1-1-2010

Construction, Operation And Evaluation Of A Compact Upright Bioreactor For The Elimination Of Nutrients (CUBEN)

Maryam Reza
Ryerson University

CONSTRUCTION, OPERATION AND EVALUATION OF A COMPACT UPRIGHT BIOREACTOR FOR THE ELIMINATION OF NUTRIENTS (CUBEN)

by Maryam Reza
B. Eng in Chemical Engineering, Ryerson University, 2008

A thesis presented to Ryerson University
in partial fulfillment of the requirements for the degree of
Master of Applied Science (M.A.Sc.) in the program of
Chemical Engineering

Toronto, Ontario, Canada, 2010

Author's Declaration

I hereby declare that I am the sole author of this thesis.

I authorize Ryerson University to lend this thesis to other institutions or individuals for the purpose of scholarly research.

Maryam Reza

I further authorize Ryerson University to reproduce this thesis by photocopying or by other means, in total or in part, at the request of other institutions or individuals for the purpose of scholarly research.

Maryam Reza

ABSTRACT

Thesis Title: Construction, Operation and Evaluation of a Compact Upright Bioreactor for the Elimination of Nutrients (CUBEN)

Master of Applied Science, 2010

Maryam Reza

Chemical Engineering

Ryerson University

Eutrophication is reported as the most important water quality issue around the world. The potential death of Lake Winnipeg, the world's ninth largest lake, is a dramatic example of this ecological disaster in Canada. Property price devaluation, tourist repulsion, and toxicity due to eutrophication cause the annual economic losses over $3 billion in Europe, South and North America. The objective of this thesis is to develop an efficient biological nutrient removal reactor to be commercialized and used in the water/wastewater treatment industry. This bioreactor has a unique configuration which is filed as a US patent technology called "Compact Upright Bioreactor for the Elimination of Nutrients", invented by M. Alvarez Cuenca and M. Reza. It consists of four stages including Deaeration, Anoxic, Anaerobic and Aerobic where DO removal, denitrification and phosphorus removal processes take place respectively. The bioreactor performs very well obtaining 100% DO removal and 98% nitrate removal efficiency. The phosphorus removal process requires much longer operational period to reach steady state. The phosphorus removal process shows variable results having a maximum of 60% removal success.

ACKNOWLEDGEMENTS

I would like to express my heartfelt appreciation and sincere gratitude to Dr. Manuel Alvarez Cuenca, my supervisor and leader throughout the course of this work. I would like to thank Dr. Cuenca for giving me the opportunity to work on such an interesting and fruitful experimental research. The accomplishment of this thesis would have not been possible without his innovative ideas, dedicated supervisions and financial support. As a result of Dr. Cuenca's extensive knowledge on bioreactor design and many years of academic experience this thesis has been filed in the US patent office on August 6, 2010. I am also sincerely grateful to Dr. Cuenca for including me as the co-author of this US patent. This is a unique opportunity for any master student pursuing a successful career and Dr. Cuenca has given me this opportunity. I am and always will be thankful to him for all he has done for me.

I also, would like to extent my thanks to our chemical engineering technicians at Ryerson University, Mr. Tondar Tajrobehkar, Mr. Daniel Boothe and Mr. Ali Hemmati for constructing the bioreactor (CUBEN) and for their support during the troubleshooting of the unit.

I would like to express my appreciations to Dr. Martina Hausner, in the department of Biology/Chemistry and my dear sister, Mona Reza, research assistant and third year student in the biology program at Ryerson for providing us with microscopic analysis of the bioreactor's samples. This analysis took them a lot of effort, time and expertise which helped us to finalize this thesis.

Finally, I would like to thank my family specially my mother for her loving support that led me to pursue my degree and accomplish this work.

TABLE OF CONTENTS

TABLE OF FIGURES

LIST OF TABLES

LIST OF GRAPHS

1.0 INTRODUCTION

The excessive concentration of nutrients such as phosphorus and nitrate in the surface and ground water is currently one of the major environmental concerns. The high nutrient concentration in lakes, rivers and surface water in general causes severe reduction in water quality and is an important threat to aquatic ecosystem. Eutrophication is the result of excess concentration of nutrients which magnifies the growth of algae and plankton which in return disrupt the normal functioning of aquatic life. The uncontrolled growth of plankton, algae and other aquatic vegetation depletes the concentration of dissolved oxygen in the water. The low concentration of dissolved oxygen in the surface water within the range of 0 to 30% saturation causes a phenomenon called hypoxia. The lack of sufficient oxygen in water is detrimental to the aquatic lives of fish, marine mammals and many other organisms.

The human induced eutrophication, and consequently hypoxia, occurs mainly due to both agricultural runoff and to incomplete treatment of industrial, municipal and domestic wastewater which is discharged into lakes and rivers. The discharge of untreated wastewater with high nutrient concentration into the receiving body reduces the quality of the water and sustainability of reuse. The major sources of phosphorus and nitrates released into the surface water as well as ground water include agricultural practices, industrial waste and household's activities. The phosphorus release from human sources is due to the use of synthetic detergents, food waste, food additives and other household products. Also, the use of fertilizers in farming increases the phosphorus build up in the soil which is ultimately washed out into the ground water (Baetens, 2001).

The economic losses due to eutrophication have become a major issue for many countries around the world. The annual loss in USA is reported over $2.2 billion per year (Dodds *et al*., 2008), and $105-$160 million per year in England and Wales. In addition, the eutrophication of the Baltic Sea as well as the State of Sao Paulo in Brazil are other instances where the economic impact of eutrophication has been extremely high (Pretty *et al*., 2003). Figure 1.1 highlights and maps 415 eutrophic and hypoxic coastal areas worldwide in which 169 are hypoxic areas, 233 are areas of concern and 13 are areas in recovery from eutrophication (Selman *et al*., 2008). This figure illustrates the dramatic global deterioration of the ecosystems.

This worldwide issue must be comprehensively assessed, managed and reduced by governments and environmental organizations

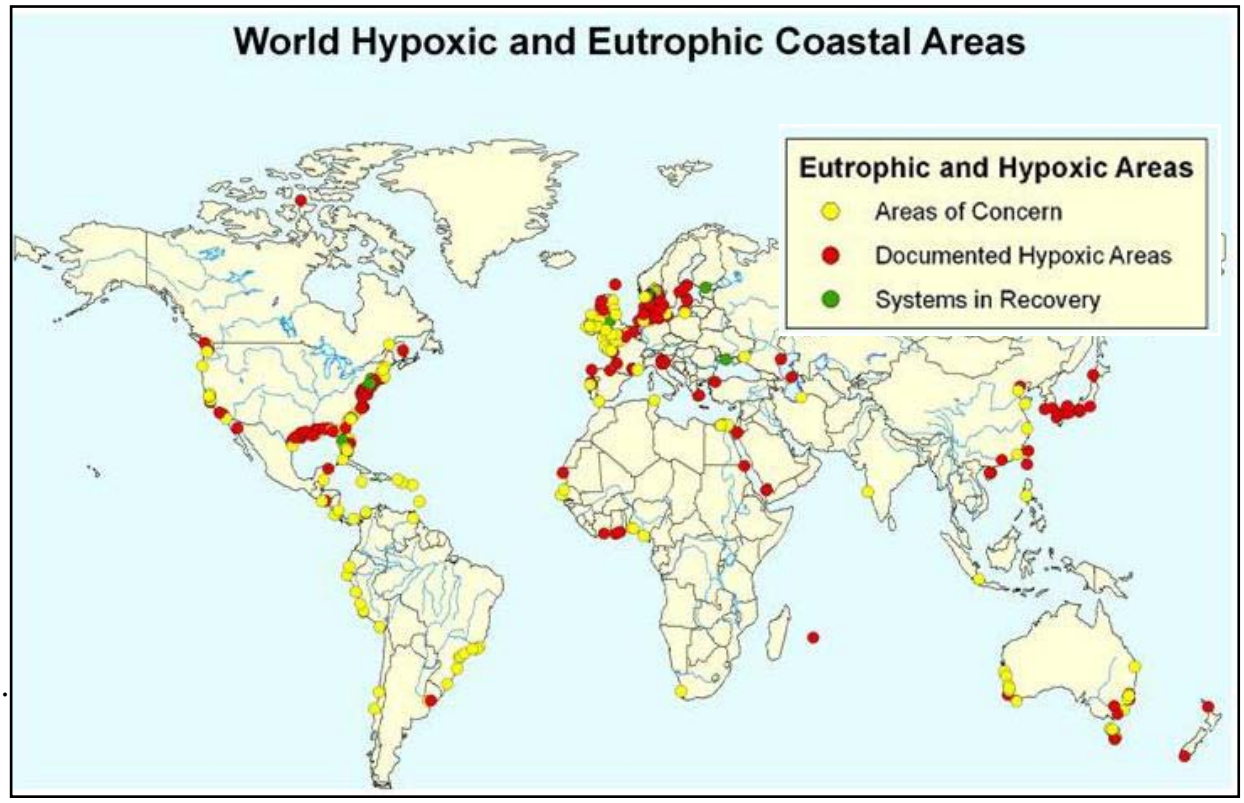


Figure 1.1: Coastal Hypoxic and Eutrophic Areas of the World (Selman *et al*., 2008)

The recovery of eutrophic water is a costly and long term process (McDonald, 2009). Only 50% reduction in total nitrogen and phosphorus concentration of dead zones costs over $3.86 and 0.436 billion US respectively. Therefore, the best solution to protect the water quality is to efficiently reduce the nutrient concentration from wastewater before it is released into the water environment. The world-wide need for the development and deployment of an efficient, reliable, cost-effective and compact technology for nutrient removal from water/wastewater is a fundamental issue. Accordingly, the objective of this thesis is to design, construct and operate an innovative biological nutrient removal reactor with smaller foot-print and higher nutrient removal efficiency compared to the conventional nutrients removal units. A full description of the technology has been filed with the US Patent Office ("Compact Upright Bioreactor for the Elimination of Nutrients" Inventors: M. Alvarez Cuenca and M. Reza, No. 26912, August 2010).

1.1 Nutrient Removal Processes

Nitrogen and phosphorus are the major nutrients causing water quality issues in which eutrophication is the most widespread and the biggest threat for the environment. Complete nitrogen removal involves two biological treatment processes including aerobic nitrification and anoxic denitrification. Normally, nitrification process which is conversion of ammonia (NH_3-N) to nitrite (NO_2^-) and Nitrate (NO_3^-) takes place in the secondary stage of wastewater treatment plants after or along with the BOD removal process. The effluent from secondary treatment contains mostly nitrate since nitrite is very unstable and normally is converted to nitrate. Further removal of nitrate requires an anoxic phase which occurs in the tertiary stage of the wastewater treatment plants. Under the anoxic condition nitrates are used by denitrifying bacteria as oxidants instead of dissolved oxygen to utilize organic matters in the water. This process is called Denitrification which can be carried out independently or in conjunction with phosphorus removal process.

Phosphorus in water or wastewater exists in either particulate phase or dissolved phase. Particulate phosphorus is insoluble in water and includes living and dead plankton and phosphorus adsorbed to particulate matters in the water. The dissolved phase includes inorganic phosphorus (orthophosphate, PO_4^{-3}, and polyphosphate) and organic phosphorus. A typical wastewater treatment plant with only secondary treatment removes about one-third of the influent total phosphorus by settling the insoluble phosphorus. Also, small portion of soluble phosphorus is removed during the secondary treatment by normal heterotrophic bacteria for their cellular growth. In order to remove soluble or dissolved phosphorus from wastewater advanced tertiary treatment must be performed. In the tertiary treatment stage, there are two ways to remove the dissolved phosphorus and reduce the effluent concentration of phosphorus to meet the discharge limit set and regulated by the Ontario Ministry of the Environment.

Traditionally, chemical addition techniques were used for phosphorus removal. These chemicals include metal salts such as $FeCl_3$, $FeSO_4$ or lime. The addition of metallic salts results in precipitation of metal-phosphorus compounds such as ferric phosphate ($FePO_4$), calcium phosphate ($Ca_3(PO_4)_2$) and struvite (NH_4MgPO_4). There are many disadvantages to chemical phosphorus removal including:

- Significant increase of the excess sludge production
- High cost of chemical addition

- Accumulation of ions may restrict the reuse of the effluent

Due to the large number of disadvantages associated to the chemical addition techniques more attention has been paid towards research on biological phosphorus removal and feasibility and optimization of this process in the last two decades. Normal microorganisms use small amounts of soluble phosphorus for their cell function and contain 1.9% phosphorus by weight. However, there are special types of bacteria called phosphorus accumulating organisms (PAOs) that have the ability to reserve phosphorus in their cells ranging from 5% up to 38% by weight when they are subjected to anaerobic and then aerobic conditions. The advantages of biological phosphorus removal include:

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

The disadvantages of the biological P removal involve high installation costs, complexity of operation and inability to achieve effluent phosphorus concentration lower than 0.5 mg/L. In general, when the Enhanced Biological Phosphorus Removal (EBPR) process is operated successfully, it has relatively lower operational costs and is an environmentally sustainable option for phosphorus removal compared to chemical removal techniques.

1.2 Status of Water Resources and Nutrient Removal Technologies in Canada

Canada holds over 20 per cent of the world's fresh water. Although, the need for electricity conservation and other sources of energy and power has become widely accepted among Canadians, unfortunately many people are not aware of the importance of water conservation. Average household consumption in Canada is about 330 liters per day which according to MACLEAN's magazine (Nancy MacDonald, 2009) this amount is more than twice that in Europe. Water and wastewater treatment is very expensive even for a country like Canada with so much water resources. If Canadians would know the fact that producing only five minutes of clean tap water is equivalent to the energy requirement to have 14 hours of light from 60Watt light bulb, they would treat water as a valuable resource.

According to Water and Wastewater Digest magazine (Schici, 2009) approximately 25% of all water pollution problems are due to nutrient related causes. Enrichment of nutrients in water is mainly caused by human activities including household usage and agricultural practices. The presence of excess nutrients in lakes and rivers is a hazardous threat for human, animal and aquatic life which needs to be carefully studied and proper measures must be taken by government officials around the globe. Wastewater treatment in general and nutrient removal from wastewater in particular is a domestic and international environmental concern. Therefore, major international agencies and organizations have invested billions of dollars for development of better and more efficient technologies in this regard. Nutrient removal is one of the most significant challenges facing both developing and developed countries like Canada due to high cost of advanced treatment. For instance, the World Bank estimates that $600-$800 billion is needed to invest on water and wastewater treatment including nutrient removal processes (Industry Canada, 2003).

Canada has over 700 small and medium-sized water and wastewater facilities with annual sales of approximately $1.4 billion. Canada is still one of the world leaders in research and development of innovative technologies in water and wastewater treatment field. These technologies, products and services include (Industry Canada, 2003):

- Ultra-violet Disinfection (for removal or inactivation of pathogens)
- Membrane Technology (water filtration)
- Biological Nutrient Removal (denitrification and phosphorus removal process)

- Anaerobic Treatment (of sludge from municipal and industrial wastewater)
- Ion Exchange
- Wet Air Oxidation
- Biosolids Treatment
- Water Information Systems and Software
- Wetland Technologies (for natural eco-system remediation)

Table 1.1 shows the environmental market in which water and wastewater constitute the second largest components of the global market.

Segment Share	Percent
Water and Wastewater	39%
Waste Management	40%
Air Pollution Control	6%
Consulting	6%
Remediation	3%
Other	6%

Table 1.1: Global Market in the Environmental Industry (Industry Canada, 2003)

Although, Canada has participated in the technology development of many environmental processes, the commercialization and application of these technologies is poor. For instance, only 151 out of 738 wastewater treatment plants across the country accomplish nutrient removal process before discharging the effluent into the surface water (National Survey of Wastewater Treatment Plants, 2001). Alberta is performing quite well compared to the other regions in the country. There are about 10 wastewater treatment plants with biological nutrient removal (BNR) reactors in western Canada with various capacities ranging from 2000 to 500,000 (m^3/day) (Oldham *et al*., 2002). However, Canada's eastern provinces still use conventional wastewater treatment processes and technologies. In order to meet the stringent discharge limits, many wastewater treatment plants in eastern provinces like Ontario use chemical treatment for the removal of phosphorus as oppose to advanced biological nutrient removal.

Based on the National Sewage Report Card prepared in 2004, the Canadian cities were graded for their performance level of municipal wastewater treatment plants. The following table

(Table 1.2) shows the grades for all major cities in which the city of Calgary provides the best wastewater treatment level and Montreal is graded as the lowest among the rest.

CITY	SUMMARY	2004 GRADE
Calgary	Upgraded to 100% tertiary treatment and UV disinfection	A^+
Edmonton	Upgraded to 100% tertiary treatment and UV disinfection	A^-
Halifax	More than 65 billion liters of raw sewage discharged into the surface water each year.	D
Hamilton	Upgraded to secondary and tertiary treatment. This city discharges 5.9 billion liters of raw sewage each year.	C^+
Montreal	Primary treatment only. No discernible progress made.	F
Ottawa	Secondary treatment with seasonal chlorine disinfection	B^+
Quebec City	Secondary treatment with seasonal UV disinfection.	B
Saskatoon	100 % secondary treatment. Minimal changes since 1999.	C^+
Regina	Enhanced secondary treatment with expanded UV disinfection.	B^+
Toronto	Secondary treatment. The city discharges 9.9 billion liters of untreated sewage and run-off into the lakes and rivers.	B^-
Vancouver	100% secondary treatment upgrades won't be completed until 2030.	D
Winnipeg	100% secondary treatment. One billion liters of combined sewer overflow per year.	B^-
Victoria	Preliminary screening, no treatment. More than 34 billion liters of raw sewage is discharge into surface water each year.	Suspended

Table 1.2: National Sewage Report Card III (2004)

The above table shows lack of proper water/wastewater management and high demand for development of more efficient pollutant removal processes and technologies in Canada. The urban population growth and the lack of sufficient and efficient wastewater and storm water treatment plants have contributed to beach closures, fishery restrictions, degraded aquatic environment, elevated nutrient level and contaminated sediments in Ontario and other eastern provinces of Canada. Most of the storm water and sewage overflows are discharged into rivers, creeks and Lake Ontario which contains high levels of bacterial and nutrients and organic chemical contaminants.

1.3 Thesis Overview

The aim of the study presented in this thesis was to built, commission and evaluate an advanced technology for biological nutrient (phosphorus and nitrate) removal from wastewater. The detailed design of this unit required a broad and thorough review, including patent reviews, of existing biological nutrient removal processes as well as study of specialized nutrient removal microorganisms, their biochemical metabolisms and kinetics. The literature review section of this thesis summarizes the important facts and information about BNR.

This new technology called CUBEN (Compact Upright Bioreactor for the Elimination of Nutrients) is based on well recognized nutrient removal processes such as separate denitrification for nitrate removal and Anaerobic/Aerobic (A/O) process for biological phosphorus removal. The separate denitrification and A/O process along with other types of BNR processes will be explained in more details in the later sections. CUBEN is designed to remove nutrients more efficiently than conventional technologies currently installed in water/wastewater treatment plants. The pilot unit built in the Laboratory of Water Treatment Technologies at Ryerson University can be simply scaled up and installed after the secondary treatment section of actual wastewater treatment plants. The enlargement of this unit is simple since it can be expanded vertically by adding more aerobic, anaerobic or anoxic stages. CUBEN has a small footprint due to its vertical configuration which is a great advantage since many treatment facilities have land limitations.

Table 1.3 presents the effluent concentration requirement for a wastewater treatment plant with advanced treatment level (tertiary treatment) in Ontario based on the MOE Policy Guidelines 08-01. Nutrient standards may range from 1 mg/L to 0.1 mg/L depending on the receiving water. The design of the CUBEN aims to meet these requirements for future commercial applications of this unit.

Wastewater Constituents	WWTP Effluent Concentration (mg/L)
BOD_5	10
TSS	5
TP	0.3
TKN	< 1.0

Table 1.3: Effluent Concentration Requirement for WWTP in Ontario (Determination of Treatment Requirements, MOE Policy Guidelines 08-01)

LITERATURE REVIEW

2.0 BIOLOGICAL NUTRIENT REMOVAL PROCESSES

Biological nutrient removal (BNR) processes provide nitrogen and phosphorus removal by incorporating anoxic, anaerobic and aerobic conditions for microorganisms to carry out cellular metabolism in response to their specific environment. An anoxic stage contains water/wastewater with nitrate (NO_3^-) as electron acceptor instead of dissolved oxygen. Therefore, this stage is concentrated with NO_3^- and contains very low or no dissolved oxygen (DO) concentration. An anaerobic stage has neither NO_3^- nor DO in the wastewater. In conventional wastewater treatment plants (WWTP), the removal of nutrients occurs after secondary treatment. That is, after the elimination of most of the carbon and ammonia. These processes are followed by both denitrification to eliminate the nitrates, and by phosphorous removal. In all BNR plants in operation, the processes take place in rectangular, horizontal tanks. CUBEN is a major departure from that configuration.

2.1 Denitrification Process

Denitrification is the reduction of NO_3^- to N_2 by certain heterotrophic bacteria commonly named denitrifiers. The denitrification process requires anoxic conditions with sufficient amount of carbon source. Anoxic conditions refer to the presence of combined oxygen in the form of nitrate, nitrite and sulfate and absence of dissolved oxygen. A properly designed anoxic zone allows the proliferation of denitrifying bacteria. Denitrifiers are heterotrophic bacteria that use nitrate/nitrite as electron acceptor in the absence of molecular oxygen. There are large numbers of bacterial genera in wastewater which are capable of denitrification and include *Achromobacter, Aerobacter, Alcaligenes, Bacillus, Flavobacterium, Micrococcus, Proteus* and *Pseudomonas*. There is uncertainty regarding the fraction of heterotrophic bacteria that can denitrify in a nutrient removal reactor, however, evidence has shown that the introduction of wastewater into an anoxic stage will give a competitive advantage to denitrifying bacteria over other heterotrophic bacteria community (Grady *et al*., 1999).

The following flow diagram shows nitrogen transformations in biological wastewater treatment. As it can be seen from Figure 2.1, denitrification completes the nitrogen cycle by returning nitrogen gas (N_2) to the atmosphere.

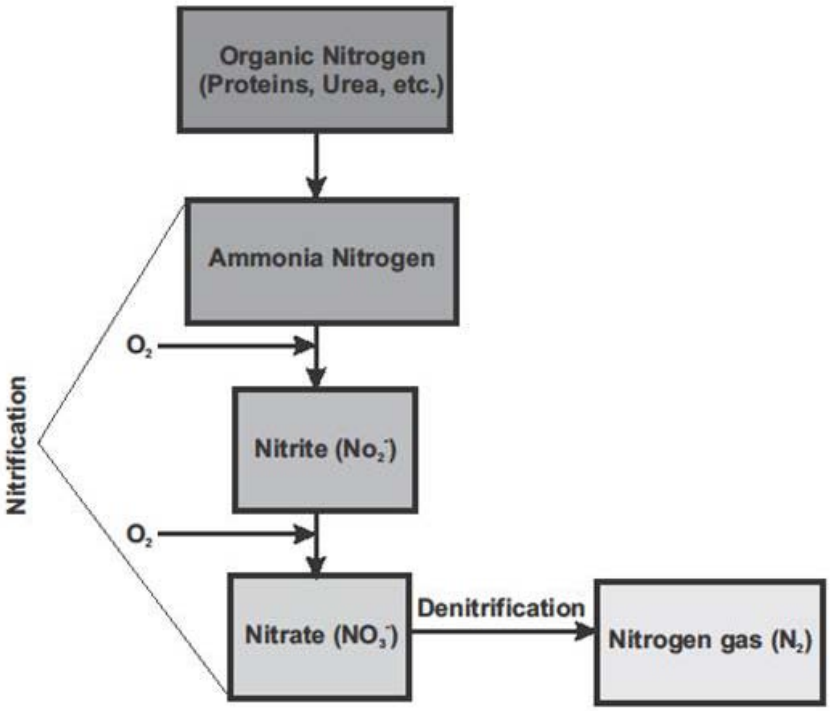


Figure 2.1: Nitrogen Transformation in Biological Wastewater Treatment (Metcalf & Eddy, 2003)

The following reaction shows the conversion of nitrate to nitrogen gas:

NO_3^- + Organic Matter (i.e.BOD) $\longrightarrow$ N_2 (gas) + CO_2 + H_2O + OH^- + New Cells [Eq. 2.1]

Organic matter is used by denitrifying bacteria as carbon and energy source. The type and amount of organic matter is a very important factor in the denitrification rate. The organic compounds that improve the denitrification process include methanol (CH_3OH) and Volatile Fatty Acids (VFA) (Jeyanayagam, 2005). Table 2.1 lists some of the common nitrogen/nitrate removal processes currently being used in wastewater treatment plants.

Nitrate removal processes	Post-Anoxic Denitrification
	Pre-Anoxic Denitrification or Modified Ludzak-Ettinger (MLE)
	Four-Stage Bardenpho
	Nitrox™
	Bio-denitro™
	Step-feed activated sludge process (SFAS)

Table 2.1: Various Nitrate Removal Processes

2.1.1 Post-Anoxic Denitrification Process

This process involves one aerobic and one anoxic horizontal reactor in series followed by a secondary clarifier. Denitrification takes place after nitrification and often an electron donor such as methanol or acetate is added in proportion to the influent to enhance both processes. The Solid Residence Time (SRT) for such a system is typically between 3 to 5 days. The anoxic hydraulic residence time (HRT) of this process can be in the range of 2 to 4 hours and the aerobic HRT is typically 1 hour.

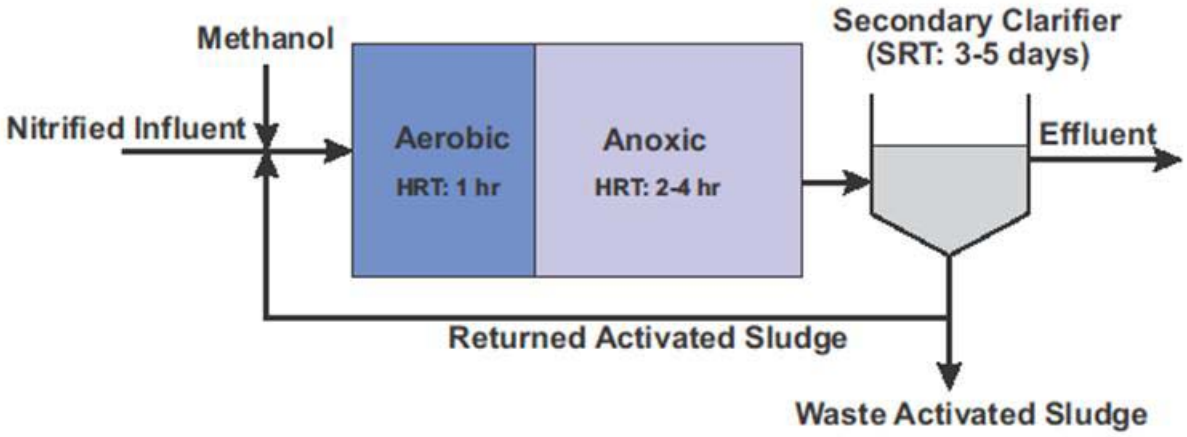


Figure 2.2: Post-Anoxic Denitrification Process (Metcalf & Eddy, 2003)

2.1.2 Pre-Anoxic Denitrification or Modified Ludzak-Ettinger Process (MLE)

The MLE process is the most common process used for biological nitrogen removal in wastewater treatment plants. The MLE process consists of anoxic reactor followed by an aerobic reactor where nitrification takes place. Nitrate produced in the aerobic stage is recycled back into the anoxic stage. The organic substrate in wastewater is used for denitrification. The MLE process is called substrate denitrification since no external carbon source is required. The MLE process is also called pre-anoxic denitrification since anoxic stage precedes the aerobic stage (Metcalf & Eddy, 2003). This process represents one of the simplest systems within which both nitrification and denitrification take place in different stages. In this system, both wastewater and recycled biomass enter the anoxic stage with a very low dissolved oxygen and high nitrate concentration. The internal recycle flow ratio (recycle flowrate/influent flowrate) is in the range

of 2-4. The following bioreactor is an aerobic stage with a dissolved oxygen concentration of approximately 2.0 mg/L.

