification and biological phosphorus removal (SNDP) requires electron donors and electron acceptors for microbial energy and growth. DO, NO_2^- and NO_3-N are major electron donors in biological wastewater processes. The rate of production of NO_2^- and NO_3-N in the aerobic stage strongly depends on the DO concentration. As well, the rate of consumption of NO_2^- and NO_3-N in the anoxic stage depends on the absence of DO.

In a SNDP process, NO_2^- and NO_3-N are both common products and oxidizing substrates. As nitrifiers convert NH_3-N to NO_2^- and then to NO_3-N, these electron donors are utilized by both denitrifiers and denitrifying PAOs (DPAOs). NO_2^- is an intermediate molecule in a simultaneous nitrification and denitrification. Recent studies have reported contradictory results on NO_2^- utilization by DPAOs. Some experiment have supported NO_2^- uptake by DPAOs such as Ahn *et al*. (2001). On the other hand, there are a number of studies reporting inhibitory effects of NO_2^- on phosphorus uptake rates (Saito *et al.*, 2004) (Kuba *et al.*, 1996). The role of NO_2^- as an electron donor in the BNR processes must be further examined and its inhibitory effects on microbial processes must be thoroughly evaluated.

NO_2^- is the intermediate nutrient for nitrite oxidizing bacteria (NOBs) and denitrifying organisms. If DO concentration is low (i.e. 0.5mg/L), full nitrification will be hindered. As a result, NO_2^- oxidation to NO_3-N via NOBs will be reduced and eventually NOB population will completely disappear. As it was mentioned in Chapter 4, molecular biology analyses of the biomass samples showed no traces of NOBs in the bioreactor.

This section describes a series of experiments conducted to investigate the rate of removal of both NH_3-N and total phosphorus (TP) by SNDP process in the Anoxic and the Aerobic stages of the bioreactor. Specifically, the inhibitory effects of NO_2^- on TP uptake rates

under anoxic and aerobic conditions were examined. Furthermore, optimum concentrations of COD to maximize TP and NH_3-N uptake were determined. In addition, this chapter discusses a series of experiments performed on the bioreactor to determine the SNDP response to the variation in inlet NH_3-N. In total three sets of experiments were conducted to achieve the following objectives:

- To investigate the impact of aerobic DO concentration on NH_3-N and TP uptake rates.
- To determine the optimum COD concentration to be added to the ALU to produce interacellular PHAs by the biomas, and
- To determine the effect of high inlet NH_3-N concentration on the overall SNDP process efficiency.

7.2 Materials and Methods

7.2.1 Reactor operations

The investigation conducted in this section was carried out in the 65 L vertical continuous flow bioreactor working in conjunction with the anaerobic lateral unit (ALU). Figure 3-2 in chapter 3 presented in detail the process flow diagram and Figure 7-1 shows a schematic of the reactor, the ALU and process flow. As discussed in chapter 3, the ALU provided a strictly anaerobic environment for microorganisms to synthesize and store intracellular PHAs and to utilize them later in both the Anoxic and Aerobic stages of the reactor. It was found that the COD added to the ALU (ranging from 1200-1600 mg/L) was not fully used up by the biomass for PHAs synthesis. Indeed, approximately 500 mg/L of soluble COD remained unused in the ALU after four hours of residence time. The stream leaving the ALU and entering the bioreactor contained a combination of the intracellular PHAs and extracellular readily available COD.

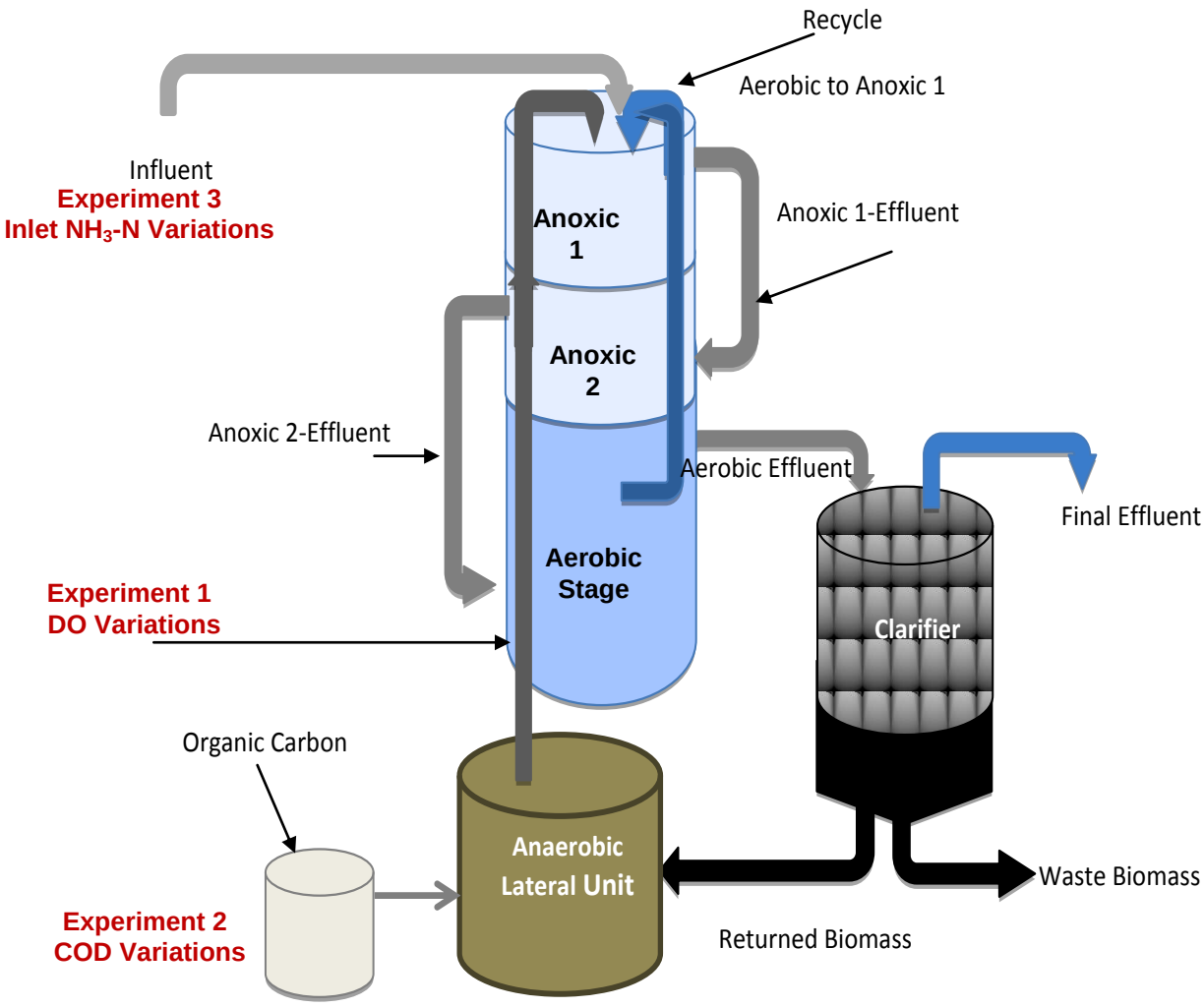


Figure 7–1 Schematic Representation of the Continuous Flow Bioreactor

(Refer to chapter 3 for more details)

The bioreactor's operating conditions were: temperature (20-25^{o}C), pH (7-7.5), Influent/effluent HRT (10 L/h), Aerobic to Anoxic recycle rate (20 L/h) and Recycled biomass from the ALU to the 1st Anoxic stage (15 L/hr), SRT (50 days), MLSS (5000 mg/L), MLVSS (4500 mg/L). Table 7-1 shows the arrangement of the experiments and operational variables used in this chapter.

Table 7-1 Operating Conditions

No. of the Experiment	No. of Runs per Experiment	Experimental Variables		
		DO Concentration (Aerobic Stage)	**COD Fed to the ALU**	**NH_3-N Concentrations Feed**
Experiment 1	Run 1 Run 2 Run 3	0.5-1mg/L 2.5-3mg/L 5.5-6mg/L	1400mg/L	45mg/L
Experiment 2	Run 4 Run 5 Run 6	2.5-3mg/L	1000mg/L 1200mg/L 1400mg/L	45mg/L
Experiment 3	Run 7 Run 8 Run 9	2.5-3mg/L	1400mg/L	49 mg/L 80mg/L 120 mg/L

Every run was carried out for three days to allow the process to adapt to the imposed conditions. The ALU was fed with different COD concentrations with a mixture of acetic, propionic and butyric acids. The synthetic feed had the composition shown in Table 7-2.