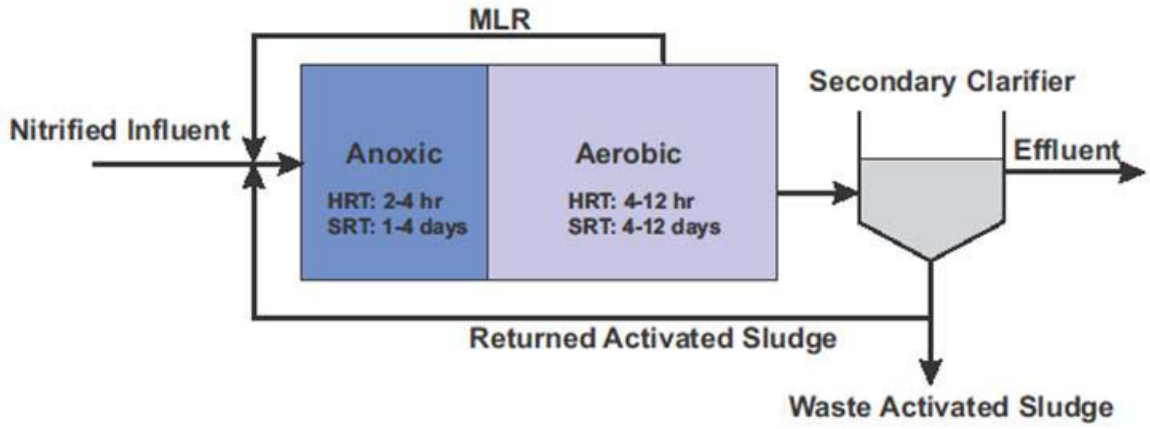


Figure 2.3: Modified Ludzak-Ettinger Process (Grady *et al*., 1999)

2.1.3 Four-Stage Bardenpho Process

The 4-stage Bardenpho process incorporates both pre-anoxic and post-anoxic denitrification. This process was modified later to include biological phosphorus removal process as well. The Modified Bardenpho process will be explained in more details in later section.

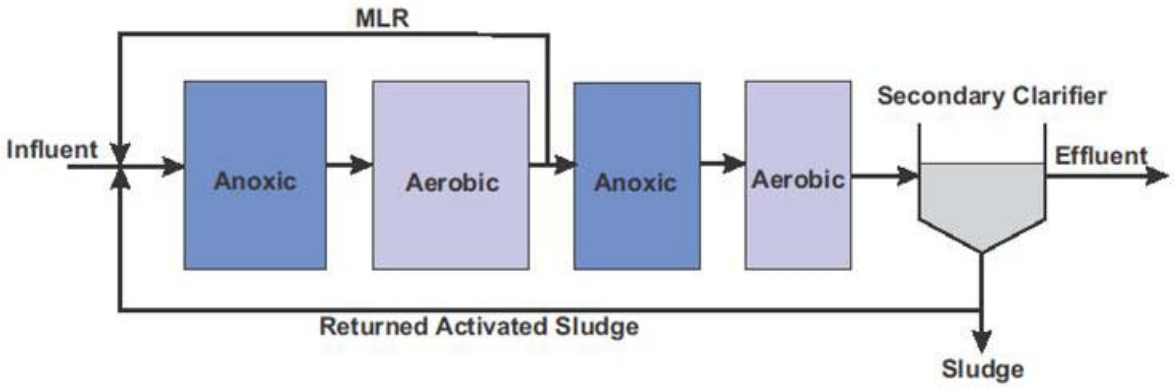


Figure 2.4: 4-Stage Bardenpho Process (Metcalf & Eddy, 2003)

2.2 Biological Phosphorus Removal

Phosphorus is a macro-nutrient required by all living cells. It is absorbed by microorganisms in the form of orthophosphates to form organic phosphates in order to build the cell structure. It is also an important part of Adenosine Tri-Phosphate (ATP) which is the energy current of all cells. The phosphate bonds in ATP are high-energy bonds and their formation and hydrolysis is the primary means by which cellular energy is stored and released (Shuler *et al*., 2002). Phosphate is also an important component of nucleic acids (DNA and RNA) and phospholipids in cell membrane. Therefore, scientists could use the concept of phosphate necessity in living cells to develop certain processes to eliminate the excess phosphorus from the environment particularly from surface water. The increase in the concentration of phosphorus in lakes and rivers causes eutrophication which is the adverse response of an ecosystem to excess nutrients. The phosphorus compounds that are considered pollutants include orthophosphates, organic phosphates and poly-phosphates. The average concentration of phosphorus both inorganic and organic in wastewater is within the range of 5 to 20 mg/L (Scheer *et al*., 1997). Several mechanisms have been proposed to explain the biological removal of phosphorus by microorganisms in wastewater treatment plants. To remove the phosphorus, biomass suspended in wastewater must first be subjected to an oxygen and nitrate free environment (Anaerobic) where no electron acceptor is present. The concept of phosphorus removal and the function of phosphorus accumulating organisms (PAOs) will be explained in more detail in the following sections.

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

Over the past two decades, various biological phosphorus removal process configurations have been developed, modified and used in wastewater treatment industry. They all consist of anaerobic and aerobic stages as well as anoxic stages if phosphorus removal and denitrification are combined. Table 2.2 shows some of the most common biological phosphorus and combined phosphorus and nitrate removal processes.

Phosphorus removal	Phoredox (A/O)
	Phostrip (combined chemical and biological phosphorus removal)
Combined nitrate and phosphorus removal processes (Enhanced biological nutrient removal)	A^2/O Process
	Modified Bardenpho (5-stage)
	Standard and Modified UCT

Table 2.2: Various Phosphorus Removal Processes

Some of the above phosphorus removal processes are described below:

2.2.1 Phoredox (A/O) Process

The term A/O stands for anaerobic and oxic (aerobic) which represent the sequence of these phases in this process. This is the basic process configuration for biological phosphorus removal which was first identified by Barnard in 1974 and then patented by Air Products and Chemicals Inc. This is a phosphorus removal sequence that is going to be used in the CABEN reactor.

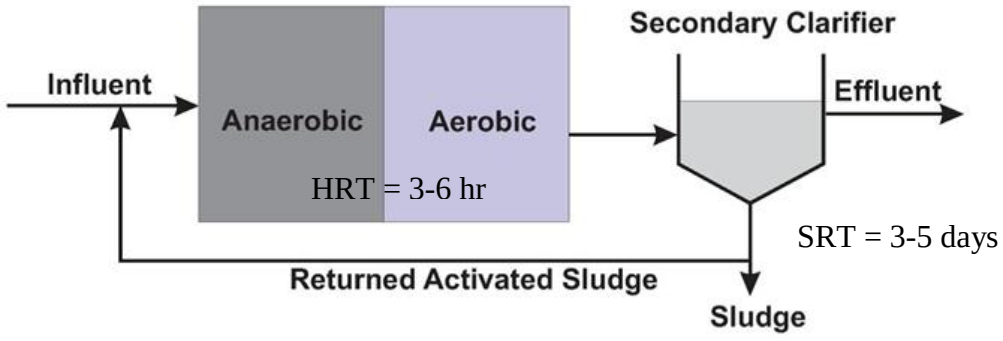


Figure 2.5: Phoredox (A/O) Process (Metcalf & Eddy, 2003)

2.2.2 A^2/O Process

The term A^2/O stands for anaerobic, anoxic and oxic (aerobic) bioreactor in sequence. It is a combination of 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 process. However, the phosphorus removal efficiency is lower than A/O.

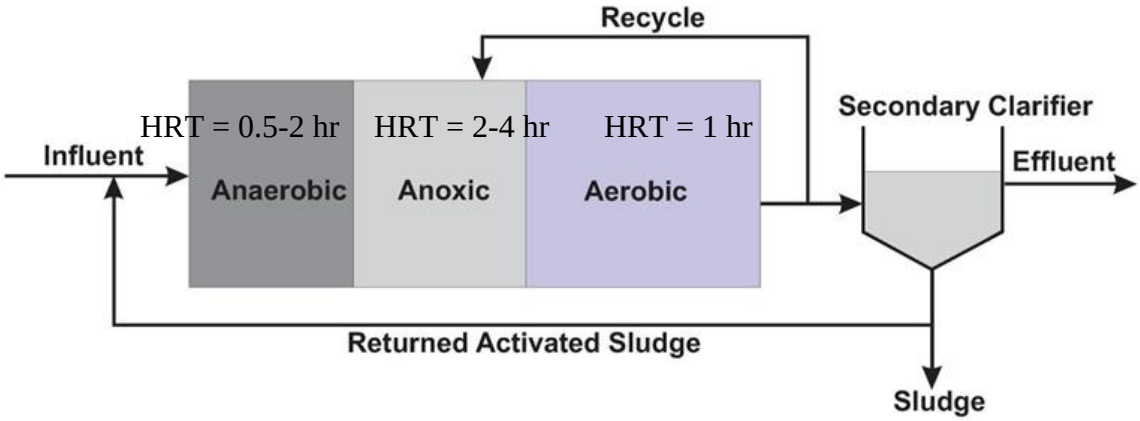


Figure 2.6: A2/O Process (Metcalf & Eddy, 2003)

2.2.3 Modified Bardenpho Process

This process is used for the removal of both phosphorus and nitrogen. The 5-stage system provides anaerobic, anoxic, and aerobic stages for phosphorus, nitrogen and carbon removal. The sequences of these stages are shown in figure 2.5.

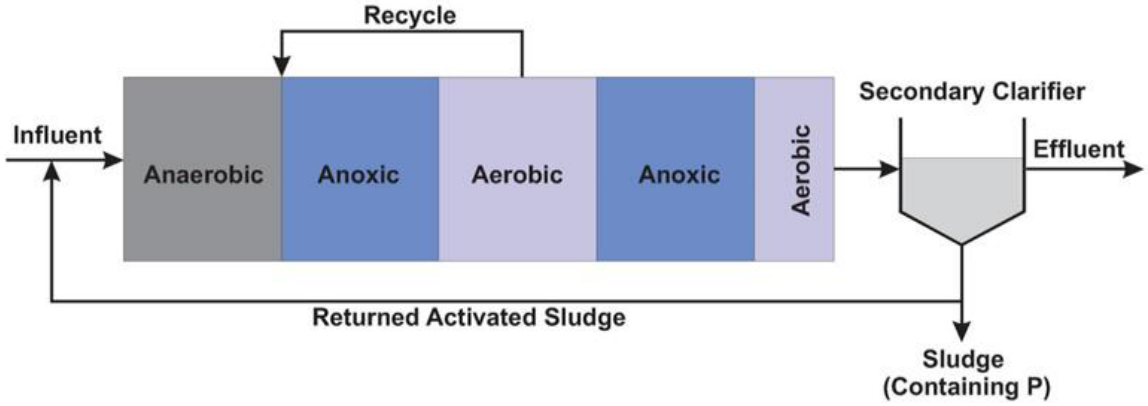


Figure 2.7: Modified Bardenpho Process (Metcalf & Eddy, 2003)

The modified Bardenpho process is very similar to the 4-stage Bardenpho process with an anaerobic stage added to achieve phosphorus removal. This process has several limitations which include high surface requirement, complicated design and control as well as moderate phosphorus removal efficiency.

2.2.4 UCT (Standard and Modified)

The UCT process stands for university of Cape Town process where it was developed. The standard UCT process is very similar to the A^2/O process with two exceptions:

1. The returned activated sludge is recycled to the anoxic stage instead of the anaerobic stage
2. The internal recycle is from the anoxic stage to the anaerobic stage

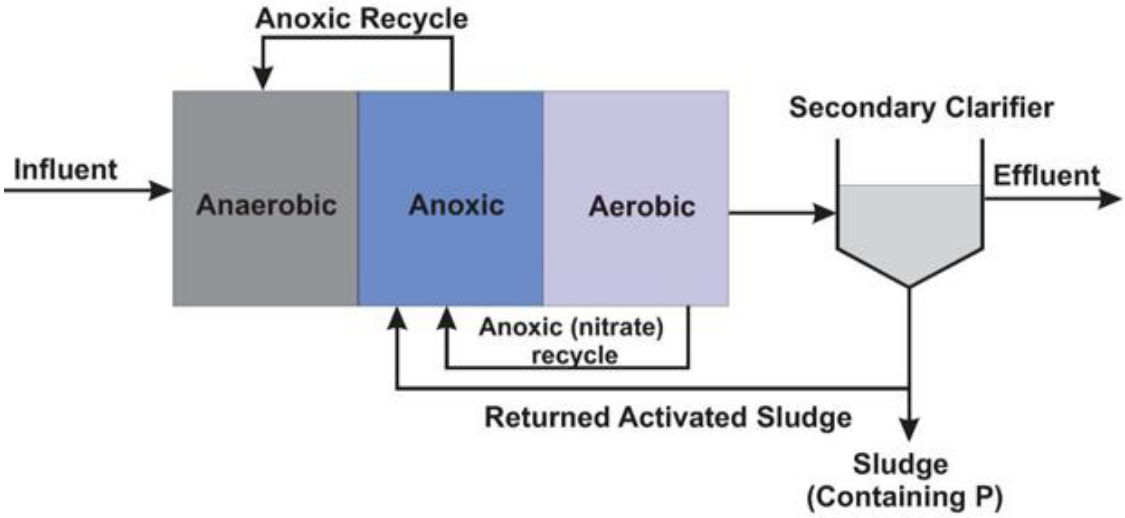


Figure 2.8: Standard UCT Process (Metcalf & Eddy, 2003)

In the Modified UCT process, the return activated sludge is directed to an anoxic reactor that does not receive internal nitrate recycle flow. The second anoxic reactor is used to receive internal nitrate recycle flow from the aerobic zone to provide a better denitrification for this system. Although Modified UCT process performs well in removing nitrogen and phosphorus, it is very difficult to monitor and control the processes taking place inside all bioreactors.

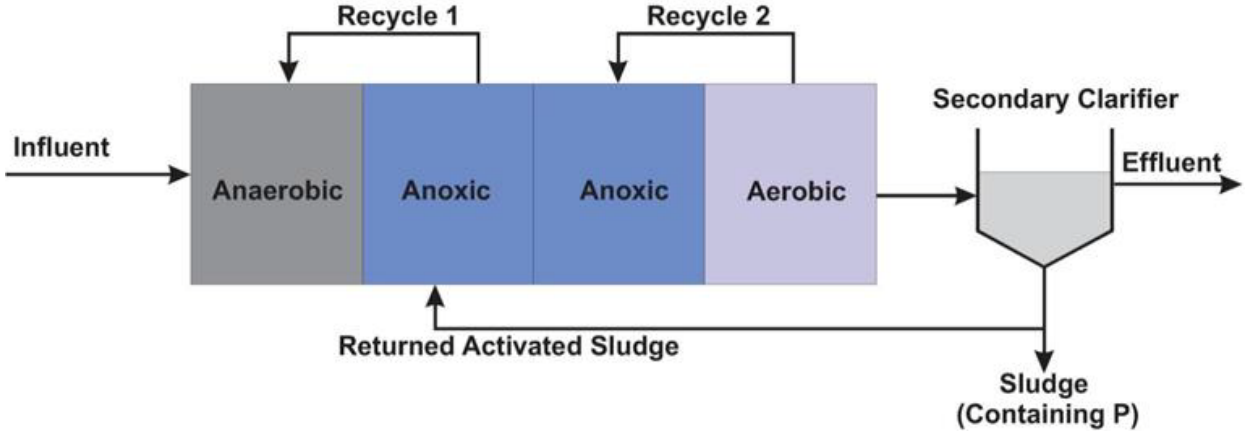


Figure 2.9: Modified UCT Process (Metcalf & Eddy, 2003)

2.3 Advantages and Disadvantaged of the Commercial BNR Processes

The use of the aforementioned BNR processes in wastewater treatment plants depends on many factors including target effluent quality, influent quality, operators experience and available funding. The following tables are summary of the advantages and drawbacks of the BNR processes explained in previous sections. The performance of all the BNR processes is site-specific and thus the tables below provide a general comparison of treatment performance of various BNR configurations.

Nitrate Removal Processes		
Process	**Advantages**	**Disadvantages**
Post-Anoxic Denitrification	• Excellent nitrogen removal • Minimum reactor volume	• Required upstream denitrification • Supplemental electron donor required • High energy requirement
Pre-Anoxic Denitrification (MLE)	• Good nitrogen removal • Moderate reactor volume • Good solid settleability • Reduced oxygen requirement • Simple control • Alkalinity recovery	• Nitrogen removal capability is a function of internal recycle • Potential Nocardia growth problem • DO control is required before recycle
Bio-denitro™	• 5-8 mg/L TN is achievable	• High construction cost (since two oxidation ditch reactors are required) • Complex operation
Nitrox™	• Easy and economical to upgrade the system	• Nitrogen removal capability is limited by higher influent TKN concentrations • Process is susceptible to ammonia bleed-through
4-Stage Bardenpho	• Capable of achieving effluent TN level less than 3 mg/L	• Large reactor volumes and footprints are required • Second anoxic zone has low efficiency

Table 2.3: Advantages and Drawbacks of the Biological Nitrogen Removal Processes (Metcalf & Eddy, 2003) & (Grady *et.al*., 1999)

Phosphorus Removal Processes		
Process	**Advantages**	**Disadvantages**
Phoredox (A/O)	• Simple operation • Low BOD/P ratio • Short HRT • Good phosphorus removal	• Phosphorus removal declines if nitrification occurs • Limited process control flexibility
Pho-Strip	• Can be incorporated easily into existing activated sludge plants • Process is flexible • phosphorus removal performance is not controlled by BOD/phosphorus ratio	• Required lime addition for phosphorus precipitation • Additional tank capacity required for stripping lime

Table 2.4: Advantages and Drawbacks of the Biological Phosphorus Removal Processes (Metcalf & Eddy, 2003) and (Grady *et al*., 1999)

Combined Biological Phosphorus and Nitrogen Removal Processes		
Process	**Advantages**	**Disadvantages**
A^2/O	• Removes both nitrogen and phosphorus • Produces good settling sludge • Simple operation	• Nitrogen removal is limited by internal recycle ratio • Needs higher BOD/P ratio compare to A/O process • Moderate phosphorus removal
Modified Bardenpho (5-stage)	• • Produces good settling sludge • Can achieve 3 to 5 mg/L TN in unfiltered effluent	• Less efficient phosphorus removal compared with A/O or A^2/O • Requires larger tank volume
Standard and Modified UCT	• Good nitrogen and good phosphorus removal • Produces good settling sludge • Nitrate loading on anaerobic zone is reduced, thus increasing phosphorus removal	• More complex operation • Required additional recycle stream

Table 2.5: Advantages and Drawbacks of the Combined Biological Phosphorus and Nitrogen Removal Processes (Metcalf & Eddy, 2003) and (Grady *et al*., 1999)

2.4 Phosphorus Accumulating Organisms (PAOs)

PAOs are able to take up high amounts of phosphorus beyond those required for normal cell growth and repair. This is partially due to their higher energy requirement to accomplish the cyclical chain reactions compared to normal heterotrophic bacteria. PAOs are often present in wastewater treatment undergoing activated sludge processes; however, they only develop the ability of phosphorus removal when they are subjected to alternating strictly anaerobic phase and aerobic or anoxic phase. During the anaerobic phase, PAOs take up and store easily biodegradable organic matters and convert them into a carbon polymer called PolyHydroxyAlkanoates (PHAs) mainly in the form of Poly-beta-Hydroxybutyrate (PHB) and Poly-beta-Hydroxyvalerate (PHV). The energy to uptake acetate and convert and store it as PHAs is obtained partially from the glycogen break down as well as hydrolysis of energy rich polyphosphate (Poly-P) into orthophosphate and finally hydrolysis of Adenosin Triphosphate (ATP) to Adenosin Diphosphate (ADP) and Adenosin Monophosphate (AMP). The hydrolysis of ATP and ADP are shown in the following equations (Baetens, Daniel., 2000-2001):

$$ATP + H_2O \longrightarrow ADP + P_i + H^+ + \text{energy} \qquad \Delta G^o = -310 \text{ kJ/mol} \qquad \text{[Eq. 2.2]}$$

$$ADP + H_2O \longrightarrow AMP + P_i + H^+ + \text{energy} \qquad \Delta G^o = -318 \text{ kJ/mol} \qquad \text{[Eq. 2.3]}$$

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

Under the anaerobic conditions, PAOs take up the easily biodegradable matters and convert them into PHB/PHV with concurrent release of ortho-phosphate into the surrounding water. The release of ortho-phosphate is due to the phosphorus concentration difference between inside and outside their cells. The concentration of phosphorus is high inside the PAO cell due to ATP hydrolysis and polyphosphate breakage compared to low phosphorus concentration in the water surrounding the cell. This concentration difference results in the release of ortho-phosphates from higher concentration (inside PAO cell) to bulk solution (low phosphorus concentration).

When PAO community leaves the anaerobic phase and enters the aerobic stage, they oxidize and utilize their cellular reserved PHAs and uptake phosphorus from water (both phosphorus content in the influent and phosphorus that is being released under anaerobic condition). 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 2.10 shows a close up photo of PAOs in the anaerobic (left) and aerobic (right) phases. The black stains display the phosphate contents of the PAO cells. This figure provides an excellent comparison of PAO cells in the influent and effluent using A/O configuration. The dark cells in the aerobic zone show high capability of PAOs in removing phosphorus from wastewater.

Figure 2.10: The Actual PAOs in the Anaerobic and Aerobic stages (Protecting our future, The City of Calgary Wastewater)

On account of the capabilities of PAOs to uptake organic materials in the anaerobic phase and store them as PHB or PHV, they have gained a competitive advantage over regular microorganisms. Under proper conditions, which include complete an anaerobic phase followed by an aerobic phase and addition of proper organic carbon source in the anaerobic zone, PAOs are capable of accumulating phosphorus in their cell up to 38% of their cell mass. In comparison normal heterotrophic bacteria are only able to store phosphorus for about 2.5% which is considerably lower than PAOs. Morphological characteristics of PAOs are described as non-motile rods or cocci, usually exist in clusters, are PHB staining positive, and contain Neisser positive granules in the cells. First, they were believed to be gram negative but later a possibility has arisen that they are gram positive (Mino, 1998). Some of the bacteria recognized as PAOs until now include: *Pseudomonas putida GM6* (high phosphate accumulating ability and rapid recovery from deteriorated system), *Candidatus Accumulibacter phosphate, Acinetobacter, Microlunatus phosphovorus strain NM-1, Pseudomonas sp, Propionibacter pelophili*.

Figure 2.11 illustrates the concentration profile of phosphorus, acetate, glycogen and PHA in the PAO cells as well as the bulk solution in anaerobic and aerobic phases.

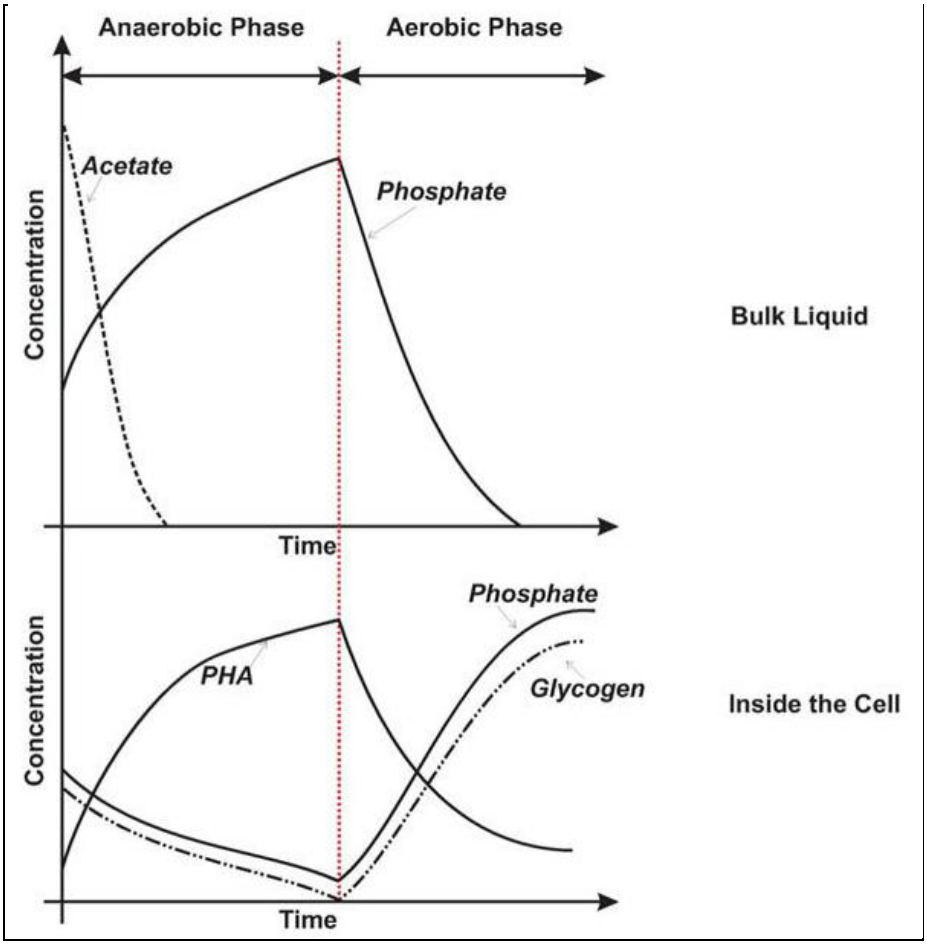


Figure 2.11: Concentration Profiles of Phosphate, Acetate, PHA and Glycogen under Anaerobic-Aerobic Conditions (Baetens, 2001)

Based on figure 2.11, the phosphorus concentration profile increases in the bulk liquid under anaerobic phase and then decreases to a very low level in the aerobic phase. In the anaerobic stage, PAOs release phosphorus into the bulk solution (increase the phosphorus concentration in the wastewater) while taking up all the acetate from wastewater. In this stage, PAOs store the carbon source as PHAs inside their cells while using the cell's glycogen storage (reduce 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 orthophosphates from the bulk solution and reserve them internally as polyphosphates.

2.5 Biochemical Mechanisms of PAOs

Many different biochemical mechanisms have been postulated to define PAO's behavior and functioning in the anaerobic and aerobic phases. Wentzel *et al.* (1991) pointed out two possible biochemical models to explain the source of the reducing power, the Mino model and the Comeau-Wentzel model. These two models form the basis of most of the research papers up until now.

Before explaining these models, it is important to mention that fermentation reaction occurs in the anaerobic phase by facultative heterotrophs. The carbon compounds that already exist in the wastewater or being added in the anaerobic phase are transformed into short chain fatty acids and then ate converted to acetate. According to some scientists the transport of acetate through the cell membrane of PAOs is an active transport which means energy is required for this transportation. For example, some scientists consider that acetate is transported across the cell membrane using 0.5 mole ATP (Baetens, 2001). On the other hand, some have proposed the passive diffusion of all short chain fatty acid across the cell membrane as a preferred mechanism. Initial models such as the Comeau-Wentzel and the Mino models relied on the active transport and most recent models account for passive transport. No conclusive answer is proposed and this concept is still under research and investigation. All models contain valuable information; therefore, old models although are not accepted completely will be explained in this section.

2.5.1 Comeau-Wentzel Model

Based on this model, acetates which are formed as a result of fermentation by heterotrophic microorganism under anaerobic condition passe through the PAO's cell membrane and get 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 hydrolysis of ATP to ADP. The PAO cell responds to the decrease in ATP/ADP ratio therefore re-synthesize ATP from break down of internal polyphosphates. About 90% of the acetyl-CoA is converted into stored PHB or PHV. The remainder of acetyl-CoA is metabolized through the TriCarboxylic Acid (TCA) cycle to provide the reducing power ($NADH^+ H^+$) for the synthesis of PHB/PHV. The breakdown of the intracellular polyphosphates increases the concentration of orthophosphates inside the cell which

are released to the bulk solution (Grady, Leslie *et al*., 1999). The overall reducing power ($NADH_2$) production in the TCA cycle can be written as (Baetens, Danielle, 2001):

Acetyl-CoA + 0.5 ATP + H2O ⟶ 2NADH2 + CO2 [Eq. 2.4]

Figure 2.12 illustrates the Comeau-Wentzel model under anaerobic condition.

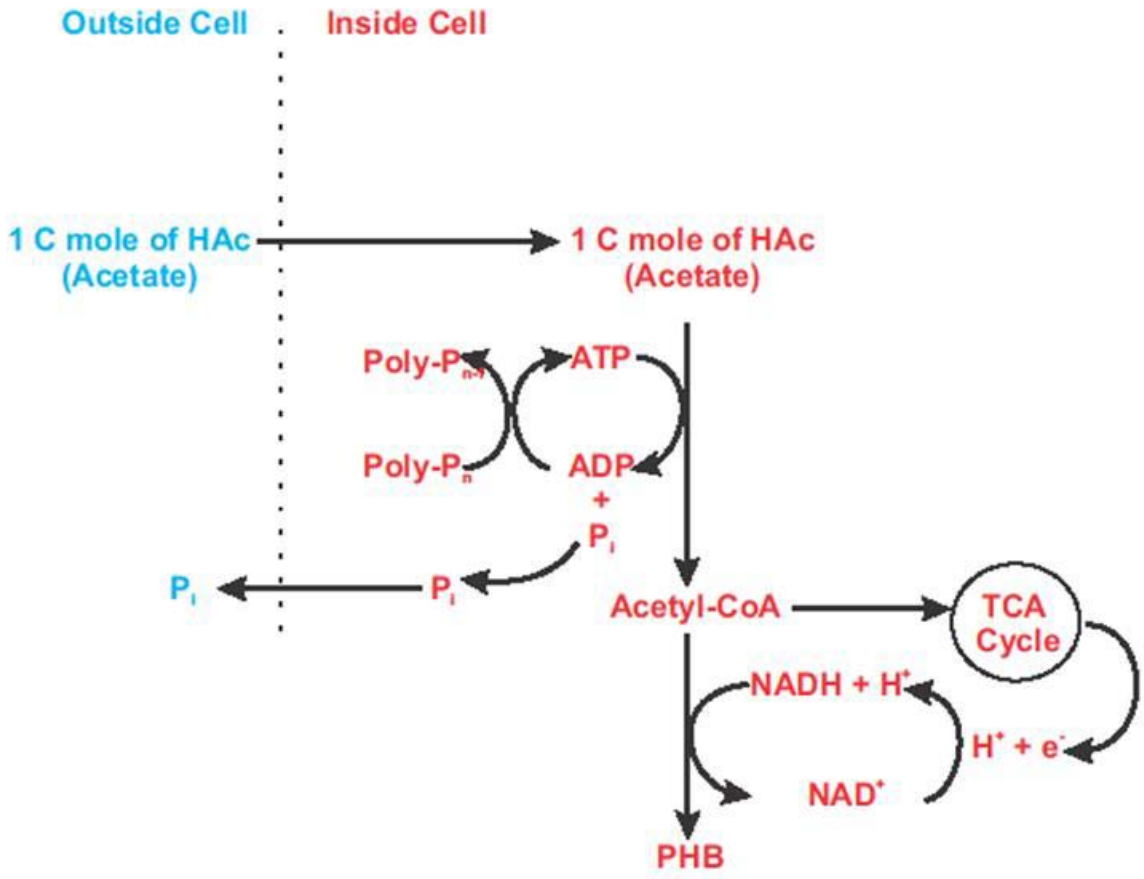


Figure 2.12: Comeau-Wentzel Model for the PAO's Anaerobic Metabolism (Grady *et al*., 1999)

When the wastewater and the suspended biomass enriched with PAOs enter the aerobic zone, the PAOs perform normal aerobic metabolism for growth by using the stored PHB and PHV as their carbon and energy source to generate ATP through electron transport chain. When the amount of ATP increases, then the polyphosphate synthesis is stimulated thereby taking up all the orthophosphate released in the anaerobic phase plus the phosphate originally present in the wastewater (Grady *et al*., 1999).

One of the major drawbacks with this model is the accumulation of $FADH_2$ (flavin adenin dinucleotide) in the TCA cycle under anaerobic condition (Seviour, R, 2010). $FADH_2$ is an electron donor that is utilized during the cellular respiration and is formed from oxidation of succinate (component of TCA cycle) to fumerate. $FADH_2$ accumulation inside PAO cells can shut down the TCA cycle under anaerobic condition in which there is no electron acceptor present to break down the $FADH_2$. Fumarate and succinate have been identified as potent inhibitors for the oxidative degradation of organic compounds such as PHAs. $FADH_2$ is produced through the following reaction:

Succinate + FAD → Fumarate + $FADH_2$ [Eq. 2.5]

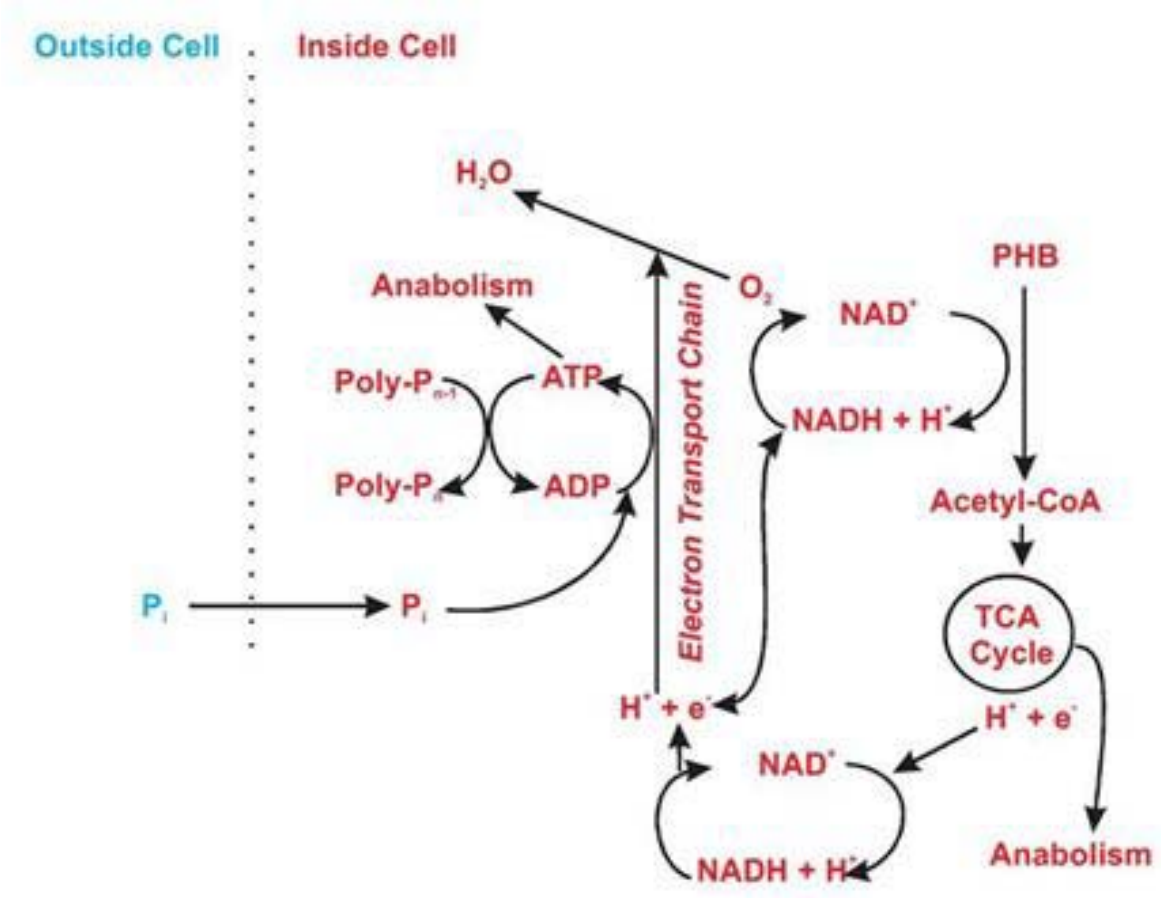


Figure 2.13: Comeau-Wentzel Model for the PAO's Aerobic Metabolism (Grady *et al*., 1999)

2.5.2 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 2.14 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 glucose released from the glycogen not 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 converts acetate to acetyl-CoA as well as some of the reducing power needed for PHB synthesis (Grady *et al*., 1999).

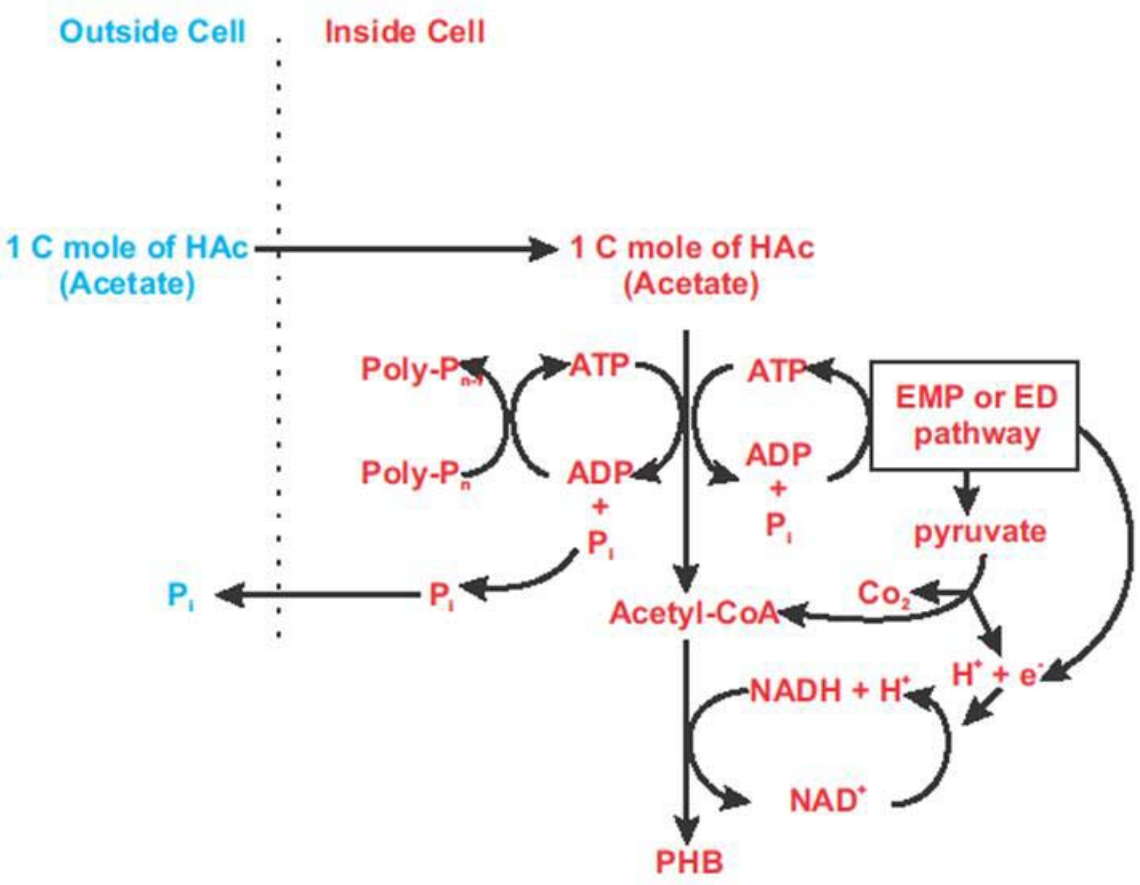


Figure 2.14: Mino Model for the PAO's Anaerobic Metabolism (Grady *et al.*, 1999)

The difference between Comeau-Wentzel and Mino model is mainly the metabolism of the PAO cell in the anaerobic zone. Based on the Comeau-Wentzel and Mino model, PHB is broken down under the aerobic phase for biomass re-synthesis as well as for phosphate uptake

and storage as polyphosphate. In addition, the Mino model suggests that the PHB break down is used to replenish the stored glycogen inside the PAO cell. Figure 2.15 shows the Mino model for PAO's metabolism under aerobic condition (Grady *et al*., 1999).

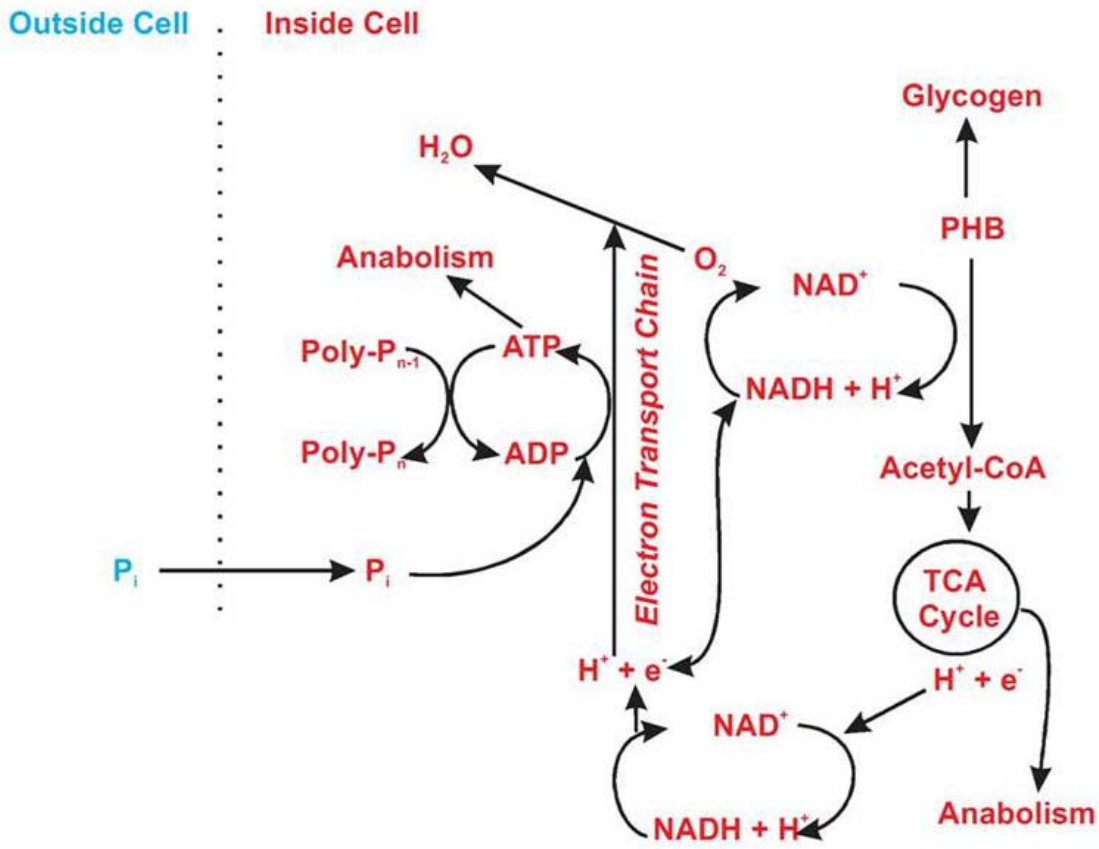


Figure 2.15: Mino Model for the PAO's Aerobic Metabolism (Grady *et al*., 1999)

Both Comeau-Wentzel and 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 phenomena in the biological phosphorus removal process. Although, this concept has been investigated by researchers for a long time still no generally accepted model exists.

2.6 Phosphorus Release and Uptake Modeling

Biological phosphorus removal is still under active experimental investigation, therefore there is little solid information about the rate expressions describing the processes in the anaerobic and aerobic phases. A complete discussion beyond the scope of this thesis is needed to fully define and describe the kinetics and stoichiometry of biological phosphorus removal.

Nevertheless, several mathematical models will be defined briefly in this section to help us comprehend this concept. The subsequent models define the following two PAO cellular mechanisms:

1. Rate of acetate uptake under anaerobic phase (modeling the anaerobic process), and
2. Rate of PAO growth under aerobic phase (modeling the aerobic process)

2.6.1 Rate of Acetate Uptake in the Anaerobic Phase

The rate of acetate uptake under anaerobic condition is an important parameter in understanding the behavior of PAOs and the overall phosphorus removal in the BNR process. Although, there is disagreement among the available models for the kinetics of acetate uptake, they all agree on the following facts (Filipe *et al*., 2001):

- VFAs are taken up by PAOs
- VFAs uptake is associated with the release of phosphate and accumulation of PHA
- The phosphate release results from the need for energy to transport acetate across the cell membrane and activate it to Acetyl-CoA
- PAOs cannot oxidize the VFAs under anaerobic condition, therefore, they store them in the form of PHA which can be used as a carbon and energy source for growth and phosphorus accumulation in the aerobic phase
- Glycogen is consumed in the anaerobic phase. The role of glycogen is to provide reducing power for the accumulation of PHA

Three different expressions have been used in the biological phosphorus removal studies to describe the rate of acetate uptake by PAOs in the anaerobic phase.

1. The first kinetic model proposed by Wentzel *et al*. in 1989 assumed that the rate of acetate uptake was independent of acetate concentration, but that it was influenced by the polyphosphate content of the biomass. Wentzel *et al.* (1989) concluded that the acetate uptake rate was zero order with respect to the acetate concentration and first order with respect to the biomass concentration. Filipe *et al.* (2001) also proposed a zero order kinetic model for the rate of acetate uptake. They found that the rate of fermentation in the anaerobic zone is very low and the acetate is immediately taken up by PAOs which means that acetate concentration remains low at all times in this stage. Using these findings they suggested that the rate of acetate uptake is

independent of the acetate concentration which makes the rate a zero order reaction with respect to acetate concentration. On the other hand, the polyphosphate content of the biomass is the most likely variable controlling the rate. The model proposed by Filipe *et al.* (2001) will be explained in the following section.

2. The second kinetic model proposed for acetate uptake was a Monod expression to make the rate of acetate uptake dependent on the acetate concentration. This model was postulated by Smolder *et al.* (1995), Kuba *et al*. (1996) and Murnleitner *et al*. (1997).

3. The third kinetic model was proposed by Romansky *et al*. (1997) which consisted of a double Monod expression with one expressing the acetate concentration and the other the polyphosphate content.

Undoubtedly, there is disagreement among the available models for the kinetic of acetate uptake. The question of whether the rate of acetate uptake is a zero order, first order or second order is a subject which still is under investigation by scientists. In this section two kinetic models for the rate of acetate uptake which are zero order expressions will be discussed.

2.6.2 Stoichiometry and Kinetics of Acetate Uptake

Model # 1

This model describes the rate of acetate uptake by PAOs as a zero order kinetic expression with respect to acetate. That is

$$r_{Acetate} = (q^{max}_{PAOs} - q_{pp}\frac{1}{f_{pp}}) \cdot \left(\frac{C_{Acetate}}{C_{Acetate} + 0.001}\right) \cdot \left(\frac{f_{Glycogen}}{f_{Glycogen} + 0.001}\right) \cdot C_X \qquad \text{[Eq. 2.6]}$$

Where,

$r_{Acetate}$ = Rate of acetate uptake (C-mmol/h)

q^{max}_{PAO} = Maximum specific rate of acetate uptake (C-mmol/C-mmol.h) which was estimated to be 0.185 $\frac{C-mmol\ Acetate}{C-mmol\ Biomass\ .h}$

q_{pp} = Proportionality constant to describe the decrease of the specific acetate uptake rate with decrease in the polyphosphate content(C-mmol/C-mmol.h) which is estimated to be $0.197\times10^{-3}\frac{C-mmol\ Acetate}{C-mmol\ Biomass\ .h}$

f_{pp} = Polyphosphate content of biomass (P-mmol/C-mmol)

$$f_{pp} = \frac{C_{pp}}{C_X}$$

C_{pp} = Polyphosphate concentration (P-mmol/L)

C_x = Biomass concentration (C-mmol/L)

$C_{Acetate}$ = acetate concentration (C-mmol/L)

$f_{Glycogen}$ = Glycogen content of the biomass (C-mmol/C-mmol)

$$f_{Glycogen} = \frac{C_{Glycogen}}{C_X}$$

$C_{Glycogen}$ = Glycogen concentration (C-mmol/L)

The above rate postulated by Filipe, Daigger and Grady (2001) reveals that if q_{pp} were a very small number, the rate of acetate uptake would equal q^{max}_{PAO} for high polyphosphate content (f_{pp}). However, when the polyphosphate content is very low, the rate of acetate uptake decreases very rapidly.

Model # 2

The simplest mathematical model for the prediction of biological phosphorus removal rate is shown by [Eq. 2.7]. There are several severe restrictions and assumptions made for the development of such mathematical model which include:

- Comeau-Wentzel model assumption
- Disability of PAOs in nitrate reduction
- PAOs can only grow using the PHB storage of the cell

This mathematical model expresses the rate of removal of substrate (Acetic acid) in the anaerobic phase. Acetic acid is a carbon source used in the phosphorus removal process. Acetic acid gets transformed into acetate by heterotrophic bacteria in the anaerobic phase. In the following equation all the organic mass is expressed as COD and all the phosphate and poly-phosphate concentrations are expressed as P (Grady *et.al.*, 1999).

$$r_{SA} = -q_A \left(\frac{S_A}{K_A + S_A}\right) \left[\frac{X_{PP}/X_{BP}}{K_{PP} + (X_{pp}/X_{BP})}\right] X_{BP} \qquad \text{[Eq. 2.7]}$$

Where,

r_{SA} = Rate of removal of AcH (C-mmol/h)

q_A = Maximum specific rate of acetic acid uptake (hr^{-1})

S_A = acetic acid concentration (mg/L)

K_A = half-saturation coefficient for acetic acid (The half-saturation constant of the Monod equation. K_A equals the substrate concentration (mg/L) at which q, specific rate of acetic acid uptake equals 1/2 of q_A, maximum specific rate of acetic acid uptake)

X_{PP} = poly-p concentration in the biomass (mgP/L)

K_{PP} = half-saturation coefficient for poly-P

X_{BP} = concentration of PAO biomass (mg/L)

Based on the stoichiometry of the reaction, by each mg/L of COD removed from the bulk solution, 1 mg/L of PHB is formed inside the PAO cell. Therefore,

Rate of PHB formation: $r_{X,PHB} = -r_{SA}$

2.6.3 Stoichiometry and Kinetics of PAOs Growth in the Aerobic Phase

The rate of PAOs growth can be described by the following equation:

$$r_{XBP} = \mu_P \left[\frac{X_{PHB}/X_{B,P}}{K_{PHB} + (X_{PHB}/X_{B,P})}\right] \left(\frac{S_P}{K_P + S_P}\right)\left(\frac{S_o}{K_o + S_o}\right) X_{B,P} \quad \text{[Eq. 2.8]}$$

Where,

r_{XBP} = rate of PAO growth

μ_P = Maximum specific growth rate coefficient for PAOs

X_{PHB} = Stored PHB concentration as COD (mg/L)

S_P = soluble phosphate concentration in (mg/L)

K_P = Half-saturation coefficient for soluble phosphate

K_{PHB} = Half-saturation coefficient for intercellular PHB

S_o = Dissolved oxygen concentration (mg/L)

K_o = Half-saturation coefficient for dissolved oxygen

$X_{B,P}$ = Biomass phosphate concentration in (mg/L)

Under aerobic conditions, PAOs grow using the stored PHB as carbon and energy source. PAOs are also capable of growth on soluble substrate (i.e acetic acid) which is ignored in this model in order to simplify the growth rate. When oxygen is absent from the bulk solution, the rate of growth will approach zero (Grady *et al*., 1999)

.

MATERIALS AND METHODS

3.0 CUBEN REACTOR DESIGN AND STAGING

The Compact Upright Bioreactor for the Elimination of Nutrients (CUBEN) is a bioreactor with unique staging sequence. No vertically-staged configuration has been found in existing BNR reactors when a complete patent search was conducted in 2008. All commercial and experimental BNR plants consist of horizontal, rectangular cross section bioreactors. Figure 3.1 shows the arrangement of CUBEN stages and associated processes in a block diagram.

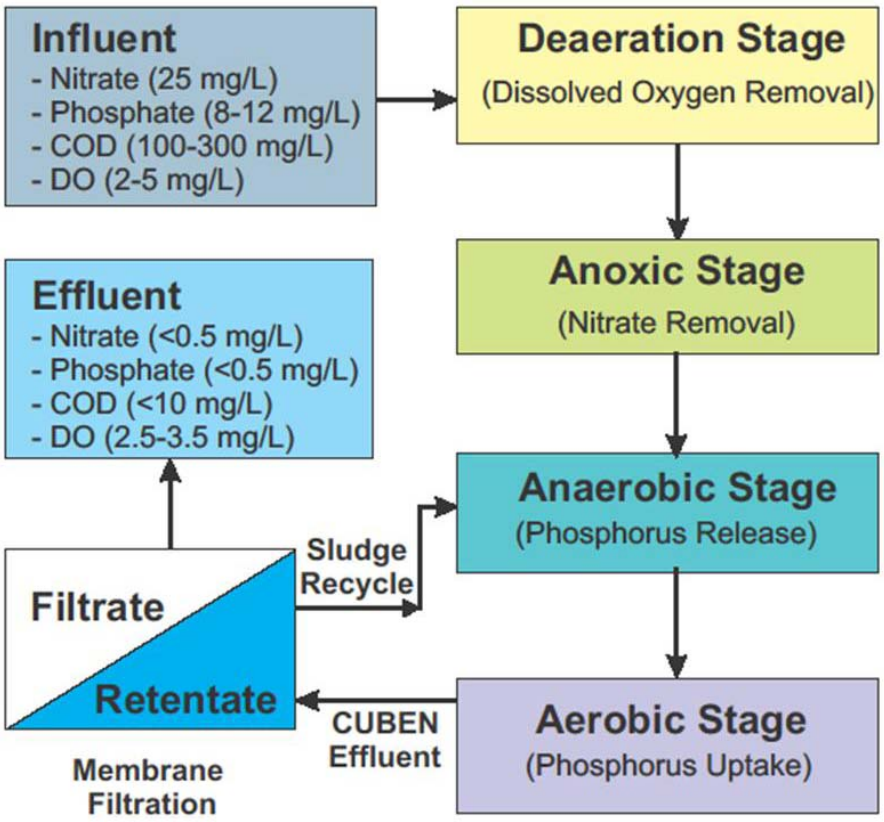


Figure 3.1: Block Diagram of the CUBEN and Membrane Filtration

The flow moves as follows: wastewater first enters from the top of the column into the Deaeration stage or vacuum stage where dissolved oxygen is rapidly removed from the bulk liquid. Then, the effluent from the Deaeration stage enters the Anoxic stage which is located underneath the vacuum Deaeration stage. In the Anoxic stage, nitrate concentration is reduced and converted to free nitrogen. A carbon source such as methanol must be added in the Anoxic

stage to provide energy for the growth of denitrifying bacteria and enhancement of the denitrification.

The effluent from this stage contains concentrations of dissolved oxygen (DO) lower than 0.1 mg/L and NO_3^- concentrations of less than 0.5 mg/L which enters the Anaerobic stage. In this stage and the subsequent Aerobic stage, Phosphorus Accumulating Organisms (PAOs) are responsible for the removal of phosphorus from the bulk solution. In the Anaerobic stage, PAOs uptake Volatile Fatty Acids (VFAs) and accumulate them in their cells in the form of Poly-hydroxyalkanoates (PHAs). As PAOs take up VFAs and store PHAs inside their cells, they also release phosphorus into the water. Therefore, the phosphorus concentration in water highly increases in this stage.

Then, PAOs enriched with PHAs enter the Aerobic stage where they oxidize the cellular PHAs as a source of energy and uptake both the phosphorus already present in the influent to the bioreactor as well as the amount released by the PAOs in the Anaerobic stage. The effluent from the Aerobic stage of CUBEN enters a membrane filtration unit or secondary clarifier to separate the sludge from the solution. The collected sludge (membrane's retentate) contains high concentration of PAOs enriched with cellular polyphosphates and the membrane's filtrate contains very low concentration of phosphorus less than 0.5 mg/L. A portion of the collected sludge (Approximately 80%) is recycled back into the Anaerobic stage to be reused in the phosphorus removal process. Sludge recycling is an important requirement for successful biological phosphorus removal process. Sludge contacting PAOs can highly improve the phosphorus removal efficiency and reduces the COD concentration in the final effluent.

3.1 Objectives of the Present Bioreactor Design

The Compact Upright Bioreactor for the Elimination of Nutrients (CUBEN), a US patent pending technology, consists of four stages each removing specific constituents from the feed (wastewater).