Table 7-2 Composition of the Synthetic Feed

Compounds	**Mass (g)**
$KH_2\ PO_4$	3.13 g
$Na_2HPO_4H_2O$	2.81g
NH_4Cl	11.25g
Urea CH_4N_2O	5g
Calcium Carbonate	5g
Minerals	
$CaCl_2.H_2O$	1.5mg/l
$MgSO_4.7H_2O$	1.5mg/L
$FeCl_3$	1.5mg /L

7.2.2 PHAs Synthesis and Extraction

PAOs and DPAOs are capable of producing intracellular PHAs. The PHAs syntheses vary in quantity and composition when BPR process involves different types of substrates. In addition, glycogen accumulating organisms (GAOs) are able to compete with PAOs and DPAOs to uptake short chain fatty acids (i.e. acetate) under anaerobic conditions and form intracellular PHAs. GAOs are generally known as the PAOs competitors and are considered detrimental for BPR. But, recent findings have shown that some of the GAOs groups are important denitrifiers which can assist PAOs under specific environmental conditions.

In the present research, *Zoogloea* has been found to be the dominant denitrifiers and has long been known to produce and store PHAs. Since 35% of the microbial population in this work comprised the *Zoogloea* group, this is an indicator that they played an important role in both denitrification and BPR process. The PHAs biosynthesis by *Zoogloea* along with several other denitrifiers have been well established by Doi (1990), and Anderson and Dawes (1990).

7.2.2.1 PHAs Extraction

The determination of PHAs in the biomass samples was a laborious operation. PHAs were determined using the following protocol:

- Collect samples ranging from 0.5 L to 1 L from all three stages of the bioreactor.
- Filter sample to separate the biomass and centrifuge for 15 minutes to completely separate the bacteria from wastewater.
- Freeze and lyophilize the cells at −30°C. In general, mild polar compounds (i.e. acetone and alcohols) solubilized non-PHA materials such as phospholipids, lipids, nucleic acids

and proteins. On the other hand, chloroform and other chlorinated hydrocarbons solubilize biopolymers such as PHAs.

- Evaporate/precipitate with a solvent such as acetone or methanol to separate the dissolved polymer.
- Suspend separated cells in an aqueous 13% (v/v) sodium hypochlorite solution and incubated for 1 hour at 25^{o}C.
- Apply external cooling to prevent a strong temperature increase as cell lysis with hypochlorite can be strongly exergonic.
- Remove the supernatant and wash the polymer with water and centrifuge (15 min, 4,000 × g, 4°C).
- Freeze-dry (lyophilize) the purified polymer in a freezer at -70°C.
- Extract the PHAs from lyophilized cells into chloroform using a Soxhlet extractor.
- Concentrate the chloroform with a rotary evaporator after a reflux period of 6 hrs.
- Dissolve the concentrated material in acetone, filter, and add into rapidly stirred methanol.
- Separate the polymer by centrifugation (15 min, 10,000×g, 4°C) and dry at room temperature.

The non-soluble portion of the extracted solid remained in the thimble and was discarded. The total amount of PHA was determined gravimetrically and calculated as the percentage of cellular dry weight. This extraction procedure was adopted from Heinrich et *al.* (2012).

7.2.3 Data Collection and Analytical Methods

Each experiment was monitored for mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), PHAs, COD, NH_3-N, TP, NO_3-N and NO_2^-. MLSS and MLVSS were analyzed according to Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Samples for ammonia, nitrite, nitrate and phosphate were pre-filtered with a 1.2 mm pore size glass fiber filter (Whatman GF/C) and filtered through 0.45 µm pore size membrane filter (Gelman GN-6). NH_3-N and TP uptake rates were studied under variable operating conditions (Aerobic DO, Anaerobic COD, and Inlet NH_3-N).

7.3 Results and Discussion

7.3.1 SNDP Performance Evaluation at Various DO Concentrations

When the DO concentration increased from 0.5-1 mg/L to 2.5-3 mg/L, the NH_3-N, TP, NO_3-N and NO_2^- uptake increased drastically in the Anoxic stage. As DO concentration was increased further from 2.5-3 mg/L to 5.5-6 mg/L the anoxic NH_3-N and TP removal rates decreased. The results obtained in this study shows that the DO concentration of 2.5-3 mg/L were optimum for both NH_3-N and TP removal as shown in Table 7-3. Summary of phosphorus, NH_3-N, NO_3-N and NO_2^- uptake rates are shown in Table 7-3.

Table 7-3 Removal Rate of TP and Nitrogen Compounds at Different DO Concentrations

DO (mg/l)	NH_3-N (mg/l) h^{-1}		TP (mg/l) h^{-1}		NO_3-N (mg/l) h^{-1}		NO_2^- (mg/l) h^{-1}	
	Anoxic	Aerobic	Anoxic	Aerobic	Anoxic	Aerobic	Anoxic	Aerobic
0.5-1	6.8	3.5	5.8	1.6	0.4	2.2	4.8	3.7
2.5-3	12.7	3.33	10.8	1.9	12.6	0.15	12.8	5.5
5.5-6	9.6	0.93	5.6	0.22	10.8	3.75	30	5

NH_3-N Oxidation

The Anoxic NH_3-N uptake rate showed an increasing trend from 6.8 to 12.7 mg/L.h^{-1} as DO concentration in the Aerobic stage was increased from 0.5-1 to 2.5-3 mg/L. The increase in the anoxic NH_3-N uptake was expected at higher DO concentration since the AOBs in the bioreactor had more DO, NO_3-N and NO_2^- available to oxidize NH_3-N. Interestingly, as DO concentration was further increased from 2.5-3 to 5.5-6 mg/L, the anoxic NH_3-N uptake was reduced from 12.7 to 9.6 mg/L.h^{-1}. NH_3-N uptake in the Anoxic stages of the bioreactor was much higher than in the Aerobic stage for all the three DO concentrations. The rate of removal of NH_3-N in the Anoxic and Aerobic stages of the bioreactor can be presented as follows:

$$\frac{dNH_3 - N}{dt}\Big|_{\text{Anoxic}} \gg \frac{dNH_3 - N}{dt}\Big|_{\text{Aerobic}}$$

At a DO concentration of 2.5-3 mg/L, the rates of removal of NO_3-N and NO_2^- in the Anoxic stages were 12.6 and 12.8 (mg/L).h^{-1} respectively. NO_3-N and NO_2^- removal continued in the Aerobic stage although at much lower rate than in the Anoxic stage. The microbial population under anoxic conditions showed similar affinity for NO_3-N and NO_2^- as electron donors. As shown in Table 7-4 and Figure 7-2, the overall NH_3-N removal efficiency reached 98.5% at DO concentration of 2.5-3 mg/L. The overall NH_3-N removal increased from 80% at a DO range of 0.5-1 mg/L to 98.5% and 98% as DO concentration increased in the Aerobic stage.

Table 7-4 Overall NH_3-N Removal Efficiency at Various DO Concentrations

Experiments	DO Variation in the Aerobic Stage	NH_3-N Removal (%)
Run 1	0.5-1 mg/L	80%
Run 2	2.5-3 mg/L	98.5%
Run 3	5.5-6 mg/L	98%

Figure 7-2 presents the NH_3-N removal efficiencies in the Anoxic stage, Aerobic stage and overall NH_3-N removal efficiency of the SNDP process at various DO concentrations.

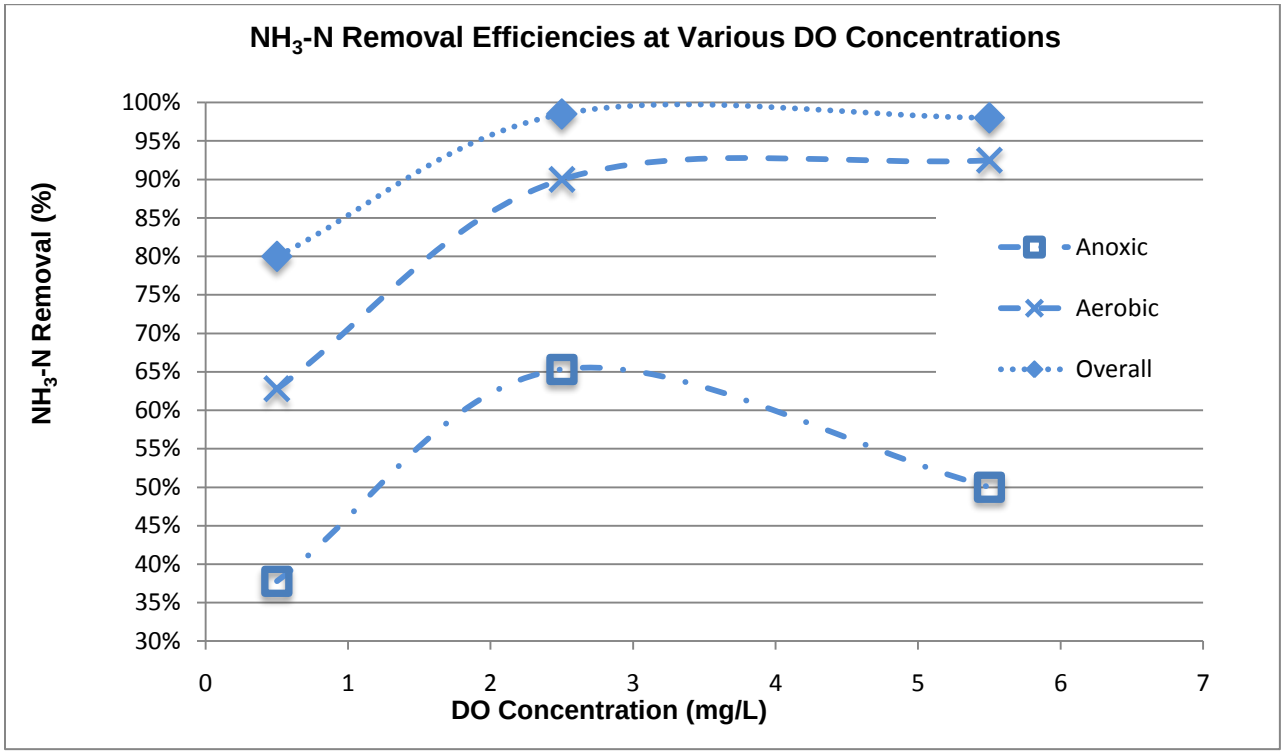


Figure 7–2 NH_3-N Removal Efficiency at Various DO Concentrations

Phosphorus Uptake

The TP uptake in the Anoxic stage increased significantly from 5.8 to 10.8 mg/L.h^{-1} when DO concentration in the Aerobic stage increased from 0.5-1 to 2.5-3 respectively. On the other hand, the anoxic TP removal rate decreased from 10.8 to and 5.6 mg/L.h^{-1} when DO concentration was further increased from 2.5-3 to 5.5-6mg/L. The rate of removal of phosphorus in the Anoxic stage was much greater than in the Aerobic stage (Table 7-3).

$$\frac{dP}{dt}\Big|_{\text{Anoxic}} \gg \frac{dP}{dt}\Big|_{\text{Aerobic}}$$

The Anoxic TP uptake rates for all the three DO concentrations were higher than the Aerobic uptake contradicting the results reported by other researchers (Kuba et *al.*, 1996; Filipe

and Daigger, 1999). The later stated that DPAOs have a disadvantage when competing with PAOs, because of the lower thermodynamic efficiency of anoxic growth compared to aerobic growth. It was not possible to estimate directly the relative ratio of aerobic to anoxic phosphate uptake and additional experiments were needed to estimate the maximum aerobic and anoxic TP uptake separately. This suggests that at DO concentration of 0.5-1, anoxic TP removal was limited by insufficient NO_3-N and NO_2^- availability. The sharp decrease in TP removal efficiency at high DO concentration of 5.5-6 mg/L may have been due to NO_2^- accumulation in the bioreactor. This is likely caused by the inhibitory effect of NO_2^- on the anoxic TP uptake.

During run 3, DO concentration increased from 2.5-3mg/L to 5.5-6 mg/L. Anoxic TP uptake rate was found to be slightly lower than run 1 and much lower than those obtained at 2.5-3 mg/L. This is likely caused by the inhibitory effect of NO_2^- on the anoxic TP uptake. At high DO concentration of 5.5-6 mg/L, NO_2^- concentration was found far greater (almost 3 times) than NO_3-N concentration in the Anoxic stages (see Table 7-3). Consequently, NO_2^- uptake in the Anoxic stage was quite significant relative to the NO_3-N uptake. As mentioned earlier, the anoxic phosphorus uptake rate was reduced at high DO level; therefore, one can realistically speculate that NO_2^- in the Anoxic stage might have been utilized by microorganisms other than the DPAOs. The aerobic NO_2^- removal comes from two different mechanisms:

1st Mechanism: NO_2^- conversion to N_2 by denitrifying organisms.

2nd Mechanism: Conventional oxidation of NO_2^- to NO_3-N by NOBs.

The microbial analysis of the biomass showed no traces of the commonly known NOBs. Detailed DNA sequencing of the biomass suggested that the first mechanism was most likely the preferred pathway. These findings cannot provide a clear understanding of the pathways involved in the production or removal of both NO_2^- and NO_3-N (mechanism 1) and whether NO_2^-

was oxidized to NO_3-N (mechanism 2) or reduced to N_2. Because of the biochemical complexity of these findings, a full study of the actual pathway requires further investigations, which are beyond the scope of this work. However, these finding are fertile material for future microbial thermodynamics and kinetics studies.

Furthermore, at high DO concentration of 5.5-6 mg/L, increase in phosphorus concentration was observed in the Aerobic stage. Since the inlet TP concentration was maintained constant, there may have been secondary phosphorus release caused by high DO concentration in the Aerobic stage. Also, several phosphorus measurements in the Aerobic stage at low DO concentration of 0.5-1 mg/L showed increase in phosphorus which suggested secondary phosphorus release. The presumed secondary P-release at low DO presented herein is unprecedented in the open literature since secondary P-release has been mainly detected at high DO concentrations. Future studies must be done to evaluate this finding in more detail.

Figure 7-3 presents the TP removal efficiencies in the Anoxic stage, Aerobic stage and overall TP removal efficiency of the SNDP process at various DO concentrations. The overall TP removal was significantly increased from 66% to 97% when DO concentration was increased from 0.5-1 mg/L to 2.5-3 mg/L. The overall TP removal was further diminished from 97% to 47% when DO increased from 2.5-3 mg/L to 5.5-6 mg/L as shown in Table 7-5.

Table 7-5 Overall TP Removal Efficiency at various DO Concentrations

Experiments	DO Variation in the Aerobic Stage	TP Removal (%)
Run 1	0.5-1 mg/L	66%
Run 2	2.5-3 mg/L	97%
Rune 3	5.5-6 mg/L	47%