As shown before, there are many different commercially available, nutrient removal technologies in the wastewater industry, trying to meet the stringent limits of nutrient discharges. Most of these technologies have various drawbacks which limits the operation of these technologies. These limitations are listed in the following table:

Disadvantages of Existing BNR Technologies
• Large construction area
• High capital costs
• Control complexity
• Excessive sludge recycle
• Undesirable sludge production
• Long residence time
• Provision of excessive carbon source requirement
• Moderate pumping

Table 3.1: Disadvantages of the Existing BNR Technologies

In addition, current environmental regulations regarding the nutrient discharge limits are becoming increasingly strict in Canada and other industrialized countries. Thus, there are strong social and economic needs for the development of a cost effective, highly efficient, easy to operate and compact, nutrient removal technology. The main goal of this thesis is to construct, operate and evaluate a nutrient removal bioreactor with unique configuration which occupies smaller foot print and has higher nutrient removal efficiency and lower pumping cost compared to conventional technologies. This new bioreactor requires less number of pumps due to its vertical alignment in which water flows by gravity from one stage to the other (Anoxic-anaerobic-Aerobic).

3.2 CUBEN Design Basis

The following table (Table 3.2) shows the feed flowrate and concentration of the constituents of the wastewater used in the design and operation of CUBEN. The CUBEN's influent contains nitrate and phosphorus concentrations which represent a wastewater that has undergone secondary treatment. The wastewater flowrate of 120 (L/day) is considered as the basis for the design of this unit.

Parameters	Design Influent Criteria	Design Effluent Criteria
Flowrate (L/day)	120	120
BOD_5 (mg/L)	50	< 5
COD (mg/L)	100-300	<10
TSS (mg/L)	0-8	< 5
NO_3 (mg/L)	25	< 0.5
Phosphorus (mg/L)	10-30	< 0.1
Dissolved Oxygen (mg/L)	4-6	2.5-3.5

Table 3.2: CUBEN Bioreactor Design Basis

The design influent and effluent criteria were developed using information available on many wastewater treatment plants with secondary and BNR process. The CUBEN effluent concentrations, once achieved steady state, are set to satisfy the discharge limits regulated by Ontario's Ministry of the Environment (MOE).

3.2.1 Consideration of Important Parameters in CUBEN Design

The parameters that can highly influence the CUBEN's operation include:

1. Hydraulic Residence Time (HRT)
2. Sludge Residence Time (SRT)
3. pH of the wastewater
4. Temperature
5. Carbon Source
6. Dissolved Oxygen Concentration

1. Hydraulic Residence Time (HRT)

The hydraulic residence time (HRT) in CUBEN is calculated to be approximately 14 hours from the time wastewater enters the Deaeration stage until it leaves the Aerobic stage. Each stage in CUBEN has different HRT associated with the contact time required for microorganisms to perform their specified tasks. In the Deaeration stage, the oxygen removal from water is very fast due to both the use of special type of water diffuser (misting nozzle) and vacuum. The size of this stage is calculated for a HRT of 6 hours before it is pumped into the Anoxic stage. The HRT of the Anoxic stage can be in the range of 1-2 hours. For CUBEN, the HRT of 2 hours is selected.

Regarding phosphorus removal, the anaerobic contact time or HRT depends on many factors such as amount of available COD in the wastewater, population of PAOs and their maximum storage capacity as well as the amount of phosphorus in the influent (Janssen P, 2002). Figure 3.2 shows the commonly applied anaerobic HRT in wastewater treatment plants with EBPR process. As it can be observed from this figure, the anaerobic HRT lays below one hour for 30% of applications, 34% between 1-2 hours, 17% in the range of 2-3 hours and 19% over 3 hours. The HRT of two hours is frequently used in successful biological phosphorus removal plants. Therefore, two hours of wastewater hydraulic residence time was selected for designing the Anaerobic stage of CUBEN. The final stage of CUBEN (Aerobic stage) has HRT of nine hours. This number was taken from successful biological phosphorus removal plants and again applied to CUBEN.

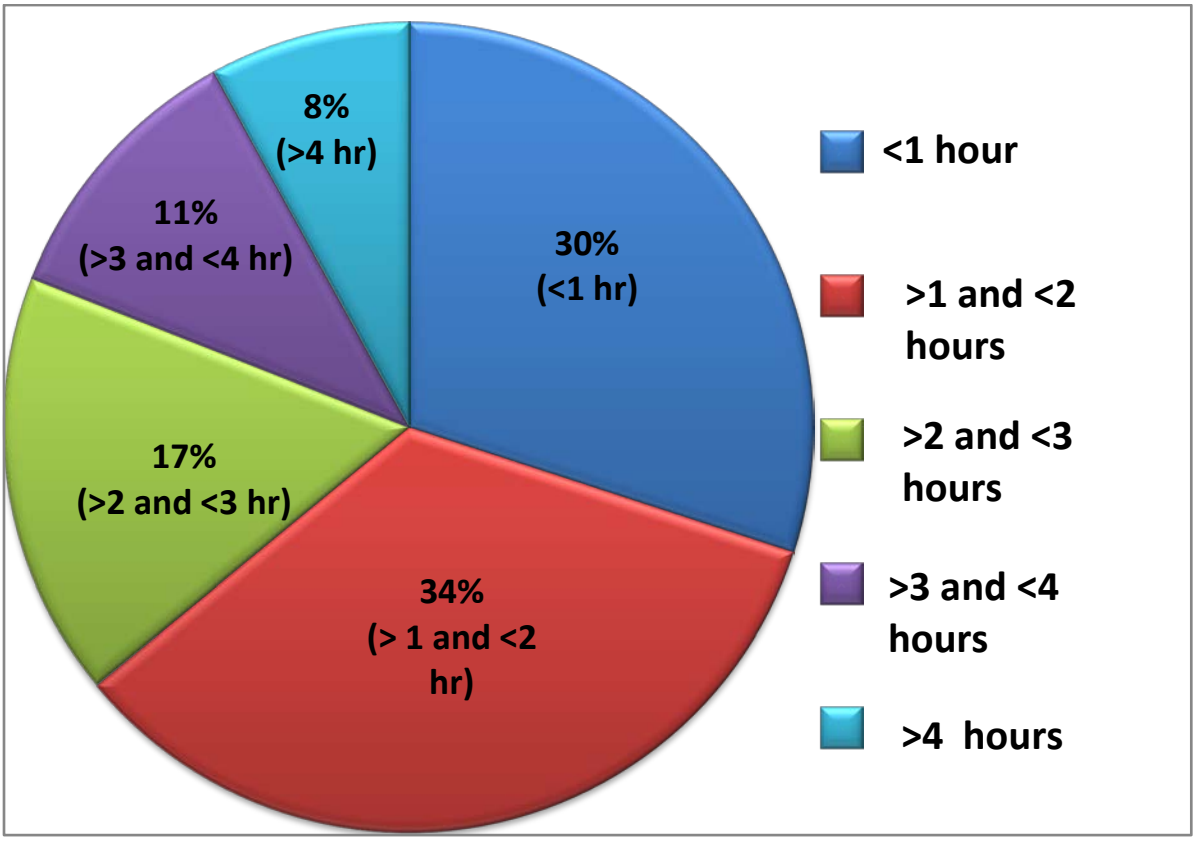


Figure 3.2: Relative Frequency Distribution of the Anaerobic HRT (Scheer *et al.*, 1996)

2. Sludge Residence Time (SRT)

One of the important factors in biological phosphorus removal is the production of excess sludge. Indeed, this is due to the reversible nature of the poly-phosphates stored inside PAO cells. PAOs collected in the sludge can break down the cellular polyphosphates and release ortho-phosphates into the environment if the SRT of the Aerobic stage is high. Therefore, adequate SRT and proper handling and recycling of the sludge dominated by PAOs is essential. The simulation results of various phosphorus removal processes such as UCT have shown that phosphorus removal efficiency reaches maximum at short SRT between 3 to 5 days. If the sludge age is shorter than this range the low sludge concentration causes incomplete conversion of biodegradable material into VFAs which reduces the availability of substrate for PAOs. For sludge older than 3 to 5 days almost all biodegradable material will be converted into VFAs and taken up by PAOs in the Anaerobic stage but the uptake of phosphorus in the Aerobic stage decreases significantly.

In terms of denitrification process in the Anoxic stage, SRT of 7 day (approximately one week) can result in high nitrate removal. The SRT of the Anoxic stage in CUBEN is higher than conventional denitrification processes. This is due to the use of packing and formation of denitrifying biofilm which produces less sludge and consequently high SRT in this stage.

3. pH of the Solution

The effect of pH on the stoichiometry and kinetics of acetate uptake by PAOs is an important element in a successful BNR process. In the Anaerobic stage, the amount of phosphorus released per acetate taken up is linearly dependent on the pH due to the additional energy requirements for acetate transport at higher pH. Also, low pH results in the production of Glycogen Accumulating Organisms (GAOs) which are PAOs competitors in EBPR process. GAOs are able to uptake acetate in the Anaerobic stage and store PHA compounds within their cells. However, they cannot uptake phosphorus in the subsequent Aerobic stage which results in the deterioration of the BPR process. In the biological phosphorus removal process, pH is a parameter that can highly affect the reproduction or deterioration of GAOs community. Based on the literature review and two months of experimental data collected herein, it can be concluded that the optimum pH for both denitrification and BNR processes in the CUBEN must be in the range of 6.5 and 8.0.

4. Temperature

The effect of temperature on nutrient removal and specially phosphorus removal is not well understood. Past studies have shown that phosphorus release and/or phosphorus uptake can increase with increasing temperature from 5^{o}C to 30^{o}C. Phosphate release rates were observed to decline at temperature higher than 35^{o}C and at temperatures higher than 45^{o}C, no phosphate release or uptake were observed. These results indicate that PAO population decays and consequently phosphorus removal deteriorates at that temperature (Baetens, Danielle, 2001). In biological P removal process, low temperatures (5^{o}C or less) can decrease the rate of P removal by negatively influencing the biochemical processes such as phosphorus release/uptake, acetate uptake, PHA synthesis and utilization. A successful EBPR process is achievable at lower temperature only through increasing the SRT of the process. Low temperature decreases the kinetics of the process therefore high sludge age in cold weathers results in better management

and utilization of the PAOs. In addition, lower temperature can shift the microbial community from Glycogen Accumulating Organisms (GAOs), PAO competitors, to purely PAO population (Oehmen *et al*., 2007). The temperature for CUBEN was within 18-25^{o}C range or room temperature. The temperature in this range does not adversely affect the denitrification nor the phosphorus removal process.

5. Carbon Source

PAOs have the capability to take up acetates and convert them into intercellular carbon polymers called PHAs under anaerobic condition. Normal heterotrophic bacteria under anaerobic conditions ferment complex volatile fatty acids (VFAs) into acetate. The ability of PAOs to take up acetate anaerobically creates a competitive advantage over normal heterotrophic microorganisms. VFAs are limited resources in biological phosphorus removal systems and their use by PAOs must be maximized to optimize the phosphorus removal process. As it was mentioned earlier, the phosphorus removal process by PAOs is a hypersensitive process and the quantity and quality of the organic carbon mixture added to the anaerobic phase directly affect the phosphorus removal efficiency. There were some instances in which phosphorus removal in a wastewater treatment plant or a bench scale experiment favored the growth of PAO's competitors called Glycogen Accumulating Organisms (GAOs). These organisms are also able to remove VFAs under anaerobic conditions; therefore, they compete with PAOs for the same substrate and thereby diminish the removal of phosphorus by PAOs (Filipe *et al*., 2001).

Other types of VFA important in BNR process are propanoic and butyric acids which are abundant in many pre-fermentation processes. In many previous studies propanoic acid has shown to be a more favorable carbon source than acetic acid (Oehmen *et al*., 2005). Other studies have also shown that the maximum rates of anaerobic acetate uptake and phosphorus release can be achieved with optimum concentrations of acetic, butyric and propanoic acids mixture (Mulkerrins *et al*., 2003). There are different ratios of mgCOD/mgP suggested by some authors to be added in the Anaerobic stage. For instance, some suggest a ratio of COD to phosphorus concentration of 15:1, 35:1 or greater is required to achieve an effluent phosphorus concentration of 1.0 mg/L or less. Randall *et al*. (1992) stated that a ratio of 45:1 (mgCOD/mgP) is necessary. Furthermore, according to Reddy (1998) 50 mgCOD/mgP is a conservative number

(Scheer *et.al*., 1997). As a result, the amount of COD (VFA or carbon source) is a parameter difficult to predict therefore, the present research protocol started the experiment with 300 mg/ L of COD or 30:1 (mgCOD/mgP) added in the synthetic wastewater. The COD concentration was varied during the commissioning of the unit in order to find the optimum value.

6. Dissolved Oxygen (DO) Concentration

In previous studies, DO concentration has shown significant effect on phosphorus removal efficiency since DO concentration impacts PAO-GAO competition. It has been frequently observed that oxygen concentrations of approximately 2.5-3.0 mg/L in the aerobic zone can favor the growth of PAOs. On the other hand, very high DO concentrations of 4.5-5.0 mg/L deteriorate the biological phosphorus removal performance (Oehmen, 2007).

Therefore, in the Aerobic stage of the CUBEN, aeration was monitored and controlled using air flowmeter and DO sensors to maintain the DO concentration within the range 2.5-3.0 mg/L. Also, presence of dissolved oxygen in the Anoxic and Anaerobic stages highly influences both denitrification and phosphorus removal processes. CUBEN has an excellent DO removal ability due to vacuum operation. Therefore, Anoxic and Anaerobic stages were continuously monitored to maintain the DO concentration of less than 0.1 mg/L.

The following diagram summarizes the ranges of the aforementioned parameters including Temperature, pH, Carbon Source Concentration, HRT and SRT in all four stages of CUBEN. These parameters and their specified ranges are very useful in understanding both the design concept and the basis for this thesis.

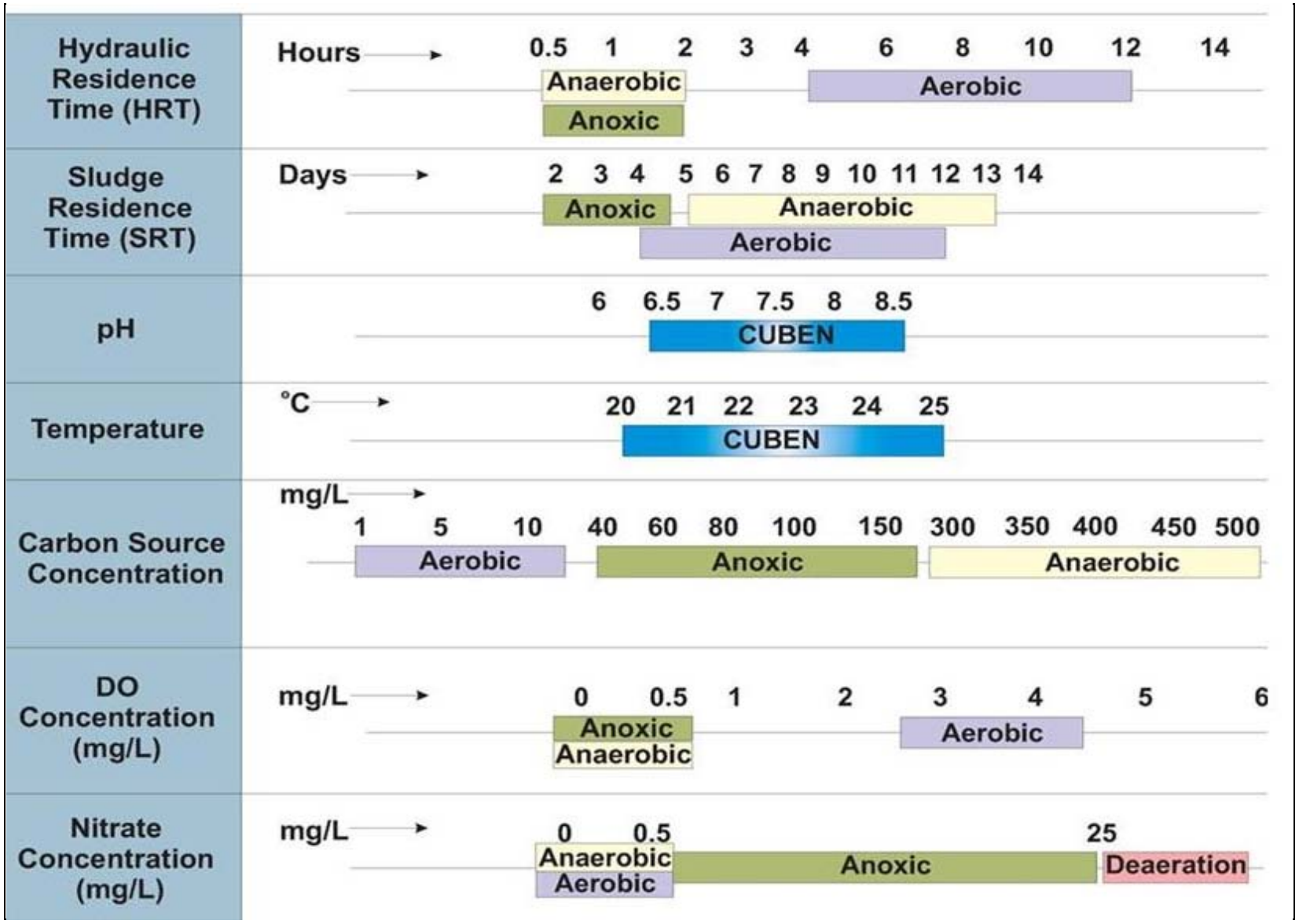


Figure 3.3: Ranges of Important Parameters in CUBEN

The following table exhibits in detail the process conditions in the four stages of CUBEN:

	Solutes in the water	Solutes to be removed	Type of Bacteria	Inlet Conc. (mg/l)	Outlet Conc. (mg/l)	Carbon Source Addition	Volume (m^3)	Height (m)	HRT (h)	SRT (days)	Vacuum and Pressure
Deaeration Stage	DO-NO_3-TP	DO	Unknown	4-6	0	Acetic + Propanoic + Butyric Acids	0.0615	0.87	6	----	50-63 cm-Hg
Anoxic Stage	NO_3-TP	NO_3	Denitrifiers	25	0.5	Methanol	0.01	0.12	2-4	2-4	1atm
Anaerobic Stage	TP	TP	PAOs	8-12	16-24	Acetic + Propanoic + Butyric acids	0.01	0.14	0.5-2	9-13 (W^{2*}) 5-7 (S^{2*})	1atm
Aerobic Stage	TP-DO	TP	PAOs	16-24	0.1	None	0.045	0.64	4-12	3-5[1]	1atm

Table 3.3: Design Parameters

1. Based on Biological Wastewater Treatment (1999), the optimum SRT for biological phosphorus removal is within the range of 3-5 days with anaerobic SRT of about 25 to 30% of the total

2. Baetens, Danielle, 2000- 2001

* “W” means winter and “S” means summer season

3.2.2 Design of the Deaeration Stage

The removal of dissolved oxygen from the wastewater entering CUBEN is a critical step for the subsequent nitrate and phosphorus removal that take place in the Anoxic, Anaerobic and Aerobic stages. In the medium and large scale plants, it is very difficult to consistently and reliably remove and control the dissolved oxygen.

The removal of dissolved oxygen from water can be achieved either physically or chemically. Chemical methods are not used due to the undesirable effects of scavengers such as sulfite or increased sludge content from the chemical addition to the water. Physical methods of oxygen removal from water include thermal degassing, vacuum degassing and nitrogen stripping. Among the above physical methods, vacuum degassing (deaeration) and nitrogen stripping are relatively fast and simple. Alvarez-Cuenca (1979) successfully applied vacuum deaeration to remove dissolved oxygen in a three-phase fluidized bed. As a result, vacuum stripping is a method that is used in the deaeration stage of CUBEN for effective and fast removal of the oxygen from the wastewater. Nitrogen stripping is more cost effective in small installations compared to the vacuum stripping which requires a greater initial capital cost. However, in the long term vacuum stripping has shown to be more economic due to lower maintenance and lower consumable costs (Landman *et al*., 2003). The performance of the deaeration stage in CUBEN is very important because the performance of subsequent stages depends on it. The lower is the oxygen concentration in the effluent leaving the Deaeration stage, the better is the efficiency of Anoxic, Anaerobic and Aerobic stages. The goal of this stage is to reduce the oxygen concentration in the wastewater to less than 0.1 mg/L and that goal was achieved.

Wastewater first enters into the bioreactor through a water distributor (misting nozzle) connected to the inlet pipe at top of the column. The misting nozzle spreads the synthetic feed in small droplets to facilitate the removal of dissolved oxygen by vacuum. The small droplets are spread on the packing section of the Deaeration stage. The type of packing used in this stage is plastic hollow spherical packing called Tri-Packs® which are commercially available. Tri-Packs® have 2.54 cm diameter and made of polypropylene (PP). The packing provides proper liquid distribution as well as more surface area for DO removal. Prior to the operation of CUBEN, the packing is dumped inside the deaeration stage and held above the deaeration reservoir with 30cm

height (Figure 3.12). A plastic rack with large openings is used to hold the packing so that deaerated feed can be easily collected in the reservoir section. Figure 3.4 shows the type of misting nozzle and Tri-Packs® packing used in the Deaeration stage.

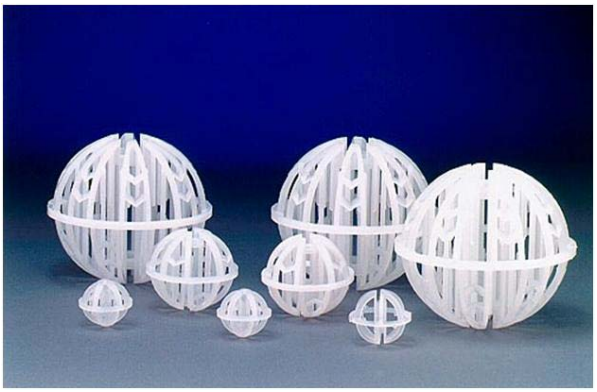


Figure 3.4: Misting Nozzle and Tri-Packs® Packing Used in the Deaeration Stage

Design of the Deaeration Stage:

Imposed diameter of the column = 0.3 m

Hydraulic residence time in the reservoir section (τ) ¼ of day or 6 hours (Alvarez-Cuenca, 1979)

Flowrate = 0.12 m^3/day

Volume of reservoir= ¼ day × 0.12 m^3/day = 0.03 m^3

Height of the reservoir:

$V = h\frac{\pi D^2}{4} \rightarrow 0.03 = h\frac{\pi 0.3^2}{4}$

Height of the reservoir = $\boxed{h_1 = 0.42\ m}$

Height of the packing zone (0.3m) plus Height of the water distributor zone (0.15m) = $\boxed{h_2 = 0.45m}$

Total Height of the Deaeration Stage:

Height of the packing + height of the water distributor + height of the reservoir= **H**

H = 0.42 + 0.30 + 0.15 = $\boxed{0.87\ m}$

Volume of the Deaeration Zone: $V = H\frac{\boldsymbol{\pi} D^2}{\boldsymbol{4}} = 0.87\frac{\pi 0.3^2}{4} = 0.0615\ m^3$

The following Figure 3.5 shows the Deaeration stage of CUBEN with the packing and water distributor zones as well as the reservoir section of this stage. ORP, pH and DO sensors are used to monitor and record the required data. Also, a vacuum gauge mounted above the reservoir section as well as a vacuum sensor above the packing zone were used to monitor the vacuum in the this stage.

Figure 3.5: CUBEN's Deaeration Stage

3.2.3 Design of the Anoxic Stage

The Anoxic stage in CUBEN must provide the necessary conditions for the denitrification process. These conditions are summarized as follows: (Haandel *et al*., 2007):

- Presence of facultative bacteria which use both oxygen and nitrate as an oxidant for organic matter. It has been established experimentally that activated sludge generated under aerobic conditions will use nitrate immediately after entering an anoxic phase. The rate of nitrate utilization doesn't change as long as the anoxic condition and availability of organic carbon sources are adequate.
- The presence of dissolved oxygen in the Anoxic stage inhibits the development of denitrification. In general, it has been observed that dissolved oxygen concentration of more than 0.2-0.5 mg/L reduces the rate of denitrification significantly.
- Temperature and pH are among the most important environmental conditions for bacterial growth. The denitrfication rate increases with temperature until 40 ^{o}C. At higher temperatures the denitrification rate is quickly reduced. Regarding the influence of pH, it has been observed that there is a maximum denitrification rate for the pH in the range of 7.0 to 8.5.
- The presence of an electron donor or biodegradable organic matter is essential for the reduction of nitrate. Methanol is among the most frequently used carbon source for denitrification.

Anoxic Stage Design Calculations: (Wiesmann *et al*., 2007)

In biological nitrate removal (Denitrification), nitrate (NO_3^-) is broken down by denitrifiers which assimilate bound oxygen to utilize the organic matter and result in the release of N_2 gas into the liquid phase. The organic matter in this study is stored separately in a tank and sufficient amount will be added to the anoxic zone. As a first approximation, a minimum BOD: TKN ratio of 3:1 was used to initiate and maintain desired denitrification.

Nitrate in the influent to the Anoxic stage generally comes from the secondary treatment where it can reach concentration of 25 mg/L. Nitrate concentration in the effluent from the Anoxic stage can be as low as 0.5 mg/L. The presence of high nitrate (NO_3^-) concentration in the Anoxic stage effluent can reduce the reliability of phosphorus removal process in the following stages. The Anaerobic stage must be protected against nitrate and dissolved oxygen so that PAOs

can carry out the phosphorus removal process. Bacteria can use nitrate compounds and oxidize part of the volatile fatty acids which must be utilized by PAOs. The presence of nitrate therefore can reduce the fraction of PAOs in the system thus the phosphorus removal capacity.

Based on the data obtained from BNR plants, the hydraulic residence time for successful denitrification normally requires 1.8 hr to be completed (Scheer *et al.*, 1997). Therefore, the volume and height of the Anoxic stage can be calculated as follows:

Wastewater flowrate (Q): 120 L/day

Diameter of the column: 0.3 m

$V = Q \times \tau = 120\ (L/Day) \times 1.8\ hr \times 1\ day/24\ hr =$ **9 L**

$$V = h\frac{\pi D^2}{4} \rightarrow 0.009 = h\frac{\pi 0.3^2}{4}$$

Height of the anoxic zone: $h = 0.12\ m$

The following table shows the design parameters associated with the Anoxic stage.

Parameters	Values
Flowrate	120 L/Day
Inlet NO_3^- Concentration	25 mg/L
Outlet NO_3^- Concentration	<0.5 mg/L
Carbon Source (Methanol & VFA)	Variable depending on the microbial kinetics
Carbon Source Concentration	300 mg/L
pH	7-8
Sludge age	3-5 days
Volume	9 L
Height	0.12 m

Table 3.4: Anoxic Stage Design Parameters

3.2.4 Design of the Anaerobic Stage

In the Anaerobic stage, under proper anaerobic conditions and sufficient VFA, PAOs break down their internal polyphosphate into orthophosphate molecules. As well, they convert Adenosine Triphosphate (ATP) to Adenosine Diphosphate (ADP) and Adenosine Monophosphate. The breakage of these bonds releases high amounts of energy required by PAO cells to uptake VFAs and convert them intercellulary into polymer compound such as 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 3.6) shows the uptake of acetate from the wastewater in the anaerobic phase and the release of phosphorus into the wastewater in the aerobic phase by a PAO cell.

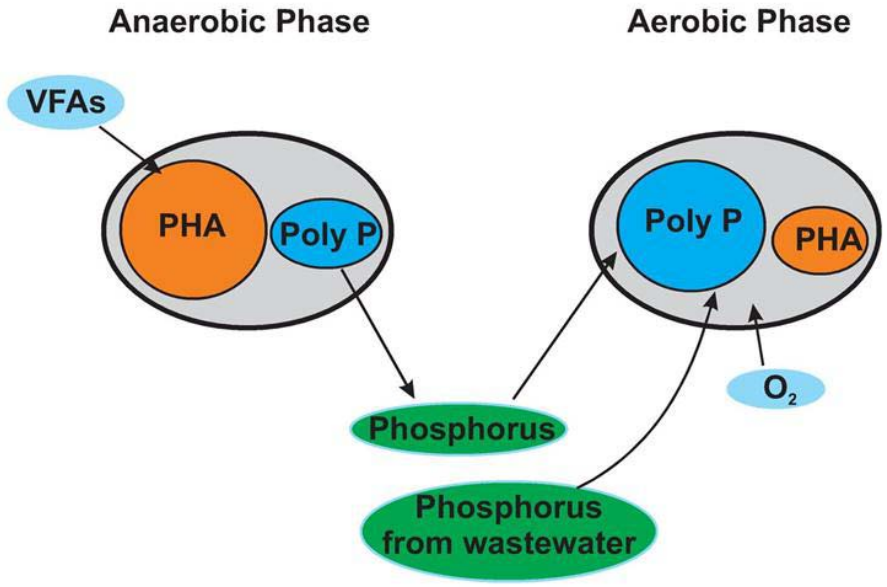


Figure 3.6: Acetate and Phosphate Uptake by PAOs under Anaerobic/Aerobic Conditions

In the anaerobic phase of a biological phosphorus removal unit, there is a relationship between the absorbed concentration of acetate and the concentration of released phosphate into the liquid as suggested by Wentzel et al (1988) to be 0.5 mgP/mgCOD.