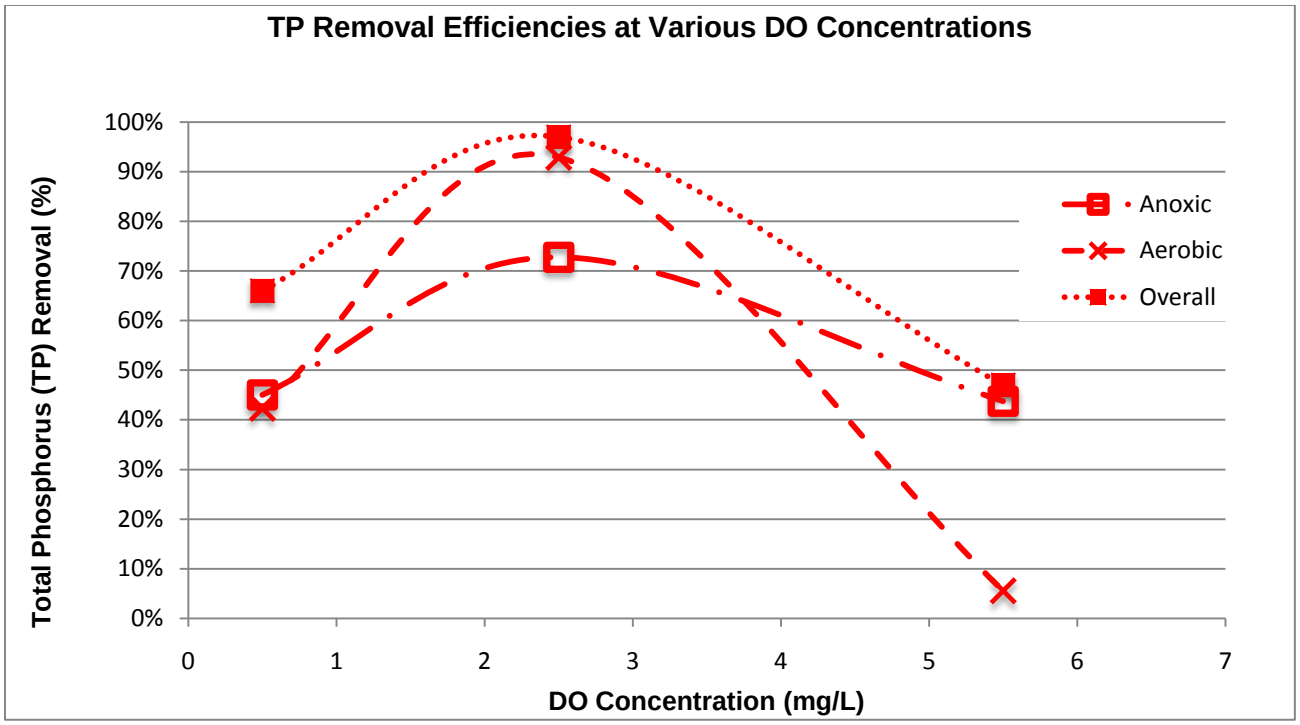


Figure 7–3 TP Removal Efficiency at Various DO Concentrations

7.3.2 Effect of COD on the Removal of Phosphorus and Ammonia

PHAs in the biomass were extracted and measured from June 2013 to November 2013 at different COD concentrations. A mixture of acetic acid, butyric acid and propionic acid was fed to the anaerobic lateral unit (ALU) containing COD concentrations which ranged from 1000 to 1400 (mg/L). The PHAs concentrations in the ALU and the three stages of the bioreactor were determined as %MLSS. As shown in Figure 7-4, PHAs concentration increased with time independently of the amount of COD added. Similarly, the TP removal rate increased during this period as previously explained in chapter 3. Also, it was expected to find an increase in the PHAs content of the biomass as the BPR process reached steady state and a maximum rate of phosphorus removal was obtained. The intracellular PHAs production did not solely depend on the concentration of COD (or VFAs). Indeed, the fermentation process in the ALU was essential

in converting the COD into more readily degradable substrates. As mentioned in chapter 4, the abundance of the hydrolyzing species of S*aprospiraceae* provided a significant advantage for PAOs/DPAOs. The symbiotic relationship between S*aprospiraceae*, *Zoogloea* (second dominant bacteria) and other nitrifiers and denitrifiers facilitated the utilization of all electron donors and acceptors involved in the process. Such unique distribution of the microbial population evolved in this bioreactor where microorganisms benefitted from simultaneous availability of COD, intracellular PHAs, nitrate, nitrite and DO (in the Aerobic stage). Figure 7-4 shows the time-dependent profiles of PHAs in the ALU and the three stages of the reactor from June to November 2013.

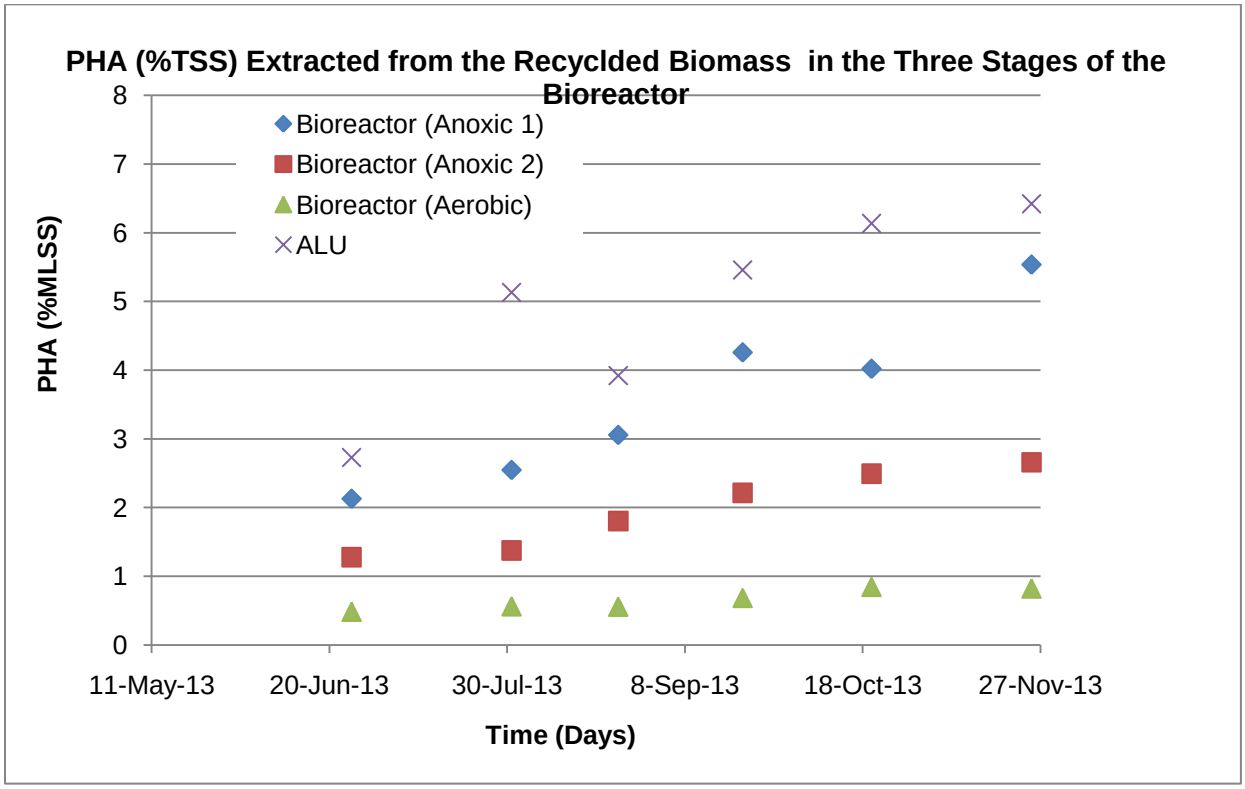


Figure 7–4 PHA (%MLSS) Extracted from the Recycled Biomass and the Three Stages of the Bioreactor

As shown in Figure 7-4, the PHAs content of the biomass gradually increased in all the samples taken over time from both the ALU and the bioreactor. The maximum PHAs content of

the biomass was approximately 6.5 (%MLSS) in the ALU. The PHAs were measured at different COD concentrations. PHAs gradually increased over time (from June to November 2013) up to 6.5 (%MLSS) when the COD concentration in the ALU was at 1400 (mg/L).

Table 7-6 Intracellular PHAs Contents (%MLSS) at Different COD Concentrations

No. of Experiments	COD Concentration	PHA (%MLSS) Extracted
Experiment 1		
Run 1	1400 mg/L	5.9% (ALU*) 4.3% (AX1**) 2.6% (AX2***) 0.97% (AE****)
Experiment 2		
Run 4	1000mg/L	3.0% (ALU) 2.9% (AX1) 1.7% (AX2) 0.52% (AE)
Run 5	1200 mg/L	5.0% (ALU) 4.3% (AX1) 2.2% (AX2) 0.69% (AE)
Run 6	1400 mg/L	5.9% (ALU) 5.3% (AX1) 3.3% (AX2) 1.1% (AE)
Experiment 3		
Run 8	1400mg/L	6.5% (ALU) 5.5% (AX1) 2.7% (AX2) 0.82% (AE)

* ALU: Anaerobic Lateral Unit, ** AX1: Anoxic 1, ***AX2: Anoxic 2, ****AE: Aerobic stage

PHA yield relative to the COD removed was calculated from June to November 2013.As shown in Table 7-7, when the COD concentration fed to the ALU increased from 1000 mg/L in August to1400 mg/L in November 2013, the PHA yield increased from 3.0 to 6.5 (%MLSS). It is well understood that PHAs can only be produced and stored during the anaerobic phase. Therefore, mass (g) PHAs yield per mass (g) COD removed could only be applied to the ALU.

Table 7-7 gPHAs yield per gCOD removed in the ALU

Date	COD (mg/L) Fed to the ALU	gPHA yield/ gCOD consumed in the ALU	PHA (%MLSS)
June 2013	1400	0.35	5.9
August 2013	1000	0.31	3.0
September 2013	1200	0.39	5.0
October 2013	1400	0.37	5.9
November 2013	1400	0.43	6.5

COD concentrations in August and September 2013 were 1000 mg/L and 1200 mg/L respectively. The results shown in Table 7-7 indicate that at 1200 mg/L, intracellular PHAs produced was 0.39, which was higher than in June and in October 2013 when COD fed to the ALU was 1400 mg/L. This indicates that there was not a linear relationship between COD added and PHAs produced. Other factors that influenced the PHAs synthesis may have been pH and SRT of the process. Indeed, the SRT of the SNDP process was increased from 35 to 50 days from June to November 2013 and that may have increased the PHAs content of the biomass at

higher SRT period (September, October, and November). The PHAs yield per COD utilized in the ALU varied for two reasons: 1) change in the COD concentration ranging 1000-1400 mg/L and 2) enrichment of the biomass with PHAs forming organism particularly PAOs/DPAOs.

The intracellular PHAs and external COD were both essential electron donors in the SNDP process. Table 7-8 shows the ratio of intracellular PHAs utilized to COD consumed (gPHAs/gCOD) in the Anoxic and Aerobic stages of the bioreactor.

Table 7-8 gPHAs utilized per gCOD removed in the three stages of the bioreactor

Date	COD (mg/L) in the recycled biomass	gPHA /gCOD Anoxic 1	gPHA/gCOD Anoxic 2	gPHA/gCOD Aerobic
June 2013	560	0.38	0.38	0.34
August 2013	523	0.44	0.45	0.25
September 2013	556	0.46	0.41	0.33
October 2013	605	0.44	0.57	0.55
November 2013	580	0.48	0.46	0.40

Figure 7-5 (a) illustrates PHAs extracted from the biomass in the ALU relative to the readily available COD concentration. Figure 7-5 (a) shows increase in PHAs yield (in the ALU) as COD concentration increased from 1000 to 1200 mg/L. Figure 7-5 (b), (c) and (d) present the amount of intracellular PHAs in the biomass (%MLSS) vs. readily available COD concentration (g/l) in different stages of the reactor.

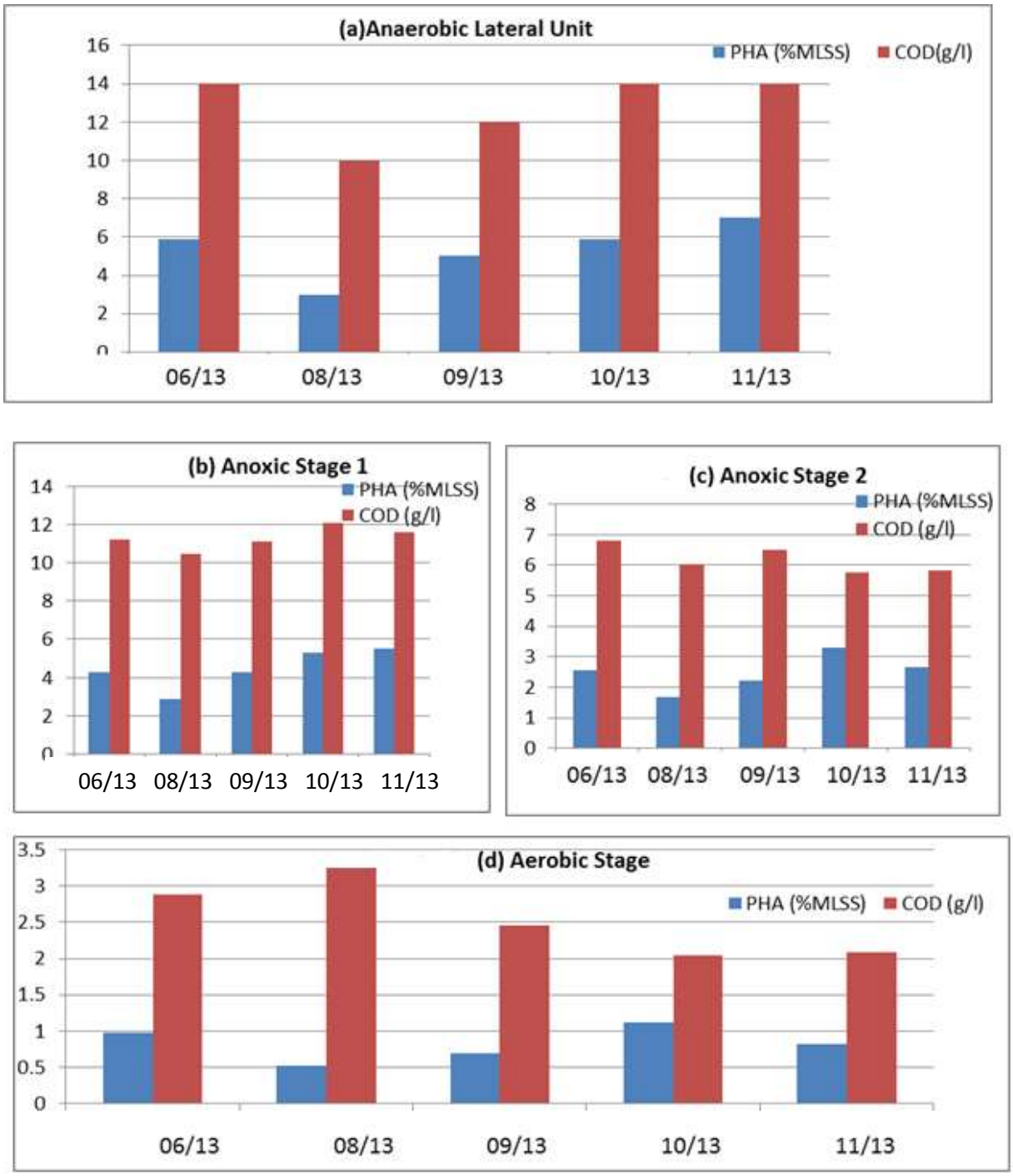


Figure 7–5 Intracellular PHA (%MLSS) vs. External COD Concentration (g/L)

7.3.3 SNDP Performance Evaluation at High Inlet NH_3-N Concentrations

As discussed before, the first step of nitrification is the oxidation of NH_3-N to NO_2^-. In the Aerobic phase, NH_3-N oxidation (uptake) can be measured directly as most of the NH_3-N fed to the reactor is oxidized and converted into NO_2^-. As seen in section 2.2.1, only a small fraction of NH_3-N is assimilated by the biomass for cellular growth. In this study, due to the alignment of the Anoxic stages followed by Aerobic stage, feed with high NH_3-N first enters the top two Anoxic stages and then flows by gravity to the Aerobic stage. Therefore, decrease in the inlet NH_3-N in the Anoxic stages cannot be associated with simple oxidation by DO. There must have been other competing processes in this bioreactor that caused NH_3-N removal in the absence of DO and in the presence of NO_2^- and NO_3-N. The influent NH_3-N was increased from 49 mg/L to 80 mg/L and finally to 120 mg/L (runs 7, 8 and 9 shown in Table 7-9). The impact of the increase in the inlet NH_3-N on both NH_3-N and TP uptake were studied and the results are summarised in Table 7-9. DO concentrations in the Aerobic stage were constant at 2.5-3 mg/L for runs 7, 8 and 9.

Table 7-9 Anoxic and Aerobic NH_3-N and TP Uptake Rates and Removal Efficiencies

No. of Runs	Inlet NH_3-N (mg/L)	% NH_3-N Removal Efficiency	% TP Removal Efficiency	NH_3-N Uptake Rate (mg/L h^{-1})	Phosphorus Uptake Rate (mg/L h^{-1})
7	49 mg/L	65% Anoxic 32% Aerobic	73% Anoxic 25% Aerobic	12.8 Anoxic Stages 2.4 Aerobic Stage	9.6 Anoxic Stages 2.8 Aerobic Stage
8	80mg/L	75% Anoxic 29% Aerobic	67% Anoxic 24% Aerobic	24 Anoxic Stages 4.7 Aerobic Stage	8.8 Anoxic Stages 3.0 Aerobic Stage
9	120 mg/L	57% Anoxic 42% Aerobic	31% Anoxic 67% Aerobic	27.2 Anoxic Stages 8.2 Aerobic Stage	4.0 Anoxic Stages 7.2 Aerobic Stage

As the influent NH_3-N concentration increased from 49 to 120 mg/L, the anoxic NH_3-N uptake increased from 12.8 to 27.2 (mg/L h^{-1}). Similarly, the rate of aerobic NH_3-N uptake increased from 2.4 to 8.2 (mg/L h^{-1}). TP uptake rate was affected differently in the Anoxic and Aerobic stages. The anoxic TP uptake decreased from 9.6 to 4.0 (mg/L h^{-1}) when the NH_3-N influent concentration increased. However, the aerobic TP uptake increased from 2.8 to 7.2 (mg/L h^{-1}). The decrease in phosphorus uptake in the two Anoxic stages was most likely due to the accumulation of NO_2^- at higher NH_3-N concentration. As discussed previously, NO_2^- inhibited the anoxic phosphorus uptake which has been also confirmed by researchers in other studies. All the raw data for chapter 7 are presented in Appendix 3.

7.4 Conclusions

Chapter 7 focuses on investigating the rate of removal of NH_3-N and total phosphorus (TP) in the Anoxic and the Aerobic stages of the bioreactor. The responses of the SNDP process were observed by varying three important operating parameters including:

1) Aerobics DO concentration ranging from 0-0.5 to 5.5-6 mg/L;

2) Anaerobic COD concentration ranging from 1000 to 1400 mg/L and,

3) Inlet NH_3-N concentration ranging from 49 to 120 mg/L.