In the phosphorus removal units, the amount of readily biodegradable substrate in the wastewater is essential for the enhancement of the process. Facultative bacteria ferment readily biodegradable materials into VFAs and subsequently acetate which can be utilized by PAOs. PAOs cannot do this fermentation themselves and can only transport and store short chain VFAs. Uptake and utilization of VFAs is a relatively rapid process but the fermentation of

biodegradable organic matter is rather slower. Fermentation can be a rate limiting reaction in the anaerobic zone if only small concentration of VFA is present in the wastewater (Grady *et al*., 1999).

Anaerobic Stage Design Calculations:

There is an optimal size for both the anaerobic and aerobic stages to achieve maximum phosphorus removal. Optimal phosphorus removal occurs when:

- HRT in the Anaerobic stage is sufficiently large to allow efficient fermentation of VFAs from COD/BOD and subsequent uptake of acetate.
- The Anaerobic SRT is small enough to prevent the growth of autotrophic nitrifying bacteria.
-
- However, the SRT in the Aerobic stage is large enough to allow the PAOs to grow.

Volume Calculation of Anaerobic Stage:

$\tau_{Anaerobic}$ = 2 hours (this is an optimum HRT as it was mentioned earlier in section 3.2.1)

Flowrate (Q) = 120 L/day $V = Q \times \tau$ = 120 L/day ×1day/24 hours × 2 hours = 10 L

$V = h\frac{\pi D^2}{4} \rightarrow 0.01 = h\frac{\pi 0.3^2}{4}$ **Height of the Anaerobic Zone: h = 0.14 m**

3.2.5 Design of the Aerobic Stage

Biological phosphorus removal is accomplished by creating conditions favorable for the growth of PAOs. As it was discussed previously, the Anaerobic stage provides selective advantage 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 and it accumulates intercellularly as carbon polymer (PHAs). When PAOs arrive into the Aerobic stage, oxidize these carbon polymers providing the energy source to take up phosphorus from the wastewater. Then PAOs use a small portion of this phosphorus to build up their internal cell structure, to grow and repopulate. The remaining phosphorus is accumulated in the form of polyphosphate inside their cells. The enrichment of the biomass with PAOs provides the biological mechanism by which phosphorus is removed from the wastewater.

The Aerobic stage in CUBEN must provide sufficient oxygen transfer to the PAOs. Compressed air is injected into this stage through a fine-bubble air diffuser located near the

bottom of the Aerobic stage. Oxygen is transferred from the rising air bubbles into the bulk solution and used by PAOs. The injected air also provides continuous mixing of this zone.

Another important parameter in regards to PAOs is the decay rate. The decay and growth rates of PAOs are significantly slower than that of normal heterotrophic bacteria. The decay and growth rates ofPAO have been experimentally found to be 0.04/day and 0.04/h respectively (Kortstee1 *et al*., 1999). Therefore, it is expected to achieve a stable and efficient phosphorus removal process after a continuous long term operation. Practical experience suggest that acclimatization of biological nutrient removal process requires at least 40 to 100 days to reach stable and good phosphorus removal yields (Patrick *et al*, 2005).

Volume Calculation of Aerobic stage:

Hydraulic Residence time: $\boldsymbol{\tau}_{\text{Aerobic}}$ = 9 hours (this is an optimum HRT as it was mentioned earlier in section 3.2.1)

Flowrate (Q) = 120 L/day

$V = Q \times \boldsymbol{\tau}$ = 120 L/day ×1day/24 hours × 9 hours = 45 L

$V = h\frac{\pi D^2}{4} \rightarrow 0.045 = h\frac{\pi 0.3^2}{4}$ **Height of the Aerobic Zone: h = 0.64 m**

3.2.6 Total Designed Height and Volume of CUBEN

$h_{\text{Deareration}} + h_{\text{Anoxic}} + h_{\text{Anaerobic}} + h_{\text{Aerobic}} = 0.87 + 0.12 + 0.14 + 0.64 = 1.77$ m

Total Height of CUBEN = 1.77 m

$V_{\text{Deaeration}} + V_{\text{Anoxic}} + V_{\text{Anaerobic}} + V_{\text{Aerobic}} = 0.0615\ m^3 + 0.009 + 0.01 + 0.045 = 0.126\ m^3$

Total Volume of CUBEN = 0.126 m^3

The following schematic diagram (Figure 3.7) illustrates the process flow diagram of CUBEN in combination with the membrane filtration unit. All the components are shown using different symbols. This drawing is scaled so the volume of each stage can be compared.

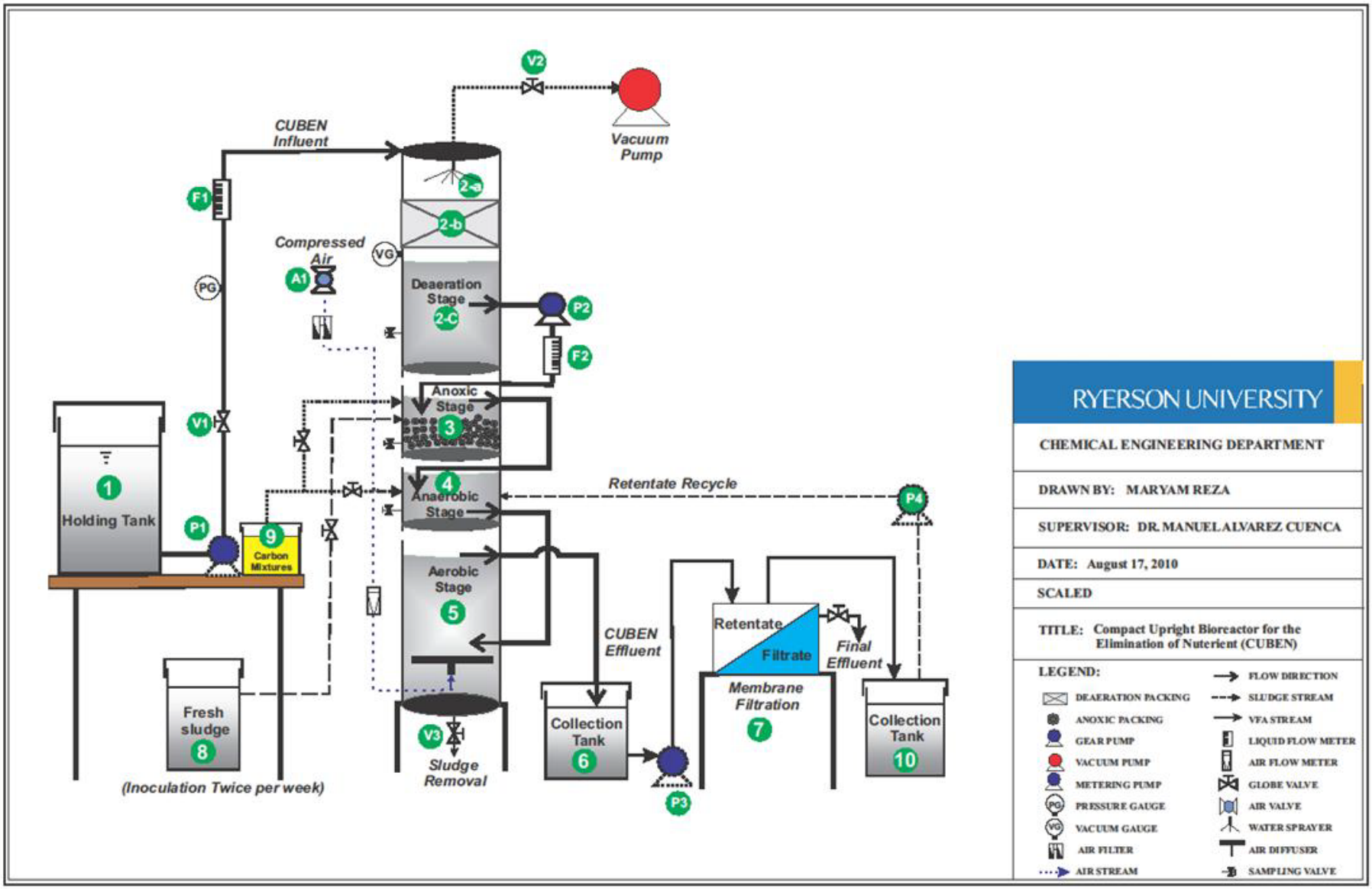


Figure 3.7: Schematic Diagram of the CUBEN

3.3 CUBEN Materials and Components

The main components of CUBEN include:

- A clear PVC column, 30cm in diameter divided in four sections
- Deaerator vacuum pump
- 2 prominent metering pumps
- 1 gear-reducer pump
- 1 FlexAir^{TM} fine bubble air diffuser
- 1 misting nozzle
- 2 liquid flowmeters and 1 air flowmeter
- 1 pressure gauge (0-200psi)
- 1 vacuum gauge (0-30 in-Hg)
- 130L feeding tank
- 30 L collection tank
- cm diameter PVC piping, elbows, valves, tees, unions
- 2.54 cm diameter packing
- 4 dissolved oxygen sensors (including 4 DO analyzers), pH sensors, ORP sensors and temperature sensors
- 1 pressure sensor and 1 vacuum sensor
- 2 Level sensors
- Data Acquisition System
- Measurement instruments for PO_4^-, NO_3^-, COD and TSS

All the above parts and equipment were set up in conjunction with a ceramic membrane filtration unit. The purpose of the membrane filtration was to separate sludge from CUBEN's effluent. The retentate from the membrane unit was collected in a 30L collection tank and was pumped back into the anaerobic stage to enhance the phosphorus removal process. The membrane's filtrate was used to determine and analyze CUBEN's final effluent concentration of phosphate, nitrate and COD.

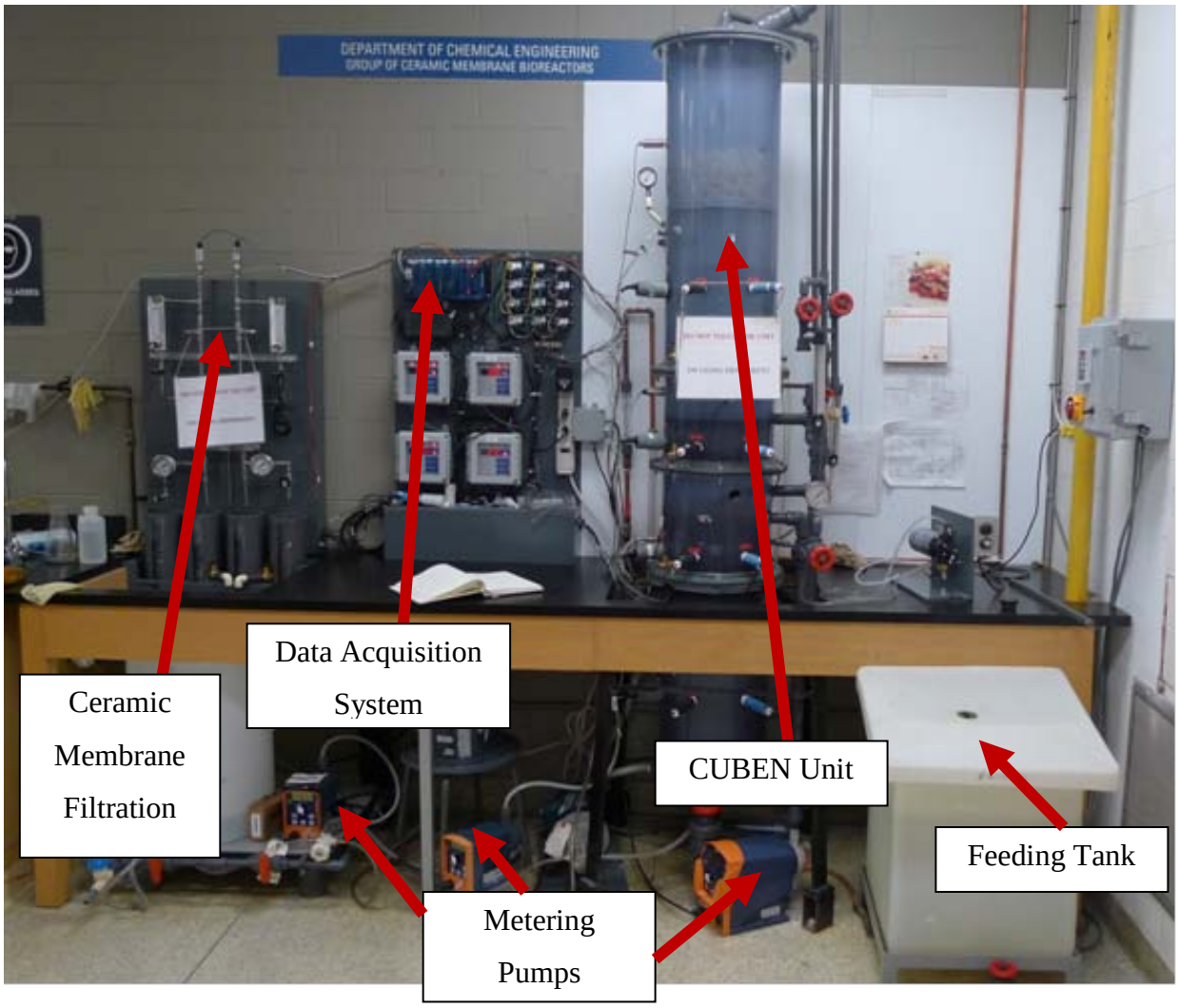


Figure 3.8: View of CUBEN, Data Acquisition System and Membrane Filtration Unit

Figure 3.8 is a picture of CUBEN (on the right), Data Acquisition System, DO monitors and Amplifiers (in the middle) and Ceramic Membrane Micro Filtration Unit (on the left). All three prominent metering pumps were located on the floor. The first metering pump from the right hand side was used to transfer water from the feeding tank to the top of the bioreactor into the Deaeration stage. The metering pump in the middle was used to recycle sludge from the membrane retentate tank into the Anaerobic stage. The third metering pump on the left hand side was used to pump the CUBEN's effluent into the membrane unit with the maximum pressure of 5 atm. The flowrate of water entering CUBEN as well as outlet flowrate of the gear pump (used to pass water from the Deaeration stage into the Anoxic stage) were monitored using two flowmeters. Also, CUBEN's inlet water pressure and vacuum suction in the Deaeration stage were monitored using the Pressure and Vacuum Gauges shown below:

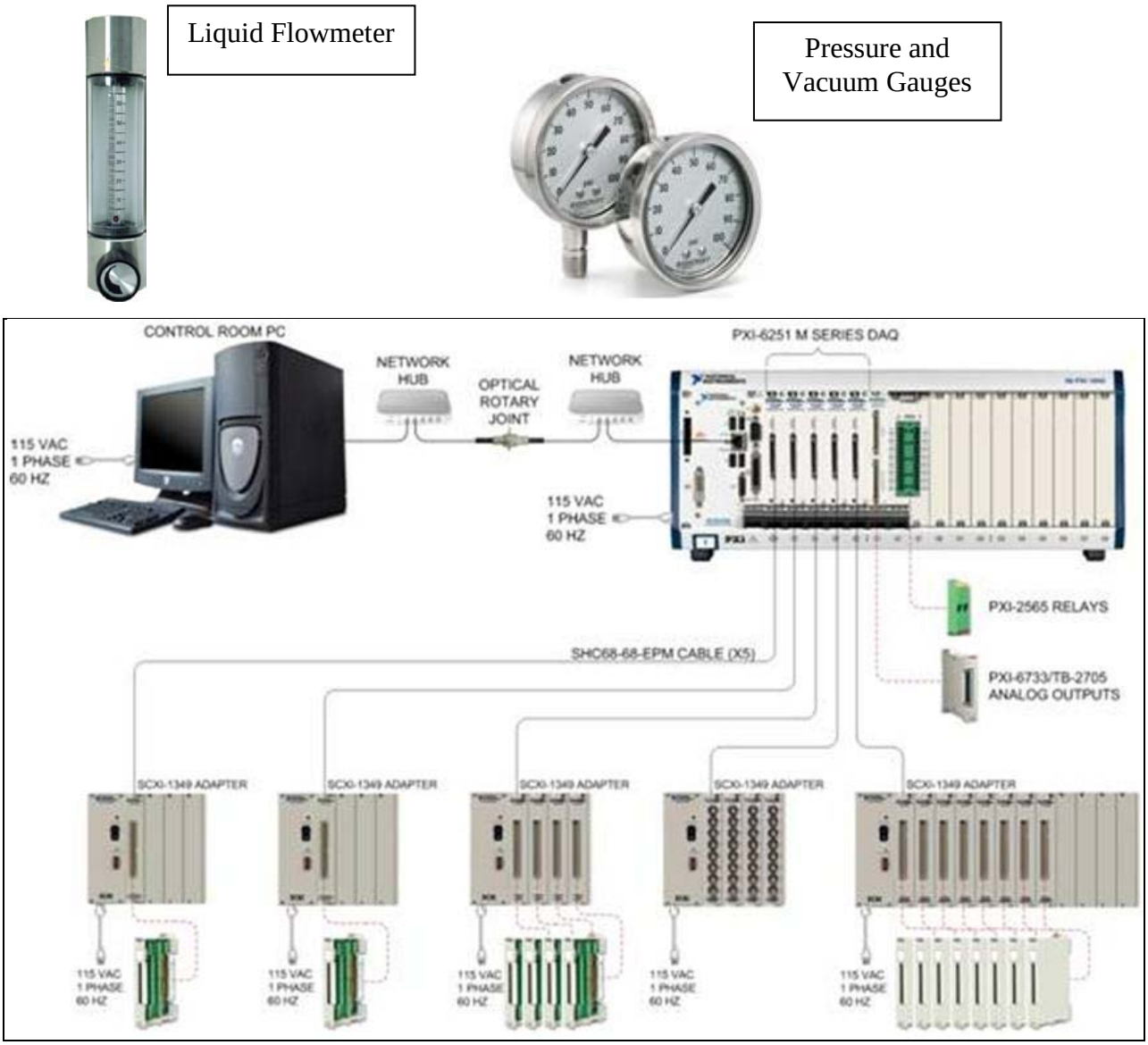


Figure 3.9: General Data Acquisition System (DAS) and Network Connections

Figure 3.9 illustrates the data acquisition hardware which was used to sample physical conditions of all the CUBEN stages. These parameters including DO, ORP, Temperature and pH were converted to electrical signals using various sensors. The electrical signals then were converted into digital numeric values by the DAS. Finally, these numeric values were processed and recorded by the computer. DAS was an excellent electronic device used to monitor the bioreactor continuously (24 hours/7 days a week).

3.3.1 Oxygen Requirement and Air Diffuser Selection

Sufficient oxygen transfer in the Aerobic stage is crucial for the high performance of CUBEN. The type of air diffuser used was FlexAir^{TM} disc with flexible membrane. The diameter of the air diffuser was 23cm which fitted perfectly inside the column. Figure 3.10 shows the FlexAir^{TM} disc and the fine bubbles produced by this type of diffuser. The oxygen transfer efficiency curves for FlexAir^{TM} air diffuser is presented in Appendix B.

Figure 3.10: FlexAir^{TM} Disc Air Diffuser

3.3.2 Pump Selection and NPSH Calculation for PUMP 2

One of the major innovations and great advantages of CUBEN is its vertical configuration which allows water/wastewater to pass through various stages (stage 2, 3 and 4) by gravity. This configuration is different from that of conventional BNR units in which wastewater must be pumped from one horizontal stage to the other. This advantage results in less power consumption and overall energy conservation. During the commissioning of the unit, we used a prominent metering pump to transfer synthetic wastewater from the feeding tank to the Deaeration stage. The metering pump was used to overcome the pressure build up by misting nuzzle at the top of the unit where water enters the CUBEN unit. However, due to the increase in pressure build up caused by particles in the synthetic solution, the misting nuzzle was frequently clogged. Finally, the nozzle was removed from the unit and the feed was raised to the vacuum stage by the pressure difference produced by the vacuum pump. Due to the pressure difference between the feeding tank located on the floor and the vacuum stage, the feed could enter the unit from the top of the column. Therefore, the need to use metering pump for feeding the unit was eventually eliminated.

The second metering pump (labeled 4 in figure 3.13) was used to recycle the membrane's retentate which was concentrated with PAO biomass into the anaerobic stage to reserve the concentration of PAOs and enhance the phosphorus removal efficiency. Another prominent metering pump was used for membrane filtration unit (labeled 3 in figure 3.13). This pump was used to increase the pressure of the fluid (CUBEN's effluent) to 5 atmosphere pressure. The high pressure (5atm) was essential for liquid to diffuse through ceramic membrane micro-filtration unit. Below is the picture of the prominent metering pumps used for this lab-scale BNR unit.

Figure 3.11: Prominent Metering pump (Prominent® Manufacturer)

Metering pumps are oscillating positive displacement pumps which extract the fluid with the back stroke of the displacer and press it into the dosing line with the pressure stroke. The liquid flowrate in the discharge point can be easily regulated using a knob to adjust the dosing strokes per time unit.

Another type of pump used in CUBEN operation was Gear Reducer pump (labeled 2 in figure 3.13). As it was mentioned earlier, the dissolved oxygen removal takes place in the vacuum deaeration stage of CUBEN. Vacuum deaeration stage with total height of 0.64 cm can hold the deaerated water only for six hours if CUBEN works in batch mode. However, continuous vacuum deaeration is fundamental in the design of this unit. The deaerated water is pumped out of the Vacuum stage using a Mag-Drive gear pump with Gear Reducer and an AC motor & controller device with variable frequency drive. The Gear pump is a positive displacement pump in which a volume of liquid is isolated in each tooth cavity. As the gear join, that cavity is reduced in volume and due to the incompressibility of liquid, the reduced cavity generates pressure and forces the liquid out of the discharge points (Dynaflow Engineering, Inc, USA). Operating the unit with continuous vacuum was one of the main challenges during the

commissioning process since there were few types of specialized pumps which work under continuous vacuum.

Figure 3.12 shows all the components of the Gear pump including gear reducer, motor and AC frequency controller. This pump was easy to install and operate. The high and low speed buttons on the AC controller were used to increase or decrease the water flowrate. This pump could easily overcome the vacuum of 20-25 in-Hg in the Deaeration stage.

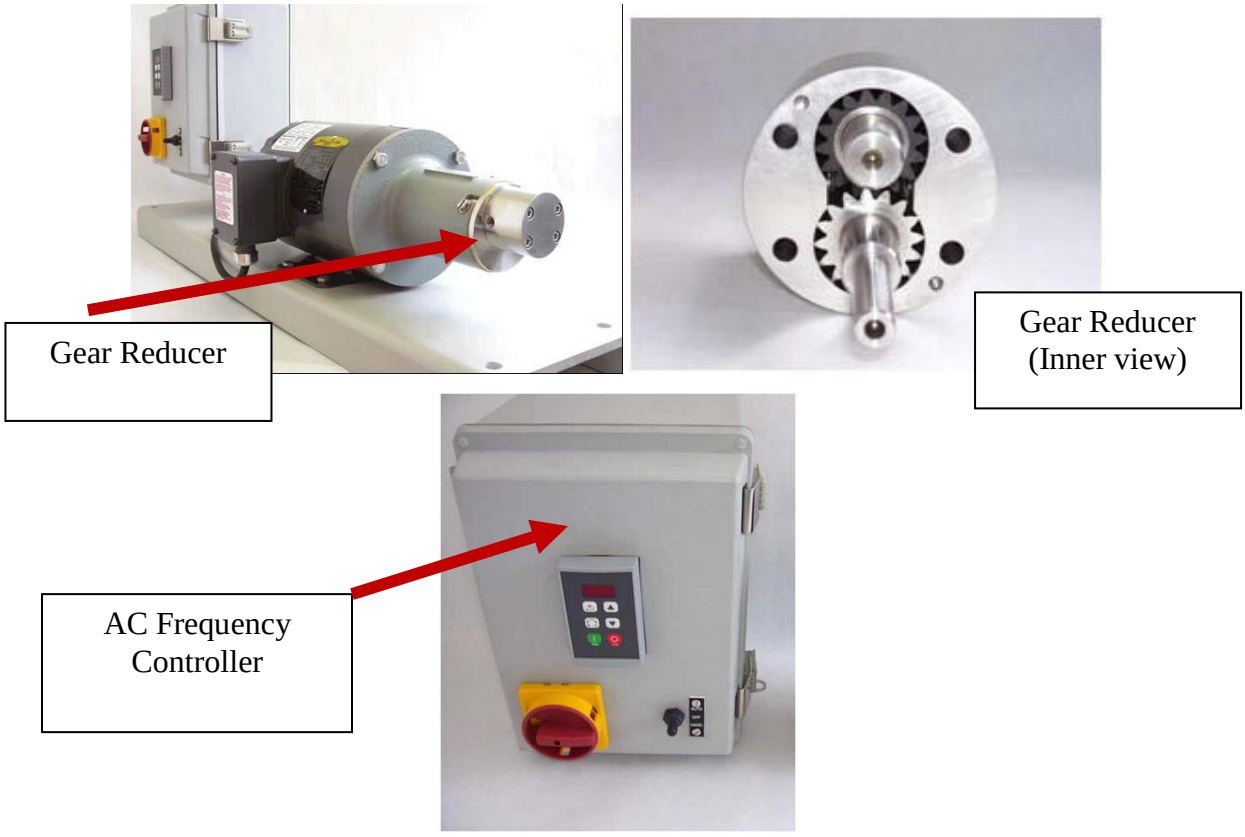


Figure 3.12: Gear Reducer Pump, Motor and AC Controller (Dynaflow Engineering, Inc, USA)

The Available Net Positive Suction Head Available $(NPSH)_A$ represents the actual pressure available before the suction side. The Required Net Positive Suction Head $(NPSH)_R$ on the other hand is the pressure provided by the pump to supply continuous flow of liquid from the Deaeration stage (under vacuum) to the Anoxic stage (under 1 atm pressure). The $NPSH_R$ must be higher than the $NPSH_A$ so that cavitations do not occur inside the pump. The $NPSH_A$ value can be determined experimentally or calculated if the system parameters are known. However, $NPSH_R$ must be provided by the pump manufacturer.