The DO of 2.5-3 mg/L in the Aerobic stage found to be the optimum concentration for NH_3-N and TP uptake. At DO of 0.5-1 mg/L, TP uptake was disrupted and episodes of secondary phosphorus release were observed in the Aerobic stage of the bioreactor. At high DO concentration of 5.5-6 mg/L, TP uptake was negatively affected when almost all NH_3-N was oxidized to NO_2^- and NO_3-N. The rate of TP uptake was hindered by sudden increase in NO_2^-

concentration in the two Anoxic stages. It was observed that PAO/DPAOs activities was suppressed, but could very quickly restore its overall phosphorus removal capacity.

Also, phosphorus uptake was particularly affected under nutrient conditions (high NH_3-N/TP) when NH_3-N concentration in the inlet was increased from 49 mg/L to 120 mg/L. The negative effects of high NH_3-N on PAO and DPAOs most likely were due to increase in NO_2^- concentration in the Anoxic stages and accumulation of NO_2^- in the bioreactor. The NH_3-N uptake rate increased in both Anoxic and Aerobic stages when inlet NH_3-N increased from 49 to 80 and then 120 mg/L.

Experimental results obtained from June to November 2013, demonstrated that both intracellular PHAs and external COD concentrations were the rate-controlling factors in the SNDP process. The stored PHAs in the biomass was fundamental for the overall process as the addition of COD to the ALU could be lower than 1400 mg/L without having negative impact on the simultaneous nitrification, denitrification and biological phosphorus removal process.

Chapter 8 – Final Conclusions and Future Work

8.1 Final Conclusions

Biological nutrient removal (BNR) is an important process in water engineering. The BNR processes developed in the last three decades are widely known as:

1. Nitrification/denitrification
2. Simultaneous nitrification-denitrification
3. Anaerobic Ammonium Oxidation
4. Biological phosphorus removal via phosphorus accumulating organisms (PAOs) and denitrification via DPAOs, and
5. Simultaneous nitrification-denitrification-biological phosphorus removal (SNDP)

The above simultaneous processes often fail in municipal wastewater treatment plants (WWTP) due to a number of factors such as flow fluctuations, variations of the influent composition, accumulation of toxic bio-products (i.e. nitrous oxide) and mass transfer limitations. Currently, there is a major knowledge gap between the analytical/microbial results obtained from lab experiments and full-fledged treatment plants. The experimental data obtained from small, bench scale sequencing batch reactors (SBRs) misrepresent large scale processes occurring in municipal and industrial WWTPs. Furthermore, microbial composition of the biomass in large WWTPs often differs from those seen in the lab scale SBRs hence many microbial and flow processes are poorly represented. Indeed many of BNR organisms are still unidentified.

The goal of this thesis was to design, develop and evaluate an effective process for the simultaneous removal of ammonia and phosphorus in a continuous flow vertical bioreactor with minimum requirements for energy and construction footprint. The results obtained in this study are remarkable in two aspects:

1. The bioreactor (design, construction and operation of a novel bioreactor), and

2. The bio-process (evaluation of the microbial structure and process performance)

A 240 L/day, vertical, continuous, and multistage bioreactor has been designed, built, and successfully tested in the Water Treatment Technologies Laboratory of the Department of Chemical Engineering of Ryerson University, Toronto. The vertical configuration of the bioreactor makes its footprint much smaller than conventional planar bioreactors used in municipal BNRs. This constitutes a significant economic advantage and a feature of special interest when refurbishing plants located in high density urban concentrations. Also, the vertical multistage configuration of the reactor makes it highly suitable for modular construction in lighter materials such as fiberglass and PVC. The excavation cost can be drastically reduced when vertical bioreactors replace the existing planar basins.

The vertical bioreactor configuration with its auxiliary units provided unique redox environments leading to the development of a highly efficient SNDP process with two hitherto largely unknown microbial groups. Using advanced molecular biology techniques the phylogenetic affiliations of the bacteria belonging to the *Saprospirasae* and *Zoogloea* species were determined. The symbiotic relationship between these two dominant bacteria may have been the key to the successful performance of the SNDP process. Furthermore, the fluid mechanics produced in the cylindrical vertical bioreactor optimized mixing, hydraulic residence time and made possible the high performance of the bioreactor (over 95% removal for both ammonia and phosphorus). The performance of the bioreactor was also evaluated in terms of the ratio Nutrient Removed/ COD required and found to be considerably higher than those ratios used by other BNR processes. The process developed in this bioreactor has a lower consumption of volatile fatty acids and therefore lower maintenance costs for equivalent rate of nutrient

removal. The hydraulic residence time (HRT) of the multistage vertical bioreactor (6.5 hours) was found to be one of the shortest HRTs among the BNR processes which make it economically favourable.

8.2 Future Work

- The scientific findings in this PhD thesis have cast doubt on the significant role of the previously known PAOs. Advanced microbial studies are needed to introduce a broader community structure and composition of the PAOs and their affiliated groups.
- In this work, three unstructured models were developed to predict Ammonia, Nitrate/Nitrite and TP concentrations in the vertical bioreactor. The validity of these models has been verified by comparing the experimental and predicted data at different substrate concentrations, SRTs and HRTs. Structured modelling of the SNDP process will be an important future contribution to this work.
- Detailed studies of the microbial groups found in this research such as *Saprospiraceae* and *Zoogloea* and understanding of their metabolic pathways are crucial for future cultivation of these groups. A better understanding of these organisms will help to identify the required cultivating parameters and to optimize the overall BNR process.
- Future studies will help to reveal the simbiotic linkages between *Zoogloea*, *Saprospiraceae* and other microbial species involved in the SNDP process.
- The SNDP process generates a type of biomass rich in phosphorus and nitrogen. Therefore, recovery and reuse of the wasted biomass from the bioreactor should be investigated. Direct industrial applications of the recovered nutrients should be further explored.

- Future investigation will demand an expansion of the experimental unit to a higher treatment capacity and improvement in the process instrumentation and control.

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Appendices

Appendix 1- Raw Data for Chapter 3 (Nutrient Concentrations, mg/l)

	Influent (mg/L)				Anoxic 1 (mg/L)				Anoxic 2 (mg/L)				Aerobic (mg/L)				
Dates	**NH_3-N**	**NO_3-N**	**NO_2^-**	**TP**	**NH_3-N**	**NO_3-N**	**NO_2^-**	**TP**	**NH_3-N**	**NO_3-N**	**NO_2^-**	**TP**	**NH_3-N**	**NO_3-N**	**NO_2^-**	**TP**	**O_2**
3/12/2013	38	21.1	64	32	35	21.9	45	-	38.7	22.1	35	-	25.3	18	27	35	4.5
3/14/2013	43	20.5	54	-	35	12.1	45	-	23.1	15	34	-	8.1	15	62	36	4.4
3/17/2013	30	10.9	12	33	26	8.6	40	-	26	15	63	-	10.2	16.1	36	-	4.3
3/18/2013	30	10.9	12	32	26	8.6	40	-	22.4	15	51	-	7.5	4.1	87	-	4.2
3/23/2013	34	14.4	15	32	30	5.2	40	-	28.7	15	15	-	6.5	3.9	88	34	4.1
3/28/2013	41	34.4	106	32	21.6	4.8	61	-	19.4	4.4	69	-	3.2	5.5	78	-	4.1
3/30/2013	52	14.5	34	33	19.3	4.8	35	-	19.4	4.4	61	-	16	3.7	51	-	4.2
4/1/2013	44	22.1	65	31	31.3	4.8	69	-	27.1	1.6	24	-	12.5	3.7	30	36	4
4/5/2013	38	27.8	79	32	25.5	4.8	91	-	27.7	1.3	85	-	22.8	4	50	34	3.8
4/6/2013	32	14	26	32	32.8	4.8	65	-	32.8	0.7	43	-	23	5	10	33	3.7
4/9/2013	37	14.5	38	32	37.5	4.8	15	-	27.6	1.5	17	-	27.6	5.4	9	35	3.5
4/13/2013	39	16	85	31	34.7	4.8	69	-	24	2.5	55	-	25	4	44	31	3.6
4/14/2013	40	14.2	80	32	26.8	2.5	37	-	18.3	2.3	29		26.5	2.3	27		3.7
4/15/2013	38	18	70	33	30.6	3.1	47	-	27	2.5	43		21.1	3.3	52		3.7
4/16/2013	32	15	22	33	30.2	1.6	26	-	30.6	1.7	29		21.8	3.5	33		3.5
4/19/2013	21	14	20	33	23.3	1.8	67	-	23.3	1.6	71		11.7	1.3	30		3.2
4/24/2013	21	14.5	30	31	13.1	3.2	46	-	12.3	2.4	57		4	1.3	27		3.3
4/25/2013	36.1	18	31	32	16.9	1.7	44	-	16.1	1.7	42		3.8	1.5	42		3.5
4/26/2013	36	21	30	32	10.7	3.1	50	-	11.9	1.3	39	29	4	1.5	49	31	3.2

	Influent (mg/L)				Anoxic 1 (mg/L)				Anoxic 2 (mg/L)				Aerobic (mg/L)				
Dates	**NH_3-N**	**NO_3-N**	**NO_2^-**	**TP**	**NH_3-N**	**NO_3-N**	**NO_2^-**	**TP**	**NH_3-N**	**NO_3-N**	**NO_2^-**	**TP**	**NH_3-N**	**NO_3-N**	**NO_2^-**	**TP**	**O_2**
4/27/2013	36	23	30	33	10	1.1	60	-	11	1.2	39	-	6.6	1.5	70	-	3.3
5/2/2013	36	25	30	32	10.9	1.1	66	-	11.6	1.2	95	-	6.8	2.1	77	-	3.5
5/16/2013	40	24	48	33	33.6	1.8	108	-	28.4	1.7	168	-	23.9	4.1	50	-	3.6
5/17/2013	40	31	34	33	19.5	0.8	104	-	26.7	0.4	133	-	25.6	2	45	-	3.4
5/31/2013	40	32	35	34	37.7	2.3	87	-	28.8	0.6	73	-	22.5	8.5	42	-	3.3
6/13/2013	41	29.5	36	32	23.7	1	78	-	18.7	0.5	58	-	14.9	5.8	66	-	3.1
6/15/2013	43	32.5	35	33	15.2	2.2	73	-	13.9	0.7	84	-	12	5.7	66	-	2.9
7/4/2013	40	31	38	32	21.9	2.2	17	29	22.1	0.7	17	26	18	0.9	17	22.6	3.8
7/10/2013	42	32	37	32	12.1	0.2	57	26	15	0.7	21	24	15	1.1	8	25.3	3
7/15/2013	43	33	35	31	8.6	0.7	91	27	8.6	0.7	60	21	16.1	1.2	14	12.7	2.5
7/17/2013	40	32	36	33	12	1.9	80	29	8	0.7	59	23	4.1	1.7	53	15.7	2.5
7/19/2013	46	31	33	32	5.2	0.6	66	25	4.3	0.7	45	19	3.9	1.3	17	12.2	2.5
7/25/2013	45	30	30	32	4.8	0.6	79	27	5.4	0.7	60	21	5.5	1.3	53	13.4	2.2
7/30/2013	46	33	34	32	5	0.8	90	26	5	0.7	60	18	3.7	1.7	19	14.5	2.2
7/31/2013	45	32	33	32	4.8	0.2	85	28	6.5	-	45	19	4.3	0.9	17	14.2	2.2
8/3/2013	44	31	33	32	2	0.7	91	23	5	0.7	60	20	9.4	1.1	14	15.8	2.5
8/24/2013	46	33	35	33	2.5	0.2	80	25	4.7	-	62	19	3.8	5.5	20	15	2.6
9/5/2013	45	32.5	33	35	1	2.2	81	28	3.5	0.7	63	16	3.2	1.8	14	11.9	2.5
9/21/2013	45	33	32	33	1.1	0.6	95	24	4.0	0.7	57	15	3	1.3	14	12.5	2.3
10/2/2013	50	34	33	32	0.9	0.5	91	16	4.5	0.7	59	10	2	1.2	14	6.5	2.2

	Influent (mg/L)				Anoxic 1 (mg/L)				Anoxic 2 (mg/L)				Aerobic (mg/L)				
Dates	**NH_3-N**	**NO_3-N**	**NO_2^-**	**TP**	**NH_3-N**	**NO_3-N**	**NO_2^-**	**TP**	**NH_3-N**	**NO_3-N**	**NO_2^-**	**TP**	**NH_3-N**	**NO_3-N**	**NO_2^-**	**TP**	**O_2**
10/6/2013	45	32	30	33	0.75	0.6	85	17	3.9	0.7	62	-	0.3	1.0	14	6.5	2.2
10/20/2013	44	32	31	32	0.8	0.8	95	16	4.2	0.7	65	10	0.5	1.2	15	1.8	2.3
10/25/2013	45	35	33	32	1.2	0.6	92	18	3.0	0.7	51	7	0.8	1.1	16	1.5	2.3
11/9/2013	46	36	32	33	0.6	0.5	92	20	2.5	0.7	48	10	0.3	1.0	13	0.8	2.3
11/25/2013	45	32	34	31	0.5	0.8	96	15	2.9	0.7	45	11	0.4	1.1	15	1.1	2.3
12/14/2013	44	33	33	33	0.7	0.4	93	19	2.1	0.7	32	8	0.3	0.9	12	1.3	2.3
12/22/2013	45	31	35	32	0.5	0.6	91	17	1.9	0.7	24	4	0.2	0.6	15	0.4	2.1

Appendix 2- Experimental Data for Chapter 6

Appendix 2a – Nitrification Model

Data obtained during batch experiment for nitrification

Test No.	NH_3-N in the Influent (mg/L)	NH_3-N in the Effluent (mg/L)	MLVSS(mg/L)	SRT (days)
1	46	15	1500	35
2	45	10	1540	35
3	45	5	1460	35
4	45	3.5	1650	40
5	45	1.1	1320	45
6	45	0.7	1410	50

$$\frac{\mathrm{SRT}}{1 + K_d \mathrm{SRT}} = \frac{K_s}{\mu_{max}} \frac{1}{S} + \frac{1}{\mu_{max}} \qquad \text{Eq. 6-10}$$

k_d=0.15

$\frac{\mathrm{SRT}}{1 + K_d \mathrm{SRT}}$	$\frac{1}{NH_3 - N}$
5.45	0.07
5.45	0.10
5.60	0.20
5.60	0.29
5.71	0.91
5.88	1.43

Figure 6-4 was plotted using the above values calculated from Eq. 6-10

Appendix 2b – Denitrification Model

The following table was used to plot Figure 6-5 for denitrification model (Eq. 6-16).

HRT (h)	NO_3-N + NO_2^- (mg/L)	$\frac{1}{HRT} ln(\frac{S_o}{S})$	$(\frac{S_o - S}{HRT})$
1.5	41.0	0.29	14.67
2.0	34.8	0.30	14.13
2.5	27.5	0.33	14.20
3.0	21.5	0.36	13.83
3.5	16.2	0.39	13.37
4.0	11.1	0.43	12.98

$$\frac{0.29X}{11.2} = -\frac{1}{11.2}(\frac{S_o - S}{HRT}) = \frac{1}{HRT} ln(\frac{S_o}{S}) \quad \text{Eq. 6-16}$$

Appendix 2c – Bio-P Model

$\frac{S_o \, HRT}{S_o - S}$	HRT (h)
3.04	2.00
3.47	2.50
3.69	3.00
3.54	3.50
4.03	4.00
4.35	4.33
4.68	4.67
5.02	5.00

$$\frac{HRT}{Removal\ Effciency} = 1.66 + 0.65\ HRT \quad \text{Eq. 6-24}$$

Appendix 3- Experimental Data for Chapter 7

Each test was conducted 3 times. The average values are shown below.

Experiment 1
Run 1: DO = 0.5-1mg/L

Reactor Stages	Time (h)	Phosphorus Uptake (mg/L) (Mean)	NH3-N (mg/L) (Mean)	NO_3-N (mg/L) (Mean)	NO_2^- (mg/L) (Mean)	COD (mg/L) (Mean)	DO (mg/L)
Anoxic	0	32	45	15	25	526	0
	30	31	42	14	23	422	0
	60	26	38	15	21	350	0
	90	22	32	22	18	320	0
	120	20	30	18	12	280	0
	150	17.6	28	16	13	260	0
Aerobic	180	18.2*	25	12	24	255	0.8
	210	16.3	21	10	22	210	0.8
	240	14	18	9.5	20	180	0.8
	270	15.5*	17	7.8	18.5	165	0.8
	300	15	15	6.6	16	150	0.8
	330	12	14.5	5.6	14.3	132	0.8
	360	12.5*	12	5.8	12.3	112	0.8
	390	11	11.3	5.7	11	98	0.8
	420	10.5	10.2	3.2	9.2	85	0.8

* Secondary phosphorus release (increase in phosphorus concentrations)