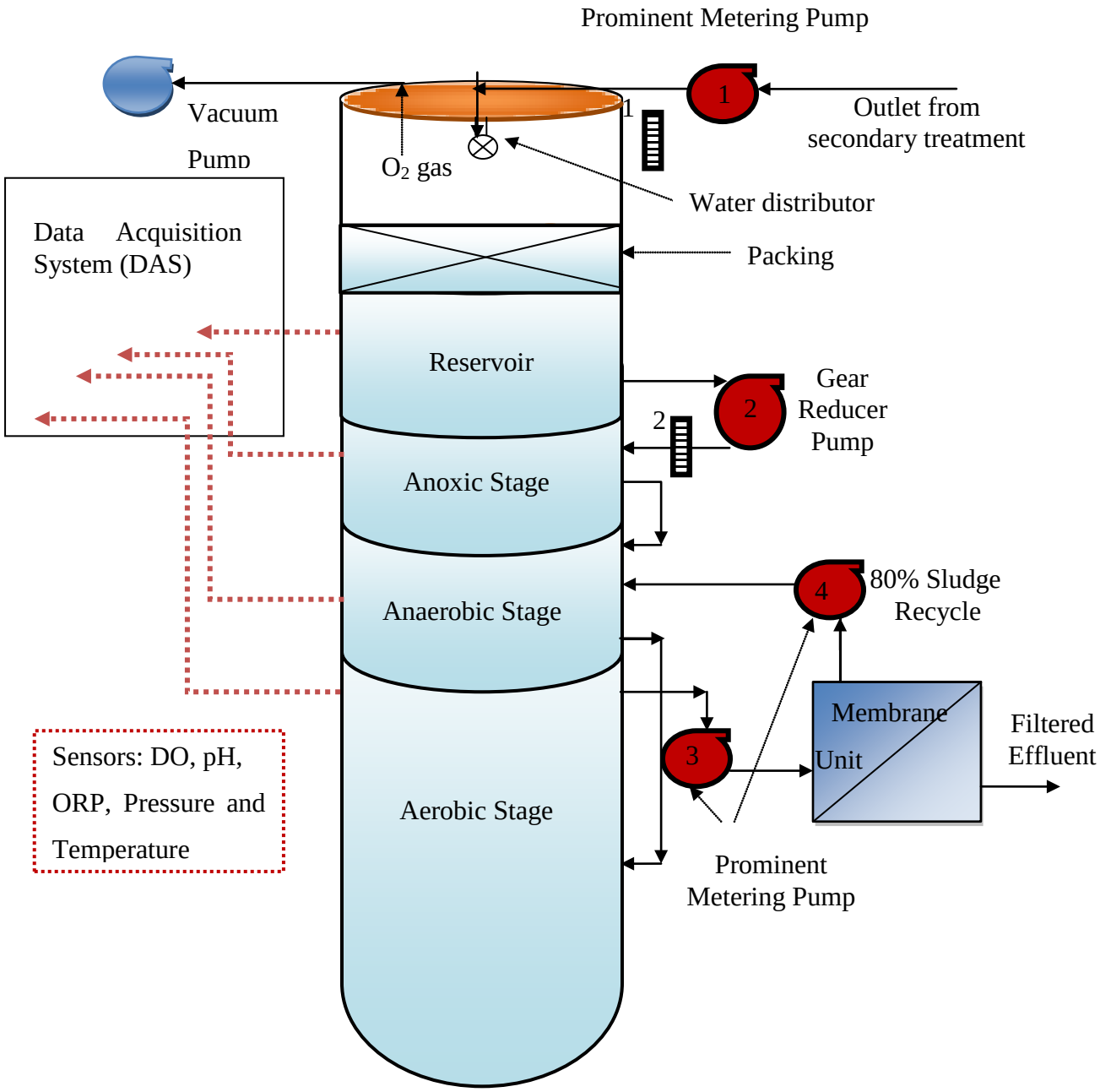


Figure 3.13: Schematic Diagram of CUBEN with Pumps, Sensors and DAS

NPSH Calculation for pump # 2:

H_{VAC}+ H_Z (hydrostatic pressure) = NPSH+ H_{ANOXIC} + H_F (Friction Head)

Assume conservatively H_{VAC} = 0;

H_Z = 0.42 m;

$H_F = (f.l.V^2)/2gD = 4.5\times10^{-6}m \approx 0$;

H_{ANOXIC} = 10.33m (1 atm)

0 + 0.42m = NPSH + 10.7m + 0 NPSH (Available) = -10.28 meter of water (-1 atm)

Negative pressure is a way of expressing pressure measurements below atmospheric pressure. The NPSH (Required) for pump # 2 was provided by the pump manufacturer so that it can overcome the vacuum in the Deaeration stage of CUBEN. Therefore, a specialty pump (gear reducing pump) was purchased to supply small flowrate of 120 L/day and to draw off deaerated water/wastewater from the reservoir section under continuous vacuum. Pump # 1, 3 and 4 shown in figure 3.13 are prominent metering pumps which work under normal atmospheric pressure.

3.4 Automation and On-line Measurements of Various Parameters

The operation of CUBEN is based on the efficiency of the biological processes. DO concentration inside each stage (anoxic, anaerobic and aerobic) is an essential parameter which can jeopardize the CUBEN's efficiency, if it is not properly monitored and controlled. Biological phosphorus removal is optimized by removing DO and nitrate from the Deaeration and Anoxic stages. Preventing the presence of these two electron acceptors in the Anaerobic stage maximizes the performance of the process.

The on-line measurements of the parameter such as DO concentrations, pH, temperature, pressure and ORP as well as spectroscopic analysis of phosphate, nitrate and COD concentrations throughout different stages of CUBEN provide insight into the reactions occurring inside the bioreactor. For instance, phosphorus release in the Anaerobic stage and phosphorus uptake in the Aerobic stage can be monitored using the phosphorus profile in both stages as a predictor of process upsets and they can be used to adjust process operating parameters. Nitrate profile through the Anoxic stage can be used to indicate incomplete denitrification that can result in the presence of nitrate in the influent to the anaerobic zone (Grady *et al*., 1999). A Data Acquisition System (DAS) was used to get in real time measurements of the aforementioned parameters except nitrate, phosphate and COD concentrations which were measured using DRB200 Digester and DR2700^{TM} Spectrophotometer. The performance of CUBEN was monitored and controlled by preparing a concentration profile using recorded data. Figure 3.14 shows the expected concentration profile in all stages of the reactor from efficient municipal BNR plants as reflected in table 3.2. In the following sections the experimental component profile is compared with the predicted profile shown in figure 3.14. The performance of CUBEN processes (i.e. DO removal, denitrification and phosphorus removal) can be determined by comparing experimental and expected profiles.

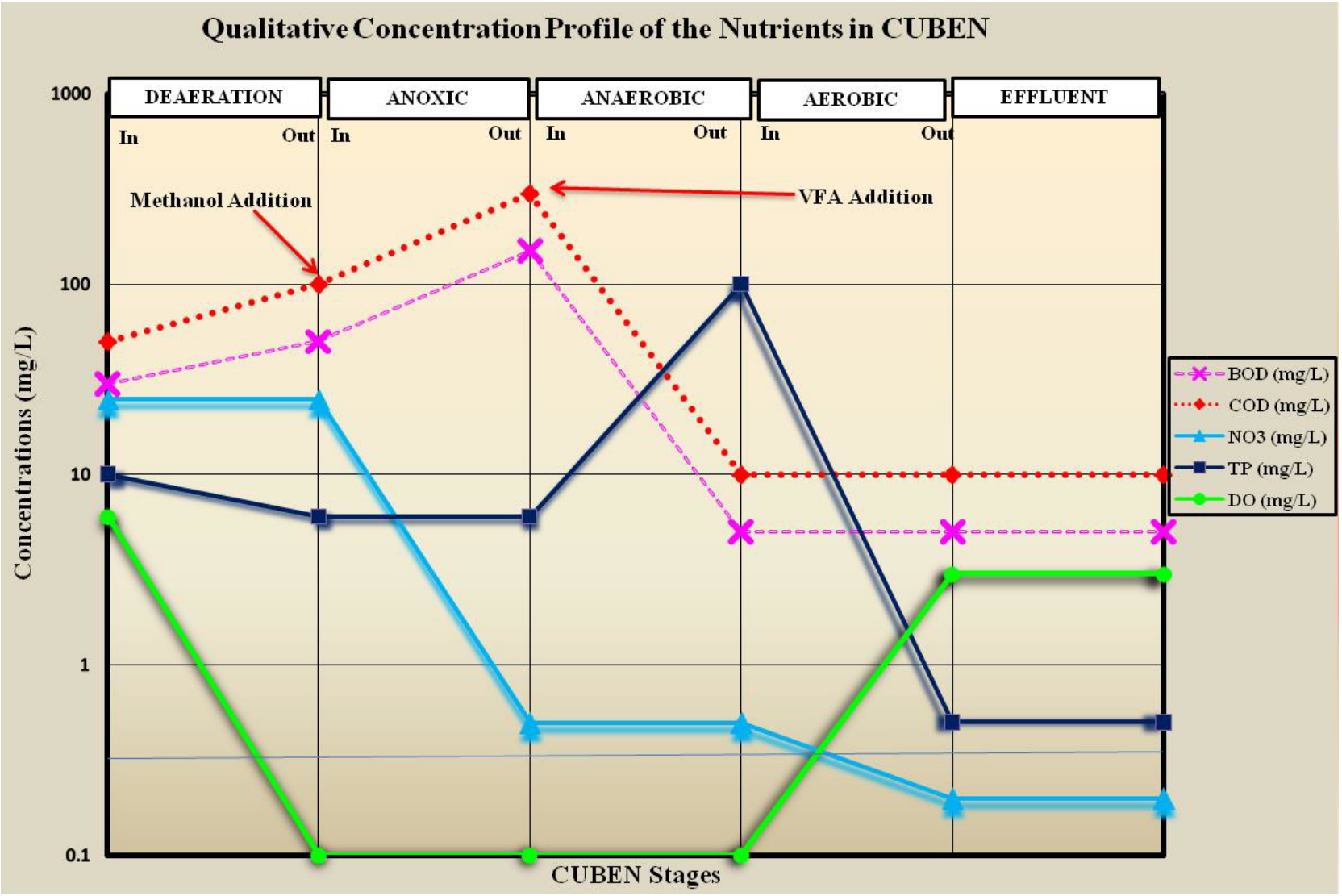


Figure 3.14: Predicted Component Profiles in all stages of CUBEN

3.4.1 Monitoring of pH, Temperature, Dissolved Oxygen (DO) and ORP

Biological nutrient removal using PAOs is a hypersensitive process. In practice, there are several factors that may reduce the efficiency, reliability and stability of this treatment process. These factors can directly influence anoxic, anaerobic and aerobic processes and indirectly influence the overall biological nutrient removal efficiency. These factors include:

- pH
- Temperature
- DO Measurement
- Oxidation-Reduction Potential (ORP)

- pH

The pH level in all four stages of the CUBEN reactor were monitored through installed pH sensors (one sensor for each stage). These sensors were connected to the Data Acquisition System (DAS) using four preamplifiers. The preamplifier device converts the high impedance mV signal of a pH or ORP electrode to a low impedance signal which can be processed by the DAS. Figure 3.15 shows the type of pH sensor and preamplifier used herein. This type of pH electrode is designed to be mounted horizontally on the outer surface of the bioreactor column and is suitable for wastewater pipeline with severe environmental conditions.

Figure 3.15: pH Sensor and Preamplifier Used to Measure the Experimental Data

The NaOH and acetic acid were used to maintain the pH in the range of 6.5-8.0 which proved to be favorable for both denitrification and biological phosphorus removal. Lack of automatic pH controller for this bioreactor was one of the challenges during the experimental period. Installation of an automatic pH controller can highly improve the efficiency of the biological processes inside CUBEN.

- Temperature

Temperature plays an important role in both denitrification and phosphorus removal processes. Temperatures higher than 35^{o}C deteriorate the biological activity of the microorganisms. Also, very low temperature of the solution can reduce the rate of both denitrification and phosphorus removal. To monitor the temperature of the bioreactor, four thermocouples were installed in the four stages of the unit. These thermocouples were also connected to the Data Acquisition System and all the values were recorded every minute during the experimental period. At the start-up, the synthetic wastewater was prepared using hot water to increase the solubility of the salts and chemicals. The use of hot water caused malfunctioning of pump # 2 (Gear Reducer Pump) due to the increase in vapor pressure on the suction side of the pump. Therefore, the temperature of the solution was kept low so that pump 2 could function properly. The bioreactor's temperature data is presented in appendix D.

Figure 3.16: Thermocouples Used in CUBEN

- DO Measurement

Dissolved Oxygen plays an important role in the effective operation of the CUBEN. High oxygen removal in the Deaeration stage (less than 0.1 mg/L) results in the enhancement of the Anoxic stage denitrification and higher phosphorus release/acetate uptake in the Anaerobic stage. Also, proper aeration of the Aerobic stage is an important factor in the overall phosphorus removal capability of the CUBEN. Therefore, continuous DO monitoring in the four stages of this bioreactor is essential. Excessive aeration or limitation of air supply in the Aerobic stage can result in phosphorus concentration increase to tens of milligrams per liter in the effluent. PAOs are in fact large reservoirs of phosphorus. Under adverse conditions, such as lack of oxygen in the Aerobic stage, the phosphate stored in their cells can be released to the water. In this situation, PAOs would perform according to our prediction in the Anaerobic stage. That is VFA

uptaken and converted into PHAs and consequently releasing phosphorus into the liquid phase. However, the uptake of phosphorus will cease in the Aerobic stage since this phosphorus uptake is linked to PHA utilization and oxygen availability. During the operation of CUBEN, DO concentrations were monitored through four DO sensors and controllers. These DO sensors are also connected to the Data Acquisition System and DO concentrations were recorded every second or minute. The following figure illustrates the actual DO meter and sensor used for this bioreactor.

Figure 3.17: Dissolved Oxygen Sensor and Oxygen Meter

- Oxidation-Reduction Potentials (ORP)

ORP is an important parameter in BNR processes and microorganisms are best measured through ORP. The on-line ORP measurement is a simple and cost effective method for process control and optimization of the CUBEN. The ORP value of a solution can be defined by the summation of all the potentials that every ion, compound or element exhibits in a solution. The ORP measurement can help to understand the bacteria's environment. This means that a reductive solution (negative ORP) is capable of donating electrons, while an oxidative solution (positive ORP) is capable of accepting electrons. The environmental condition can be a critical limiting factor in a BNR process and it can be beneficial to know the ORP of the different stages of CUBEN to optimize the processes inside each stage (Dabkowski, 2006).

The ORP value shows if the wastewater is oxidative (positive milli-volt values) or reductive (negative milli-volt values). For bacteria to respire, they need to donate an electron to a final electron acceptor. In the Aerobic stage, this final electron acceptor is dissolved oxygen (an oxidant). In the Anaerobic stage, due to the lack of dissolved oxygen and nitrate, the electron

acceptor is a reducer such as sulphate and/or organic matters. In the Anoxic stage, nitrate (NO_3^-) is the electron acceptor. Therefore, ORP values for the Anaerobic stage must be negative and the ORP of the Aerobic and Anoxic stages of CUBEN must be in the positive range. However, the results obtained throughout the continuous operation of the unit highly contradict the above predictions. Next section of this thesis provides all the details and discussions about the ORP measurements and results obtained during the operation period.

Figure 3.18: CUBEN's pH, ORP and DO Sensors Connected to DAS and Labview Software

3.4.2 Phosphate Measurements Technique

Phosphorus analysis carried out includes two steps:

- Conversion of all type of phosphorus compounds into orthophosphates (PO_4^{3-}) in a digester at 150^oC, and
- Colorimetric determination of (PO_4^{3-}) using a spectrophotometer

The first step was completed by a digestion method using Hach's DRB200 instrument. The second step in phosphorus analysis was completed using Hach DR2700 spectrophotometer instrument. Samples were taken from each stage of the unit including the synthetic wastewater at the inlet, Anoxic stage, Anaerobic stage and Aerobic stage as well as the ceramic membrane's filtrate. Due to regular fouling of the ceramic membrane unit, samples taken from the Anoxic and Anaerobic stages were filtered through 0.45µm pore diameter filter paper to separate sludge from the liquid. The separation through filter paper was not a very precise method however it was acceptable for collecting sufficient experimental data.

A Phosphate Test Kit was used to measure Phosphate concentration of the samples. The test kit included sampling vials which contained a small amount of Acid (the type of acid was not disclosed by the manufacturer). Acid inside the vials were mixed with potassium persulphate powder pillow and 5 ml of samples taken from various stages of the bioreactor. The sampling vials were heated at 150 ^oC to convert the dissolved and particulate phosphates to dissolved orthophosphate (PO_4^{3-}). The digestion of the samples was done using a DRB200 Digester shown in Figure 3.19. After digesting the samples for 30 minutes and cooling them to room temperature, 2ml of NaOH and 0.5ml of Molybdovanadate Reagent were added to each sampling vials and allowed the reaction to continue for 7 minutes. Then digested sampling vials were placed inside DR2700 Spectrophotometer and the concentrations of both orthophosphates and phosphorus bound in orthophosphate molecules (PO_4^{3-}) were measured and recorded. Figure 3.19 shows the type of spectrophotometer that was used for analytical measurements of Phosphorus, Nitrate, COD and Total Suspended Solids (TSS). DR2700 is an advanced instrument with touch screen user interface that can accommodate a wide variety of water/wastewater analytical techniques. A USB stick was also used to transfer data in the Excel sheet format for further study.

DR2700^{TM} Spectrophotometer

DRB200 Digester

Figure 3.19: DR2700TM Spectrophotometer, DRB200 Digester & Test Kits (HACH Instruments)

3.4.3 Nitrate Measurements Technique

NO_3^- concentration in the influent synthetic wastewater was between 24-25 mg/L. This was achieved by dissolving potassium nitrate in water. Nitrate measurement was performed once a day when samples from all stages of CUBEN were taken in small beakers and DR2700 Spectrophotometer (shown in figure 3.19) was used to measure the concentration of nitrates in each sample. Nitrate Test Kit (shown in figure 3.19) was used for nitrate measurement. The method included the addition of 1.00 ml of samples taken from the feed, Anoxic stage, Anaerobic stage and Aerobic stage as well as the ceramic membrane's filtrate and/or 0.45µm filter paper into the NitraVer X Reagent A test tubes. Then NitraVer X Reagent B powder pillow was added to the sampling vials. After five minutes reaction period each vial was inserted into the DR2700 cell holder for concentration reading in mg/L. The chemical mixture inside the

sampling vials (NitraVer X Reagent A and B) and powder pillows could not be identified due to business protection policy of the Hach Company.

3.4.4 COD Measurement Technique

Chemical Oxygen Demand (COD) is an essential parameter in both denitrification and biological phosphorus removal. Excess of COD in the synthetic wastewater can cause a negative effect on phosphorus accumulating organisms by reducing the level of dissolved oxygen in the Aerobic stage. Based on the past experiments conducted by Mahendraker *et al*. (2005) on the impact of influent nutrient and COD concentration ratio on oxygen transfer in biological phosphorus removal process the influent COD to phosphorus ratio of (COD:P=51) proved to achieve better oxygen transfer efficiency (OTE_f) than higher or lower COD:P ratio (Mahendraker *et.al.*, 2005) During the start up, synthetic wastewater included approximately 300 mg/L of COD and 30 mg/L of phosphorus which gives a ratio of 10 (COD:P = 10). This ratio was increased after few weeks of operation to 50 (COD: P=50). The reason for increasing the ratio was to improve the oxygen transfer efficiency (OTE_f) of the BPR process. The results of all the concentration variations are presented in section 4 of this thesis. The COD measurement was conducted using DRB200 (Digester) and DR2700 (Spectrophotometer) shown in figure 3.18. The measurement procedure involved addition of 2ml of sample to a COD digestion reagent vial. Then, the vial was heated using DRB200 Digester at 150^{o}C for two hours. After the digestion, the sampling vial was cooled to room temperature and then was placed inside the DR2700^{TM} spectrophotometer to measure the COD concentrations in mg/L with respect to a blank solution.

COD concentration in the solution came from a mixture of acetic acid, butyric acid, propanoic acid and methanol. The amount of inlet organic carbon was varied as it was the COD concentration during the experimental period. The reason for this variation was to find the optimum COD concentration relative to the population of Denitrifiers and PAOs in the bioreactor. Sodium Hydroxide (NaOH) solution was also added to neutralize the acidic solution and to maintain the pH level of the synthetic wastewater in the range of 7.0-8.5.

3.5 Synthetic Wastewater and CUBEN Inoculation

Synthetic wastewater was prepared daily to enrich the microbial community and analyze CUBEN's performance for nitrate and phosphorus removal. The synthetic solution was made to mimic the effluent from secondary treatment. The solution was a mixture of tap water, various chemicals, minerals and a minor fraction of activated sludge. The bioreactor was first inoculated with sludge taken from the aerobic digester of the Conestoga Meat Packers Inc. wastewater treatment plant in Breslau, Ontario. During the second month of experiments, the bioreactor was seeded with sludge taken from the secondary treatment stage of the Ashbridges Bay Wastewater Treatment Plant in Toronto. Table 3.5 shows the ingredients of CUBEN's feed. The calculations of the amount of chemicals and their concentrations in the solution are provided in Appendix B.

Chemicals	Concentration (volume or mass)	COD	Nitrate (mg/L)	P (mg/L)
Acetic Acid	5-10 ml	Variable	-	-
Butyric Acid	5-10 ml	Variable	-	-
Propanoic Acid	5-10 ml	Variable	-	-
Methanol	10 ml	Variable	-	-
NaOH (Salt)	15 grams in 2L			
KNO_3	4.109g	-	25	-
$KH_2 PO_4$	5.535g	-	-	10
$Na_2HPO_4.H_2O$	5.614g	-	-	10
Na_2HPO_4	5.776g	-	-	10
Minerals				
$NaHCO_3$	34.7 g	-	-	-
KCl	4.5g	-	-	-
$CaCl_2.H_2O$	1.512g	-	-	-
$MgSO_4.7H_2O$	1.512	-	-	-
$FeCl_3$	1.5g /L	-	-	-
Na_2SO_4	0.1 g/L	-	-	-
$ZnCl_2$	0.12g/L	-	-	-
Total Concentration	-	100-400	25	10-30

Table 3.5: Synthetic Wastewater for CUBEN's Operation

Approximately, 126 L of solution was prepared daily to feed the bioreactor with a flowrate of 120 L/day. The synthetic solution in the feeding tank was first pumped to the top of the Deaeration stage and then passed through the Anoxic, Anaerobic and Aerobic stages. Inoculation of the bioreactor was done twice per week during the experimental period. About 450 ml of MLSS were injected into the Anoxic and Anaerobic stages of CUBEN to enhance the denitrification and phosphorus removal processes. The seeding sludge was first filtered to separate the large particles and compounds and then was added to the bioreactor.

Figure 3.20: Conestoga Meat Packers Inc., Breslau, (Aerobic Digester) and Elmira WWTP, ON

The bacterial growth of denitrifiers and PAOs was indirectly measured by the determination of nitrate and phosphate concentrations. The reduction in NO_3^- and P concentrations proved the presence of denitrifiers in the Anoxic stage and PAOs in the Anaerobic and Aerobic stages. However, for precise understanding of the bacterial community inside the bioreactor, samples from the Anaerobic and Aerobic stages as well as the inoculums were sent to the Microbiology Laboratory of the Department of Biology and Chemistry at Ryerson University for microbiological assessment. Fluorescence In Situ Hybridization (FISH) techniques were used to identify and semi-quantify the PAOs community inside CUBEN. Section 4.7 of this thesis provides a detailed and complete protocol and results of the FISH analysis on the anaerobic and aerobic samples taken from the bioreactor on June 21, 2010.

3.6 Start-Up Procedure

The equipment selection, procurement, construction, start-up and troubleshooting of the CUBEN took approximately one year. In the preliminary start-up period only tap water was used to monitor the continuous flow inside the reactor. During the start-up period (January-May 2010) all the sections, valves, pipes and pumps were continuously checked for any flow problems or leakages.

3.6.1 Commissioning of the Experimental System

- The start-up and commissioning of CUBEN involved the following steps: (Please, see figure 3.7 for equipment numbers given below)
- Fill up CUBEN's feed tank with tap water (126 L) [Tank # 1]
- Inspect all the liquid outlet valves and open them fully
- Inspect all the pipes and make sure they are properly connected
- Inspect the air valve[A1] and vacuum valves [V2] and ensure they are properly closed
- Inspect pumps P1, P2, P3 and P4 and make sure their inlets and outlet pipes are properly connected
- Turn on pump P1
- Adjust flowmeter F1to read 120 L/day (4.99 L/h)
- Monitor the inlet pressure gauge (PG) and make sure pressure does not exceed 120 psi (For the flowrate of 120 L/day, the pump P1 pressure must be lower than 120 psi (or 8.2 atm)
- Open the main vacuum valve [V2]
- Fill the Deaeration stage [2C] just below the vacuum gauge [VG]
- Turn on pump [P2] to pump the liquid from the Deaeration [2C] into the Anoxic stage [3]
- Adjust the pump so that flowmeter [F2] reads 120 L/day
- Fill up the Anoxic stage [3]
- Fill up the Anaerobic stage [4]
- Fill up the Aerobic stage [5]
- Turn on the main laboratory air valve [A1]and CUBEN's air valve when the Aerobic stage [5] is filled with liquid
- Inspect the column for any leaks or damages

- Ensure water leaves the column and goes to the drain [V3] through the piping system
- Turn on the main switch for DO meters and Data Acquisition System
- Ensure all the sensors attached to the column are immersed in the water at all times
- Take all the readings (DO, ORP, pH, Temperature) using LABVIEW software
- Ensure CUBEN is reliable and consistent for continuous operation without supervision for at least 3 days

3.6.2 Experimental Protocol

After two months of troubleshooting, CUBEN was fed with synthetic wastewater (table 3.5) and was inoculated with sludge from Conestoga Meat Packers Inc., Breslau, ON wastewater treatment plant.

The experimental protocol follows:

- Prepare the solution in the feeding tank [1] using tap water and the chemicals listed in table 3.5
- Empty the water completely in both CUBEN and the membrane unit
- Turn on pump [P1]
- Open valve [V2] to deaerate the water using Ryerson's central vacuum pump
- Fill the Deaeration stage [2C] just below the vacuum gauge [VG]
- Start pump [P2] and allow water to pass through other stages by gravity
- Turn ON the air valve [A1] when the water level in the Aerobic stage [5]is 3/4 of the maximum level
- The Aerobic stage must be filled with synthetic wastewater up to the outlet of the pipe connected to the membrane
- Inoculate the bioreactor with fresh sludge [8] into the Anoxic [3] and Anaerobic [4] stages
- Add carbon source [9] to into the Anoxic [3] and Anaerobic [4] stages
- Collect the effluent in the collection tank [6]
- Turn ON pump [P3]
- Monitor the membrane unit [7] and collect retentate in the collection tank [10]
- Turn ON pump [P4] to recycle the retentate from the collection tank [9] back into the Anaerobic [4] stage

- Monitor both CUBEN and the Membrane unit and record (DO, P, PO_4^3, NO_3^-, COD, TSS) concentrations from the following stages: Feed tank [1], Deaeration stage [2C], Anoxic stage [3], Anaerobic stage [4], Aerobic stage [5], Membrane's Permeate and Retentate [7]

During the operation of CUBEN combined with membrane unit, it was necessary to resolve a number of problems listed below:

1. **Problem:** Obtaining a pump to be able to overcome the continuous vacuum and provide the right flowrate (120 L/Day)

2. Solution: This problem was resolved after contacting over 50 different pump manufacturers in Canada and USA. Only one company could provide us with the appropriate type of pump (gear reducer)

3. Problem: Nozzle and pipe clogging due to the accumulation of large particles

Solution: Removal of the nozzle to prevent accumulation of solids

4. Problem: Pipe leakage

Solution: Adjust Fittings

5. Problem: Frequent Membrane fouling due to high TSS concentration of CUBEN's effluent

Solution: Diluting the CUBEN's effluent to reduce TSS concentration

6. Problem: Sludge collection tank overflow or lack of feed solution due to high flowrate of pump # 1 or low flowrate of pump # 2

Solution: Proper adjustment of pumps and flowrates

7. Problem: Sludge bulking which was an indicative of settlement problems

Solution: Exclusion of east extract from the chemicals used in synthetic solution

8. Problem: Presence of struvite (Magnesium Ammonium Phosphate) inside the Bioreactor and the pipelines

Solution: pH reduction within the range of 6.5-7.5 could reduce the formation of struvite. Also, elimination of ammonium salt from synthetic wastewater reduced the production of struvite in the solution.

9. **Problem:** Low phosphorus removal due to slow growth rate of PAOs

Solution: Longer operational period is required to establish a successful and stable BPR

EXPERIMENTAL RESULTS AND DISCUSSIONS

4.0 EXPERIMENTAL RESULTS AND DISCUSSIONS

After the completion of the commissioning, the proper experimental work began. The CUBEN operation was tested for about two months under varying inlet flowrates (90L/day-300 L/day), varying inlet phosphorus (30mg/L-10 mg/L) and different organic loadings. At the beginning, it was very difficult to find an appropriate pump that could work against continuous vacuum and provide targeted 120 L/day of flowrate. Resolving this flow issue to achieve consistent and steady state flow demanded equipment and operational modifications for several months. The unit was started up with synthetic wastewater and was inoculated with actual sludge from the aerobic digesters of the onsite wastewater treatment plant belonged to Conestoga Meat Packers Ltd. in Breslau, ON. During the second month of operations, the unit was inoculated with the sludge taken from secondary treatment stage of the Ashbridges Bay Wastewater Treatment Plant.

An excellent degree of denitrification was observed throughout the experimental operation. The inlet concentration of nitrate was kept constant at 25 mg/L. Approximately 10 ml of pure methanol was added directly to the anoxic stage during the start-up period in order to maintain the high denitrification performance. Due to the excellent performance of the Vacuum stage, nitrate concentrations were reduced drastically from 25 mg/L to less than 1 mg/L in the Anoxic stage and reached to less than 0.1mg/L in the lower stages.

On the other hand, as expected the phosphorus removal process in CUBEN was difficult to sustain and improve during such a short operational period. This was due to the hypersensitivity and slow growth rate of the PAOs involved in this process. The adequate PAOs concentration largely determines the phosphorus removal capacity of a BNR unit. As it was mentioned earlier, it practically takes 40 to 100 days (Patrick *et.al.,* 2005) for a biological phosphorus removal process to become stable, reliable and efficient. CUBEN was only operated for about two months during which numerous operational modifications were conducted to improve the P removal performance. This is a limited time to achieve high phosphorus removal efficiency above 90%. Although, the objective to show that CUBEN could carry out biological phosphorus removal was achieved but efficient and stable phosphorus removal required a much longer operational period. For many researchers, the enhanced biological phosphorus removal process is viewed as a black box whose behavior can only be determined after many months of

operation. The microbial decay and deterioration of PAOs may occur unexpectedly which consequently results in decline of the biological phosphorus removal.

In order to improve the phosphorus removal process inside the unit, a series of microbiology analysis including identification and quantification tests were performed on the samples taken from Anaerobic and Aerobic stages of the CUBEN as well as the original sludge sample. Fluorescence in Situ Hybridization (FISH) analysis with rRNA-targeted probes was conducted on the samples to identify the type of PAOs inside the unit. Furthermore, the quantification of the desired bacteria was performed by image analysis (microscopic) of the hybridized fixed cells. The results of the aforementioned analysis and techniques are explained in the following sections.

4.1 ORP, pH, Pressure and Temperature Experimental Results

The Oxidation-Reduction potentials of all the stages of the bioreactor were recorded using ORP sensors attached to the column and Data Acquisition System. The purpose for using ORP sensors was to optimize the parameters affecting the microbial community inside each stage among which the presence of a carbon source was the most important parameters of concern. In general, the ORP of a solution is an indication of its ability to oxidize or reduce another solution. It can also be defined as the sum of all the potentials in the water which can be represented by positive or negative milli-volt values. For bacteria to respire, they need to donate an electron to a final electron acceptor. In the Anoxic phase, the electron donor is nitrate molecules and the electron acceptor is usually the carbon source. In this experiment, the carbon source was the combination of Butyric, Propanoic, Acetic Acids as well as Methanol. The ORP in the Anoxic stage should be between – 100 to +100 milli-volts (mv) to ensure a neutral environment for denitrification to take place. However, the recorded ORP values in the Anoxic stage of the CUBEN were lower than -100 mv during the several months of experimental work. During the start up period the ORP values in this stage were approximately -110.7 mv but as the time passed it decreased to -500 mv. This was an indication of highly reductive solution due to the excess amount of carbon source compared to the denitrifying bacterial community in this stage. Even though, the Anoxic stage was highly reductive (acidic), there was a complete denitrification process throughout the experimental operation of the unit.

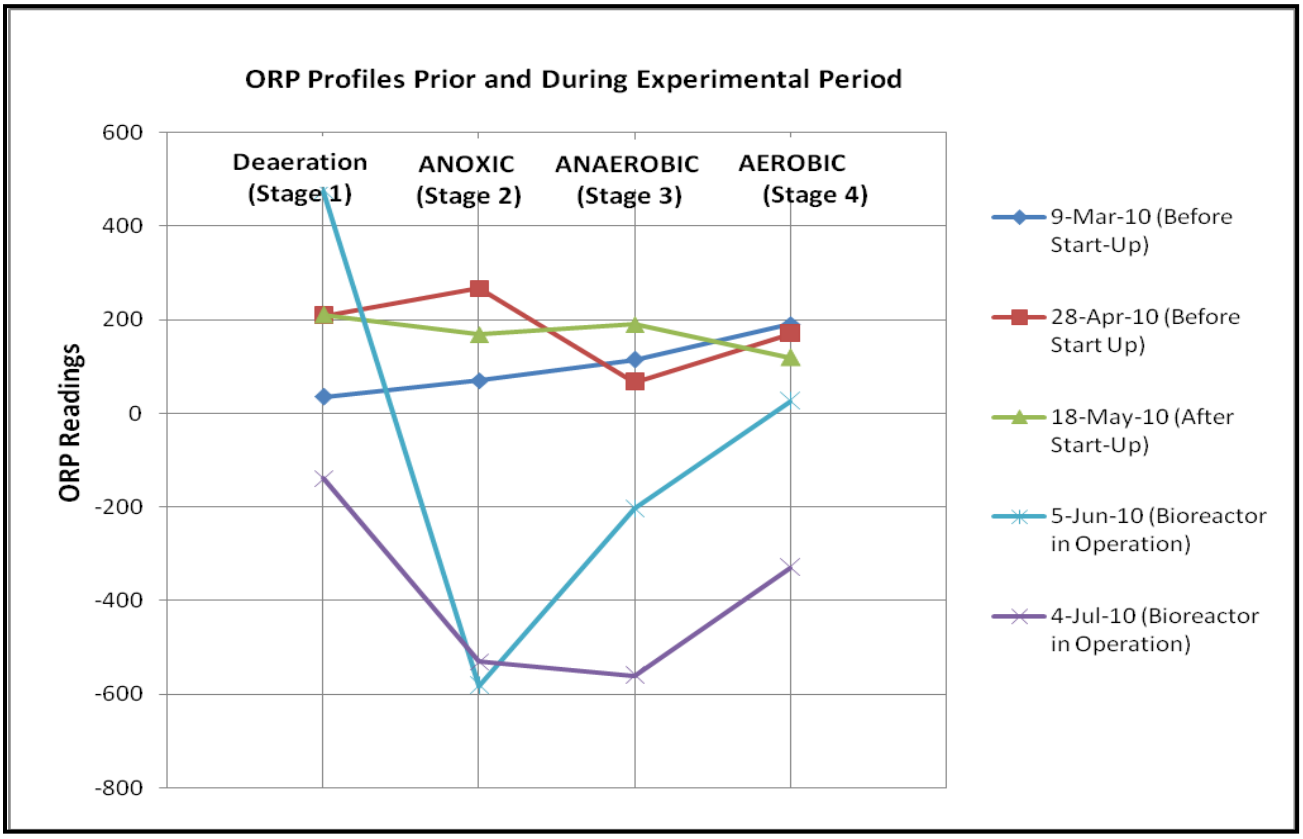
In regards to the Anaerobic stage, where acetate uptake took place, the recorded ORP values from May (start-up month) until July were well below -150 mv which was recommended by the ORP sensor manufacturer. This is an excellent result indicating the presence of a high amount of carbon source for PAOs in this phase. ORP sensors are not normally used in the Aerobic stages of industrial BNR plants since Dissolved Oxygen (DO) sensors are better and more efficient indicators of the environmental conditions in this phase. However, an ORP sensor for the Aerobic stage of CUBEN was included to improve and maintain the proper ranges of parameters such as COD concentration. The following table (Table 4.1) summarizes the ORP reading from stages one to four during March until July 2010. Since ORP values were recorded every minute for 24 hours every day, the table below shows only a small portion of the collected data. In March and April, CUBEN was operating continuously with the tap water for troubleshooting and pump calibration purposes. This is the reason for having positive ORP values for all four stages of the bioreactor. The unit was started up in the month of May when synthetic wastewater was used and the unit was inoculated with sludge from the wastewater treatment plant. ORP values in this month showed high inconsistency and values sharply increased or decreased within a short time period that is due to the unsteady state condition of the processes (Denitrification and phosphorus removal) during this month. In the months of June and July, we see negative values which are indications of reductive solutions in those stages.

Dates → / Process Stages ↓	**9-March-10**	**28-April-10**	**18-May-10**	**5-June-10**	**4-July-10**
Deaeration	35.5	209	211	475.7	-138
Anoxic	71	268	169	-581.8	-530
Anaerobic	114.7	66.5	190.4	-204	-560
Aerobic	191	170	120	205.8	-330

Table 4.1: Oxidation Reduction Potentials (March till July 2010)

Graph 4.1 illustrates the profiles of the average ORP data collected during March until July 2010 with values provided in Table 4.1. During the month of June, ORP values were drastically reduced in the Anoxic stage and then moved up in the later stages which are a sign of reduction in the amount of carbon source as the solution passed from the Anoxic to the Aerobic stages. These trends are indications of both denitrification and phosphorus removal processes. In the Aerobic stage, ORP value is above zero which means that there was no COD concentration

available in this stage. This is one of the requirements (Lack of COD in the Aerobic stage) for biological phosphorus removal and ORP sensors could confirm the occurrence of BNR inside the unit. However, in July 2010, ORP data collected in all stages of the bioreactor is in the negative side. Especially, in the aerobic stage, the negative data show excess amount of carbon source with respect to the number of PAOs which consequently indicates the decrease in phosphorus removal efficiency of the bioreactor.



Graph 4.1: ORP Profiles in CUBEN (March-July 2010)

Table 4.2 and 4.3 summarize pH, Temperature, and Pressure data recorded in all four stages of CUBEN during March-July 2010. Pressure sensors were only installed in the Deaeration and Anoxic stages. The Anaerobic and Aerobic stages were under atmospheric pressure therefore pressure sensor for these stages weren't necessary. The temperature in stages 1 to 3 seems to be constant but there is a small reduction in the aerobic stage. This is due to the aeration of this stage which lowers the temperature of the effluent. This change in temperature is normal and did not interfere with the biological process taking place in this stage. 50-63

Dates → / Process Stages ↓	Parameters	9-March-10	28-April-10	18-May-10	5-June-10	4-July-10
Deaeration	Temperature (°C)	27.1	24.2	27	25.7	24.7
	pH	8.2	9.2	7.5	8.7	8.5
	Pressure (atm/In-Hg/cm-Hg)	(0.84/25/ 63.5)	(0.70/21/ 53.3)	(0.75/22.5/ 57.1)	(0.73/22/ 55.9)	(0.4/12/ 30.5)
Anoxic	Temperature (°C)	26.3	26.0	29.7	27.1	26.5
	pH	7.7	8.3	5.3	8.6	8.5
	Pressure (atm)	0.98	0.8	1.03	0.83	0.9
Anaerobic	Temperature (°C)	25.3	25.7	29.4	25	25.4
	pH	7.2	7.7	5.2	7.7	7.6
Aerobic	Temperature (°C)	14.5	24.6	28.3	21	18
	pH	7.9	8.3	6.4	9.5	5.8

Table 4.2: Temperature, pH and Pressure Recorded Data (March-July 2010)

4.2 Dissolved Oxygen (DO) Concentration Results

Dissolved oxygen concentration plays a central role in BNR process. DO concentrations in all four stages of CUBEN were recorded every minute before the start-up of the unit to ensure that oxygen is removed in the deaeration stage and well supplied in the aerobic stage. DO Concentrations were also recorded throughout the experimental operation period from May 17 until July 16, 2010. Dissolved Oxygen concentrations were logged every minute using 4 oxygen sensors connected to the Data Acquisition System. DO concentrations in stages one through four are well represented by the predicted profile shown in figure 3.14. Below (Table 4.3) represents the average DO concentrations in the four stages of CUBEN from February until July 2010.

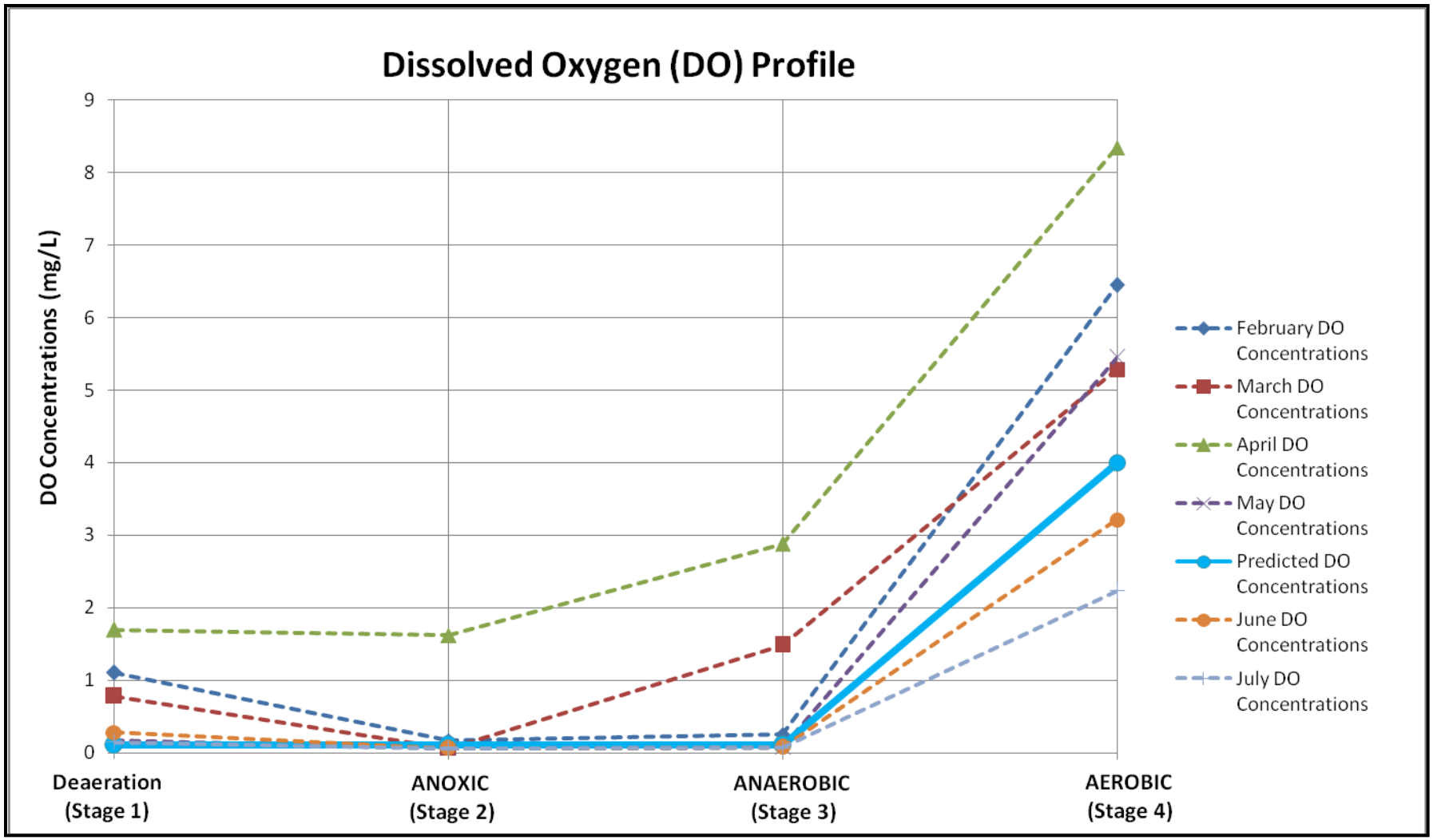
Dates → / Process Stages ↓	18-Feb (mg/L)	10-Mar (mg/L)	27-Apr (mg/L)	20-May (mg/L)	9-June (mg/L)	9-July- (mg/L)	Objective
Deaeration	1.1	0.78	1.70	0.17	0.28	0.15	0.1
Anoxic	0.17	0.10	1.6	0.06	0.06	0.06	0.1
Anaerobic	0.25	1.5	2.9	0.07	0.07	0.07	0.1
Aerobic	6.5	5.3	8.4	5.5	3.2	2.2	2.5-3.5

Table 4.3: Dissolved Oxygen Concentration (February to May 2010)

Based on the literature review of various papers published on EBPR, there is a general correlation between DO concentrations, intercellular stored PHA formation in the anaerobic phase and phosphorus uptake in the aerobic phase. Experience in numerous full scale plants has shown that very high DO concentrations (4.5-5.0 mg/L) in the aerobic stage results in low phosphorus removal. However, DO concentrations of approximately 2.5-3.5 mg/L have exhibited greater abundance of PAOs and consequently higher phosphorus removal (Oehmen, 2007). Therefore, initial high DO concentration might be one of the reasons for low phosphorus removal performance of the unit. During the second month of the experimental operation, the DO concentration was reduced from approximately 5 mg/L to about 3 mg/L. It was very difficult to adjust the DO concentration to remain within the range of 2.5-3.5 mg/L due to the large size of installed air diffuser relative to the volume of the aerobic stage. Oxygen transfer in the aerobic stage was influenced by many variables such as high concentration of soluble and particulate contaminants as well as biological entities. Also, oxygen transfer was highly affected by the biochemical reactions that took place in this stage. In addition, solids retention time (SRT), hydraulic residence time (HRT), organic loading rate and biomass recirculation rates affected oxygen transfer in the aerobic phase (Mahendraker, 2005). Due to the complexity of the process involved and the time constraints of an MASC. thesis, oxygen uptake rate (OUR) by PAOs and oxygen transfer testing could not be accomplished. These can be the basis for future research studies.

The DO concentrations in other stages of CUBEN including Deaeration, Anoxic and Anaerobic stages were very satisfactory. This is due to the excellent performance of the vacuum

stage in removing DO from the inlet synthetic wastewater. Graph 4.2 illustrates the dissolved oxygen profiles from February to July 2010 provided in Table 4.3. The DO profiles show that the experimental trends approach closely the predicted DO profile over time. This is due to several months of troubleshooting of the bioreactor. Before the start-up period (February to April), CUBEN was continuously running with tap water. The reason was to calibrate the pumps, find leaks in the piping system and monitor oxygen removal using vacuum pump. The maximum vacuum available using Ryerson's central vacuum pump varied from 50-63 cm-Hg. The average oxygen concentrations in the Deaeration, Anoxic, Anaerobic and Aerobic stages before the start-up period were 1.2, 0.62, 1.54, and 6.7 mg/L respectively. However, after start-up and inoculation of the unit there was a dramatic reduction in the oxygen concentrations in all four stages of CUBEN. This reduction was expected since inoculums were full of heterotrophic bacteria and oxygen is the primary means for their respiration. The average DO concentrations after the start up period in the Deaeration, Anoxic, Anaerobic and Aerobic stages of CUBEN were 0.14, 0.06, 0.07 and 3.6 mg/L respectively.



Graph 4.2: Dissolved Oxygen Profile in CUBEN

4.3 Denitrification Process Results

Nitrate is one of the nutrients of concern which was present in the synthetic wastewater as a result of dissolution of Potassium Nitrate in water. The influent concentration of NO_3^- was maintained between 24-25 mg/L throughout the experimental period. Denitrification process in the anoxic stage (2^{nd} stage) began approximately three days after start-up of the unit. The Anoxic stage was inoculated with fresh sludge collected from Conestoga Meat Packers Ltd., Wastewater Treatment Plant. The effluent NO_3^- concentration at the beginning was about 4-5 mg/L which showed almost 80% removal. After one week from start-up date, denitrification efficiency reached 98-100% removal. The denitrifiers responsible for the denitrification process showed a remarkable adaptability to the new environment caused by synthetic wastewater, carbon source, temperature and pH. To maintain the high nitrate removal efficiency of the unit, pure Methanol (about 5-10ml) was added directly to the Anoxic stage. Another important factor in the high denitrification rate was the inclusion of packing in this stage. The presence of Hydroxyl-Pac media in the Anoxic stage in other words, denitrification via biofolm formation offered several advantages compared to suspended growth denitrification. The following advantages of the biofilm development in the Anoxic stage were found to be the key elements in the successful denitrification process in CUBEN.

- Protection against washout of slow growing bacteria under high inlet flowrate or low hydraulic residence time
- Attached microbial community on the surface of the packing have interspecies interaction that is beneficial for the individual denitrifying bacteria
- Presence of packing in the anoxic stage provides higher surface area and consequently increases the concentration of the denitrifiers in this stage
- Maintain the population of denitrifiers in this stage which results in high nitrate removal
- The biofilm formation of denitrifiers on the surface of the packing reduces their flow to the Anaerobic stage thus avoiding the interference of the denitrifiers in the phosphorus removal process
- It provides an extremely cost-effective retrofit solution for future expansion of the unit
- Existence of high-density population of fixed film bacteria requires less Mixed-Liquor Suspended Solids (MLSS) which consequently reduces the sludge loading generation

The wastewater passing through the Anoxic stage in CUBEN facilitated by the biofilm undergoes complex chemical and biochemical transformation. The biofilm consists of several layers of bacteria which attach themselves to the surface of the packing and absorb from the fluid the nutrient essential for their survival. Since, there was no oxygen available in the Anoxic stage, the biofilm made of denitrifiers broke down the oxygen molecules from NO_3^- and released nitrogen gas. The following figure (Figure 4.1) shows the type of packing used inside the Anoxic stage. Hydroxyl-Pac media provides integrated fixed-film activated sludge (IFAS), provides high interfacial area for biomass growth therefore reducing the volume of the stage.

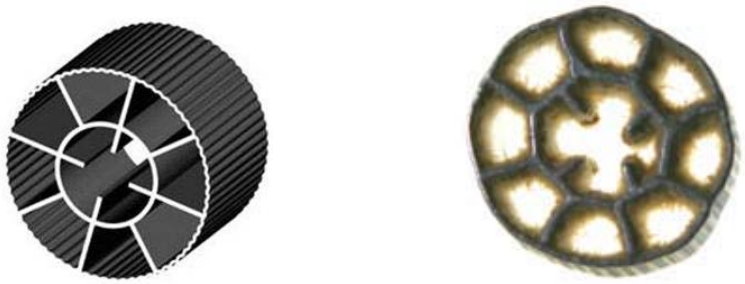


Figure 4.1: Hydroxyl-Pac Media (Headworks® BIO, IFAS)

Hydroxyl-Pac media is a polyethylene biofilm mobile carrier which was fully immersed in the fluid and covered by the biofilm. As it was mentioned earlier, this structure of the packing provided significant treatment performance within the Anoxic stage and yielded outstanding overall denitrification performance.

The following Table 4.4 illustrates some of the data collected during the course of experimental work. The denitrification process started soon after the inoculation of the bioreactor with sludge and showed excellent performance day by day to maximum of 98-100% efficiency. The nitrate concentration tests were conducted daily starting May 17, 2010 until July 12, 2010. All the data points collected during the two months of bioreactor operation are given in Appendix D.

As it can be seen from Table 4.4, denitrification took place not only in the Anoxic stage but also in later stages of CUBEN (Anaerobic and Aerobic) which resulted in the overall nitrate removal of 98-100%. This proves the presence of denitrifiers along with the presence of PAOs in both Anaerobic and Aerobic stages. These results reveal three outcomes:

- Integration of both denitrifying bacteria and phosphorus accumulating organisms (PAO) in the same environment (anaerobic and aerobic phases)

- Presence of denitrifying PAOs in the Anaerobic and Aerobic stages responsible for both denitrification and phosphorus removal processes
- Integration of both denitrifying PAOs (DPAOs) and normal PAOs in the two later stages

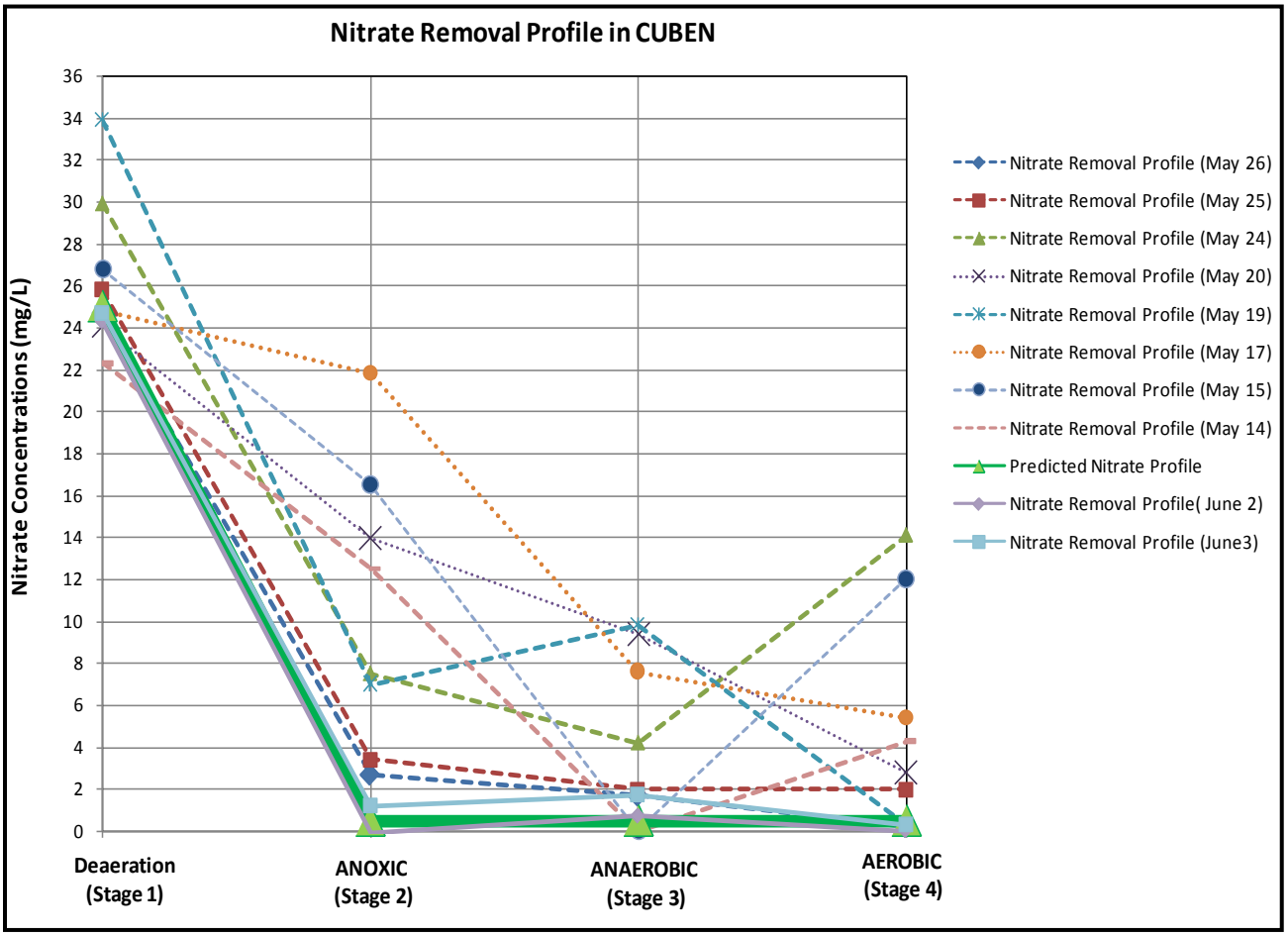
Dates → / Stages ↓	17/5/ 2010	18/5/ 2010	19/5/ 2010	21/5/ 2010	22/5/ 2010	24/5 2010	25/5/ 2010	26/5/ 2010	4/6/ 2010	6/6/ 2010
Feed Nitrate Concentration (mg/L)	24.8	33.9	24.1	25.8	24.7	25	24.3	24.7	23.7	21
Anoxic Stage Concentration (mg/L)	21.8	7	14	3.4	2.7	13.5	0	1.2	0	2.6
Anaerobic Stage Concentration (mg/L)	7.6	9.8	9.4	2	1.7	4.2	0.7	1.7	0.6	0
Aerobic Stage Concentration (mg/L)	5.4	0.3	2.8	2	0.2	3.9	0	0.3	0.1	0.3
% Removal	78	99	88.4	92.2	99.2	84.4	100	98.8	99.6	98.6

Table 4.4: Nitrate Concentrations throughout all stages of CUBEN

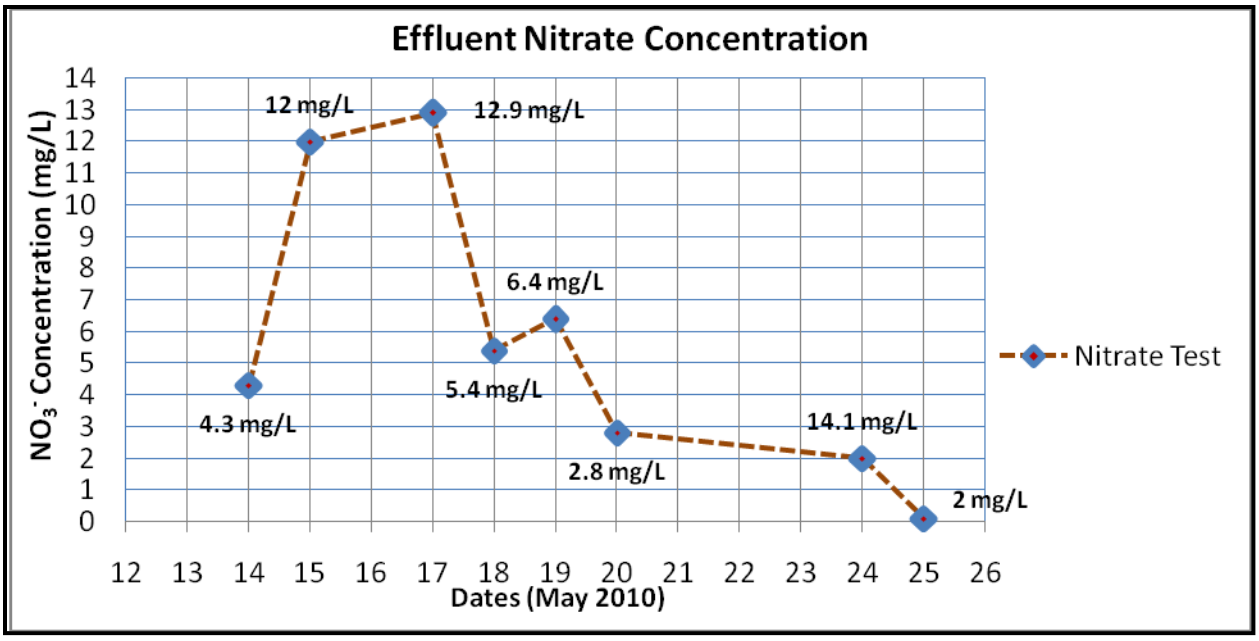
Phosphorus accumulating organisms are capable of using nitrate as electron acceptor when there is no oxygen available. Dissolved oxygen is completely removed from the solution in the Deaeration stage and small concentration of nitrates is present in the Anoxic stage effluent which is the Anaerobic stage influent. The denitrification in the Anaerobic stage might have been due to the presence of DPAOs. Based on past experiments, DPAOs are not as efficient as PAOs in removing phosphorus from wastewater but are excellent denitrifiers. Also, DPAOs utilize more carbon source for their cellular metabolism than normal PAOs. The presence of DPAOs in

the bioreactor might affect the phosphorus removal deficiency of the unit. This will be discussed in more details in section 4.3.