Experiment 1
Run 2: DO = 2.5-3 mg/L

Reactor Stages	Time (h)	Phosphorus Uptake (mg/L) (Mean)	NH3-N (mg/L) (Mean)	NO_3-N (mg/L) (Mean)	NO_2^- (mg/L) (Mean)	COD (mg/L) (Mean)	DO (mg/L)
Anoxic	0	32	47.8	31.8	60	526	0
	30	23.9	36.5	14	55	423	0
	60	17.2	29	9	49	350	0
	90	13	23.9	4	41	320	0
	120	9.7	21	0.4	35	280	0
	150	5.1	16	0.2	28	260	0
Aerobic	180	4.7	14	1.2	25	255	2.8
	210	4.3	13	2.8	21	210	2.8
	240	3.8	10	2.6	22	180	2.8
	270	3	8	2.4	26	165	2.8
	300	2.6	7.5	1.3	23	150	2.8
	330	2	4.6	1.2	19	132	2.8
	360	1.6	3.3	0.9	14	112	2.8
	390	1.2	1.3	0.6	8	98	2.8
	420	0.9	0.7	0.6	3	85	2.8

Experiment 1
Run 3: DO = 5.5-6 mg/L

Reactor Stages	Time (h)	Phosphorus Uptake (mg/L) (Mean)	NH3-N (mg/L) (Mean)	NO_3-N (mg/L) (Mean)	NO_2^- (mg/L) (Mean)	COD (mg/L) (Mean)	DO (mg/L)
Anoxic Stage	0	32	48	59	110	550	0
	30	30	42	55	102	510	0
	60	25	35	53	79	460	0
	90	22	32	45	62	420	0
	120	21	29	41	49	400	0
	150	18	24	32	35	338	0
Aerobic	180	18	4	33	36	315	5.8
	210	15.2	1.2	38	33	287	5.8
	240	14	0.9	39	31	277	5.8
	270	18*	0.5	31	30	243	5.8
	300	21*	0.5	26	25	240	5.8
	330	22*	0.3	26	24	220	5.8
	360	21	0.3	22	22	194	5.8
	390	19	0.3	20	18	186	5.8
	420	17	0.3	18	16	178	5.8

* Secondary phosphorus release (increase in phosphorus concentrations)

Experiment 2

Results of PHAs extraction in the samples taken from the ALU

Dates	COD (mg/L)	PHA (%TSS)	MLSS (g/L)
25/06/2013	1300	2.7	10
31/07/2013	1400	5.1	9
24/08/2013	1400	3.9	7.5
21/09/2013	1560	5.5	5.5
20/10/2013	1620	6.1	6.6
25/11/2013	1690	6.4	5
14/12/2013	1500	5.8	6.5
22/12/2013	1600	6.7	5

Results of PHAs extraction in Anoxic 1

Dates	COD (mg/L)	PHA (%TSS)	MLSS (g/L)
25/06/2013	560	2.1	10
31/07/2013	540	2.5	9
24/08/2013	523	3.1	7.5
21/09/2013	556	4.3	5.5
20/10/2013	605	4.0	6.6
25/11/2013	580	5.5	5
14/12/2013	524	3.8	6.5
22/12/2013	547	2.6	5

Results of PHAs extraction in Anoxic 2

Dates	COD (mg/L)	PHA (%TSS)	MLSS (g/L)
25/06/2013	340	1.3	10
31/07/2013	289	1.4	9
24/08/2013	301	1.8	7.5
21/09/2013	326	2.2	5.5
20/10/2013	288	2.5	6.6
25/11/2013	292	2.7	5
14/12/2013	270	2.1	6.5
22/12/2013	294	1.4	5

Results of PHAs extraction in Aerobic stage

Dates	COD (mg/L)	PHA (%TSS)	MLSS (g/L)
25/06/2013	144	0.49	10
31/07/2013	178	0.56	9
24/08/2013	166	0.56	7.5
21/09/2013	123	0.69	5.5
20/10/2013	102	0.85	6.6
25/11/2013	104	0.82	5
14/12/2013	85	0.64	6.5
22/12/2013	79	0.49	5

Experiment 2
Run 4, 5 and 6: COD 1000-1400 mg/L

Run 4

ALU and Reactor Stage	COD (mg/L)	PHA (%TSS)	MLSS (g/L)
ALU	1000	3	8
Anoxic 1	520	2.8	6.5
Anoxic 2	290	1.7	6
Aerobic	120	0.52	6.2

Run 5

ALU and Reactor Stage	COD (mg/L)	PHA (%TSS)	MLSS (g/L)
ALU	1200	5	6
Anoxic 1	556	4.3	5
Anoxic 2	236	2.2	5
Aerobic	120	0.69	5.2

Run 6

Date: 25/06/2013

ALU and Reactor Stage	COD (mg/L)	PHA (%TSS)	MLSS (g/L)
ALU	1400	5.9	10
Anoxic 1	560	4.3	8.5
Anoxic 2	340	2.6	6
Aerobic	144	0.97	7.2

Date: 20/10/2013

ALU and Reactor Stage	COD (mg/L)	PHA (%TSS)	MLSS (g/L)
ALU	1400	6.1	5
Anoxic 1	605	5.3	5
Anoxic 2	288	3.3	6
Aerobic	102	1.1	5

Date: 25/11/2013

ALU and Reactor Stage	COD (mg/L)	PHA (%TSS)	MLSS (g/L)
ALU	1400	7.0	10
Anoxic 1	580	5.5	8.5
Anoxic 2	292	2.7	6
Aerobic	104	0.84	5

Experiment 3 – Run 7
Inlet NH_3-N = 49 mg/L
COD added to the ALU: 1400 mg/L

Reactor Stages	Time (h)	Phosphorus Uptake (mg/L) (Mean)	NH_3-N (mg/L) (Mean)	NO_3-N (mg/L) (Mean)	NO_2^- (mg/L) (Mean)	COD (mg/L) (Mean)	DO (mg/L)
Anoxic	0	33	49	35	33	515	0
	30	25	41	26	27	453	0
	60	19	31	20	21	423	0
	90	16	25	12	16	378	0
	120	13	20	8	12	335	0
	150	9	17	6	9	310	0
Aerobic	180	7	12	4.3	17	287	2.8
	210	6	10	3.2	16	224	2.8
	240	3	11	2.5	14	203	2.8
	270	1	9.5	2.1	11	186	2.8
	300	0.5	8.2	2	9	166	2.8
	330	0	6.1	1.8	5	143	2.8
	360	-	5.2	1.7	2	132	2.8
	390	0	4.3	1.3	1.5	120	2.8
	420	-	2.1	0	0	114	2.8
	450	0	1.2	-	-	78	2.8

Experiment 3 – Run 8
Inlet NH_3-N = 80 mg/L
COD added to the ALU: 1400 mg/L

Reactor Stages	Time (h)	Phosphorus Uptake (mg/L) (Mean)	NH_3-N (mg/L) (Mean)	NO_3-N (mg/L) (Mean)	NO_2^- (mg/L) (Mean)	COD (mg/L) (Mean)	DO (mg/L)
Anoxic	0	33	80	43	56	558	0
	30	30	65	31	47	466	0
	60	24	52	22	39	408	0
	90	20	41	13	32	376	0
	120	16	34	7	26	336	0
	150	11	20	4	18	302	0
Aerobic	180	6	14	11	12	278	2.8
	210	3	9	12	19	250	2.8
	240	1	5	8	17	244	2.8
	270	0.2	2.3	5	16	229	2.8
	300	0	0.8	2	15	198	2.8
	330	0	0.1	1	11	176	2.8
	360	0	0	0.5	8	159	2.8
	390	-	-	0.4	7	123	2.8
	420	-	-	0	4	106	2.8
	450	0	-	-	-	88	2.8

Experiment 3 – Run 9
Inlet NH_3-N = 120 mg/L
COD added to the ALU: 1400 mg/L

Reactor Stages	Time (h)	Phosphorus Uptake (mg/L) (Mean)	NH_3-N (mg/L) (Mean)	NO_3-N (mg/L) (Mean)	NO_2^- (mg/L) (Mean)	COD (mg/L) (Mean)	DO (mg/L)
Anoxic	0	32	120	81	79	540	0
	30	31	104	65	72	415	0
	60	28	85	53	66	363	0
	90	27	71	40	56	314	0
	120	24	68	28	47	278	0
	150	22	52	19	41	229	0
Aerobic	180	18	37	18	31	181	2.8
	210	11	28	18	26	165	2.8
	240	4	20	16	22	147	2.8
	270	2	13	12	20	132	2.8
	300	0.3	8	11	18	120	2.8
	330	0	5	9	13	108	2.8
	360	-	3	6	10	95	2.8
	390	-	0.8	4	8	79	2.8
	420	-	0.3	3	5	63	2.8
	450	0	0.1	1	3	46	2.8