Graph 4.3 illustrates the nitrate removal profile throughout various stages of CUBEN for different dates. It also compares the removal profiles with the predicted nitrate profile which is highlighted with a solid, thick green line. The experimental nitrate removal results closely represent the predicted profile as the operating conditions are established. The inlet concentration of nitrate was varied to investigate the effect of the inlet nitrate concentration on the performance of denitrifying bacteria. Initially, high inlet nitrate concentration decreased the efficiency of the unit. However, denitrifiers quickly adapted to the high concentration and after few days the unit performance experienced a remarkable improvement. During the last month of the experimental period, the inlet nitrate concentration in the feed was fixed at 25 mg/L and denitrification process continued to take place efficiently. The end results showed a close trend between the predicted profile (solid green line on the graph) and the experimental nitrate profile.

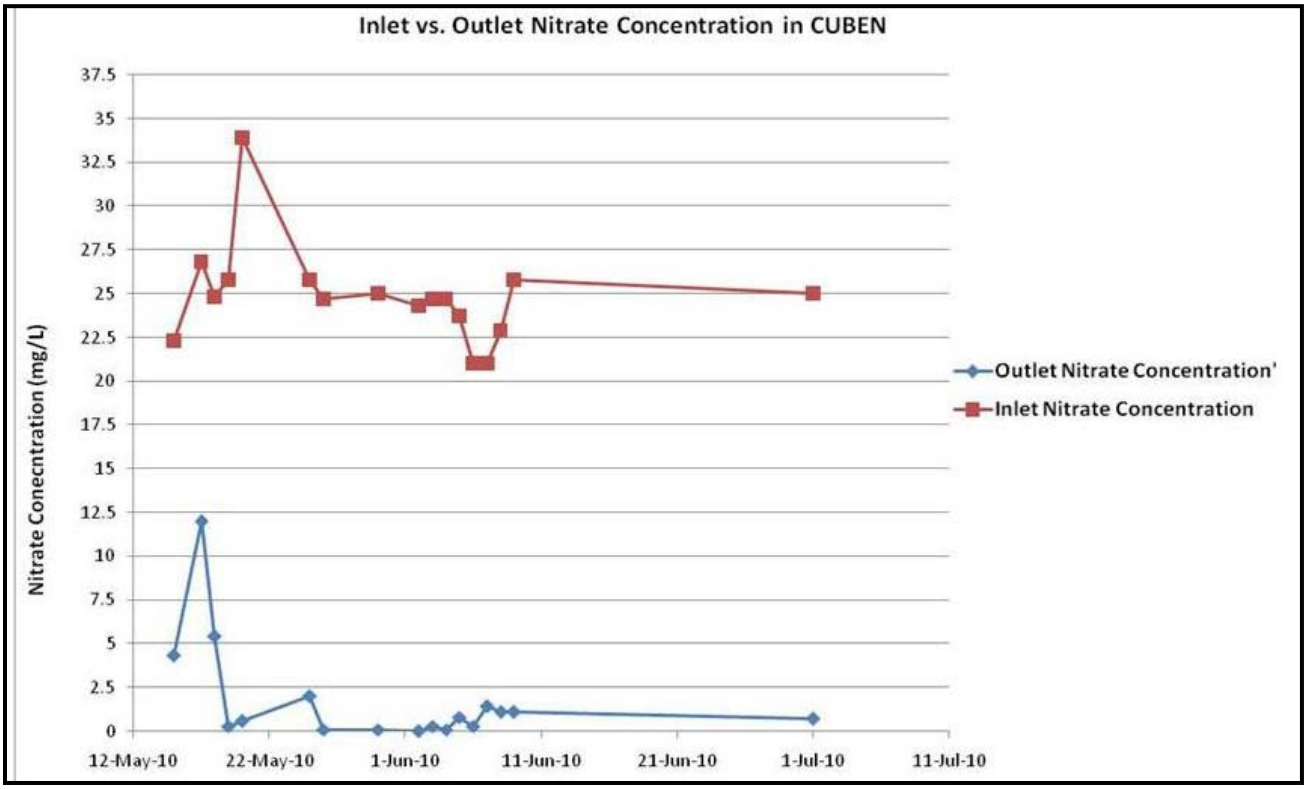


Graph 4.3: Nitrate Removal Profile in CUBEN



Graph 4.4: Effluent Nitrate Concentration Profile in CUBEN (May 2010)

Graph 4.5 highlights inlet and outlet nitrate concentration. There is a similarity between influent and effluent nitrate concentration trends. Also, this graph shows a tremendous reduction in outlet nitrate concentration which ultimately approached the predicted value of 0.1 mg/L.



Graph 4.5: Inlet vs. Outlet Concentration of Nitrate in CUBEN

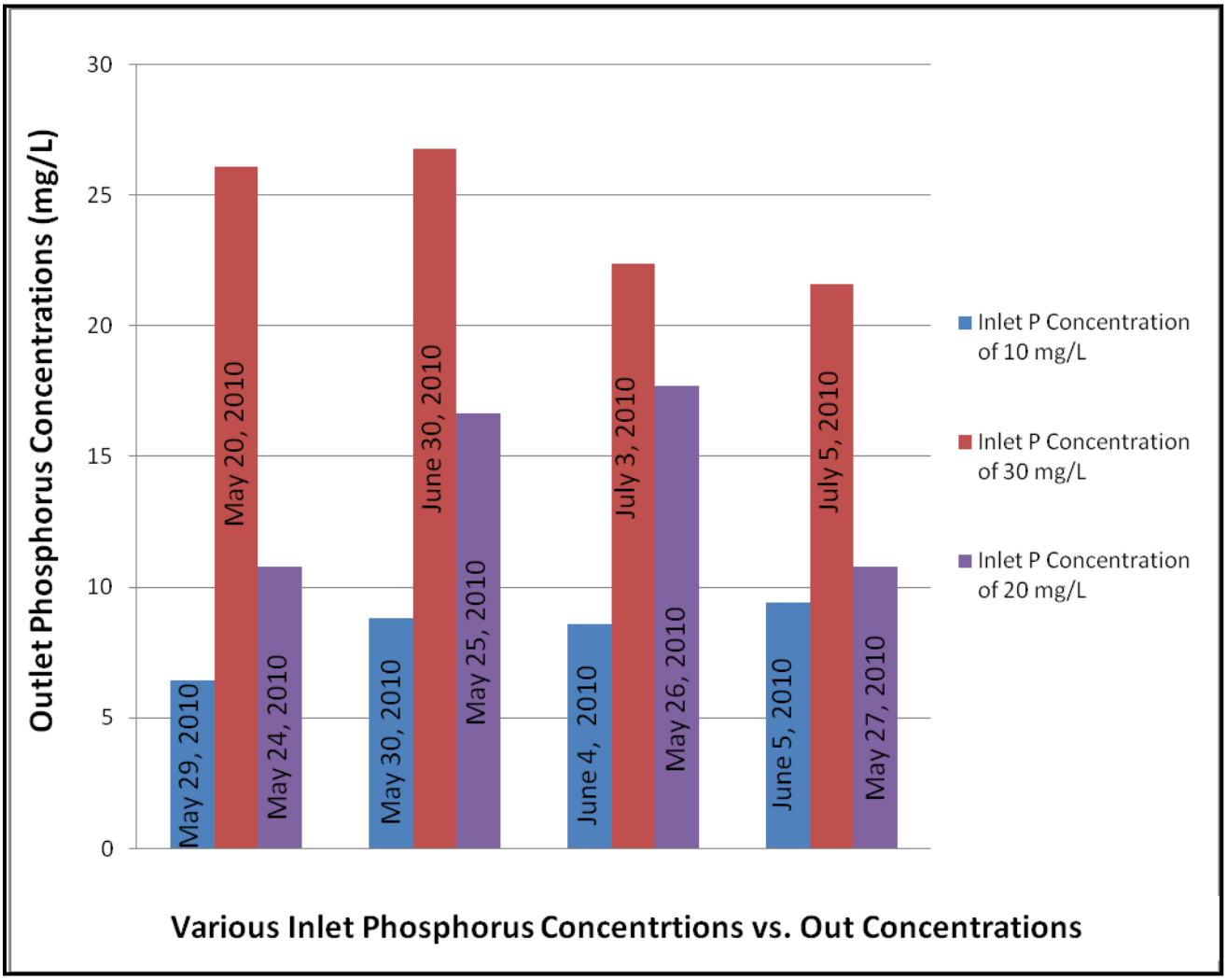
4.4 Phosphorus Removal Results

Biological phosphorus removal in the Anaerobic and Aerobic stages of CUBEN took place several days after the inoculation of the bioreactor with fresh sludge. The phosphorus removal development was much slower than denitrification since Phosphorus Accumulating Organisms (PAOs) slowly adapt to the new environmental conditions in the Anaerobic and Aerobic stages. The inoculum used for the start-up of the bioreactor was taken from the aerobic digester of Conestoga Meat Packers Inc. wastewater treatment plant. It consisted of mixed microbial community which was not enriched with PAOs culture. The enrichment of PAOs after bioreactor's inoculation was a long term process and could not be fully accomplished during the commissioning of the unit. As it was mentioned earlier, biological phosphorus removal process and in particular PAOs responsible for phosphorus removal are hypersensitive and require long term operation to reach steady state. This is due to their slow growth rate of 0.04/day and selective behavior. Many of the environmental parameters such as inlet phosphorus, pH, COD and DO concentration were manipulated during the commissioning period to enrich the PAOs and improve the overall phosphorus removal. Table 4.5 shows some of phosphorus concentration results.

Dates → / Stages ↓	19/05/ 2010	24/05/ 2010	29/05/ 2010	1/06/ 2010	2/06/ 2010	3/06/ 2010	4/06/ 2010
Fresh Feed	30	20	10	16.8	16.45	16.1	10
Stage 2 (Anoxic)	27.3	27.5	11.6	17.55	41.5	16.4	10.6
Stage 3 (Anaerobic)	29.3	10.5	9.6	39.2	47.9	15.6	10.7
Stage 4 (Aerobic)	26.8	12.8	8.6	12.3	13.1	13.7	10.1
Membrane's Permeate	n/a	10.8	6.4	8.4	6.4	-	8.6
% Removal	13.5	46	36	50	61	15	12.2

Table 4.5: Phosphorus Concentration in all stages of CUBEN with Various Phosphorus Inlet Concentrations

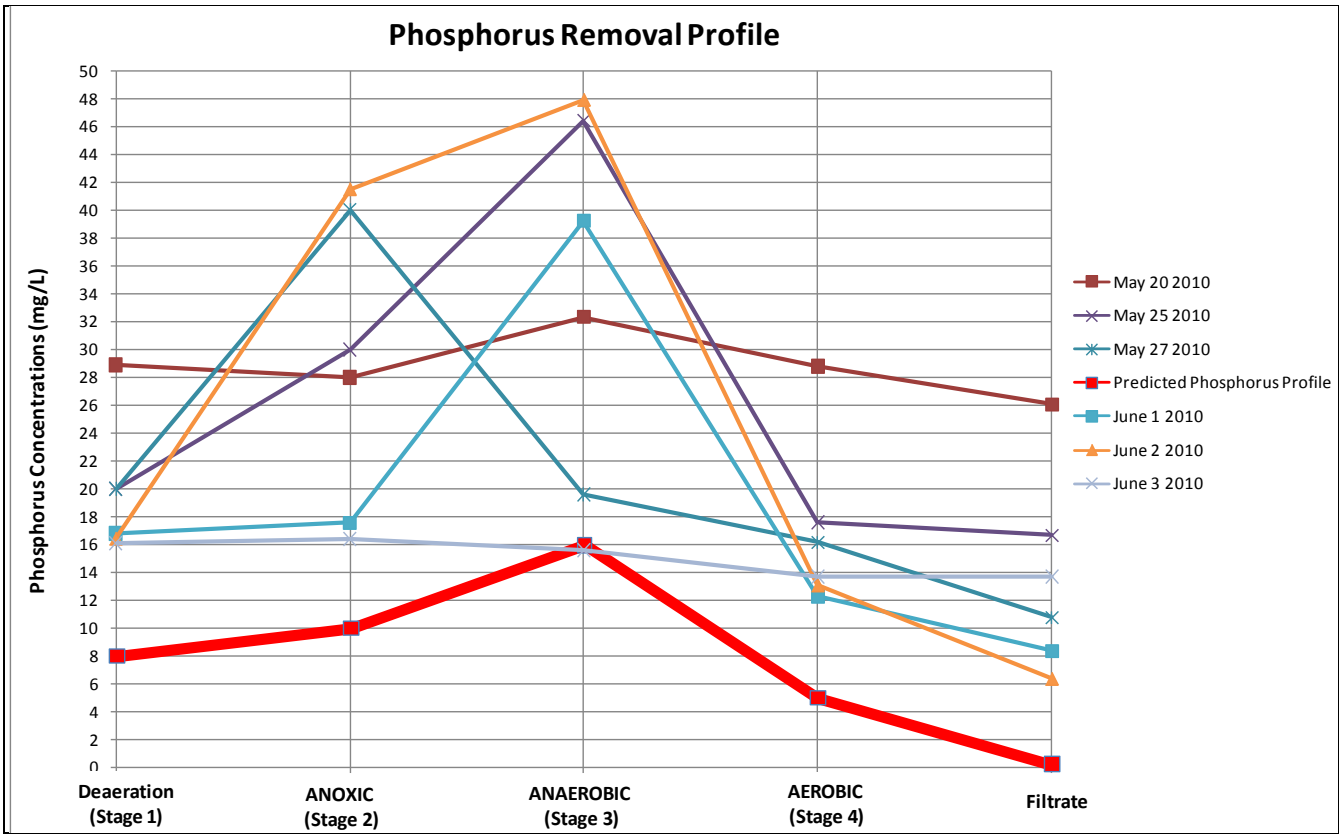
The phosphorus inlet concentrations were varied to obtain the optimum phosphorus concentration required for the PAOs enrichment. Table 4.5 illustrates the effluent phosphorus concentration relative to the inlet phosphorus concentrations of 10, 16, 20 and 30 mg/L. All the other parameters such as COD (300-350mg/L), Aerobic stage DO concentration of 4 mg/L, pH (6.5-7.5), temperature (21^{o}C) and synthetic feed constituents were kept constant for proper comparison. Based on Graph 4.6 and Table 4.5, inlet phosphorus concentration in the range of 16-20 mg/L provided higher removal efficiency compared to 10 and 30 mg/L inlet concentrations.



Graph 4.6: Inlet Phosphorus Concentrations Effect on Phosphorus Removal Efficiency

As it was mentioned in the literature review, there is a correlation between the inlet P and the amount of COD and/or VFA added to the Anaerobic stage. The experimental studies on biological phosphorus removal process have shown various results for optimum ratio of

COD/Inlet Phosphorus (mgCOD/mgP). These studies include 10 mgCOD/mgP (Wong et al., 2004), 15 mgCOD/mgP (Ohmen *et al*., 2005), 27 mgCOD/mgP (Zeng *et al*., 2003) and 50 mgCOD/mgP (Beer *et al*., 2004). All of these studies have shown excellent phosphorus removal efficiency with outlet phosphorus concentration of <0.5 mgP/L. Therefore, it can be concluded from all of these experiments that the optimum COD/inlet P concentration is variable and is specific to the experimental conditions. This ratio depends on many factors such as types of dominant PAOs population, pH, temperature and type of COD mixture used for the process. As a result, phosphorus removal process in CUBEN was carried out with different COD/inlet P ratios to find the optimum value.



Graph 4.7: Phosphorus Removal Profile in CUBEN with Various Phosphorus Inlet

Graph 4.7 illustrates the phosphorus concentration profiles starting from the Deaeration stage where wastewater entered the bioreactor and passed through the Anoxic, Anaerobic and Aerobic stages. As it was mentioned earlier, the inlet phosphorus concentration was varied from 10mg/L up to 30 mg/L to analyze the optimum inlet P concentration relative to the number of

PAOs inside the bioreactor. Also, many researchers have recommended initiating and running the phosphorus removal process with high inlet phosphorus concentration. The abundant P concentration in the feed can enrich the PAO population and consequently enhance the process.

Regardless of the various phosphorus concentrations in the influent, there was a similarity of trends among all phosphorus concentration profiles. In the Anoxic stage, there was a small increase in phosphorus concentration. This result can suggest the following possibilities:

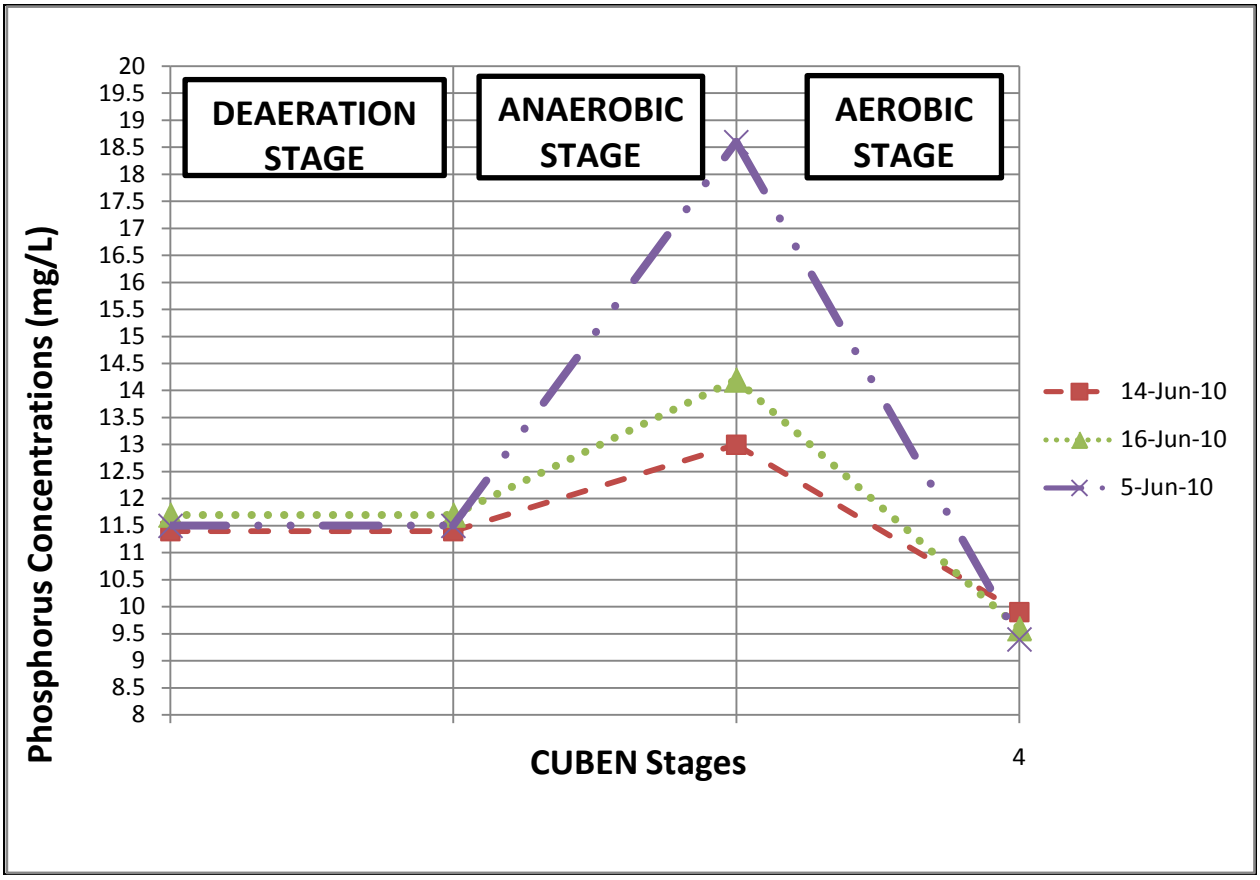
- The presence of PAOs in the Anoxic stage since PAOs are the only type of bacteria which are capable of phosphorus release under a deoxygenated feed.
- The presence of normal denitrifying bacteria and denitrifying PAOs (DPAOs) which can perform denitrification and phosphorus removal simultaneously.
- The presence of normal denitrifying bacteria, normal PAO and denitrifying PAOs in the Anoxic stage.

In the Anaerobic stage, all phosphorus concentration profiles show sharp increase of the phosphorus concentration. This result was expected since PAOs release phosphorus into the wastewater under anaerobic condition. The highest phosphorus release in both Anoxic and Anaerobic stages was observed on June 2nd. The phosphorus release by PAOs has a direct relationship with acetate uptake and ultimately intercellular PHA production. As it was mentioned in previous sections, phosphorus release is due to the energy requirement by PAOs to uptake acetate and convert it carbon polymer (PHA). The energy for this biochemical activity is obtained by breaking down internal polyphosphate bonds as well as hydrolysis of ATP to ADP. Both biochemical processes result in the release of orthophosphate into the liquid phase. Therefore, the phosphorus release in the Anaerobic stage confirmed the presence of PAOs within the CUBEN reactor. Graph 4.8, shows clearly the increase in phosphorus concentration in the Anaerobic Stage due to PAO's phosphorus release.

In the Aerobic stage, phosphorus concentration decreased drastically (as it can be seen in graph 4.8) compared to its concentration in the Anaerobic stage. According to our expectations, the Aerobic stage provided the environmental conditions including sufficient dissolved oxygen concentration, neutral pH level and completes mixing. An air diffuser installed near the bottom of the bioreactor provided the oxygen for PAOs to utilize their internal PHAs and consequently uptake phosphorus from the wastewater. Although, there was a significant difference between the concentration of phosphorus in the Anaerobic and Aerobic stages of CUBEN, the overall

phosphorus concentration difference between influent and effluent was not remarkable. The overall phosphorus removal process could not meet the objectives of CUBEN design due to many factors including:

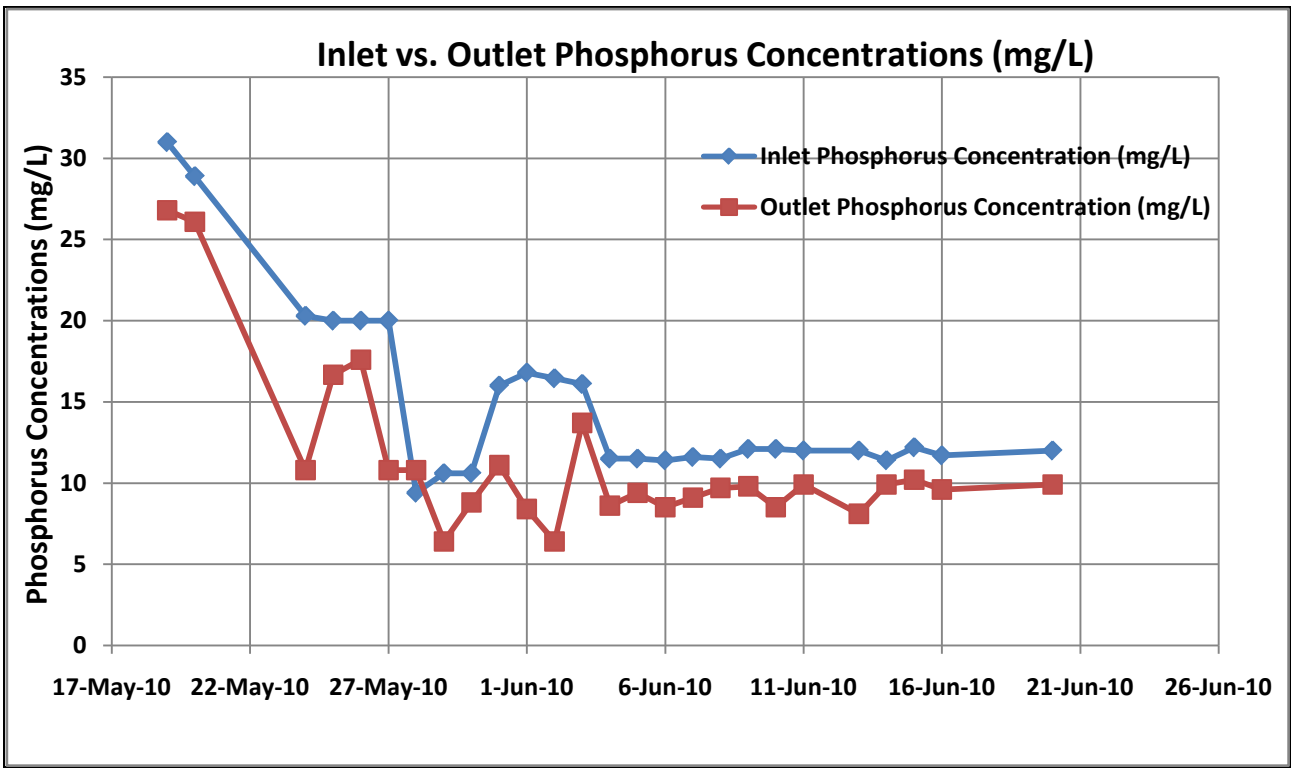
- Instability of the growth and reproduction of PAOs due to limited operational time
- Small PAOs population in the inoculums
- Lack of proper mixing inside Anaerobic stage
- Low pH of the synthetic wastewater during the start-up period
- Presence of Glycogen Accumulating Organism (GAO) in the bioreactor biomass



Graph 4.8: Phosphorus Release in the Anaerobic and Uptake in the Aerobic Stage

The results obtained for the phosphorus removal process did not reach the target value of 0.1 mg/L in the effluent. In addition to the factors mentioned before, inadequacy of the phosphorus removal in CUBEN could also be due to the fact that PAOs could not completely adapt to the environmental conditions provided in CUBEN and therefore could not reach the steady state.

As it can be observed from graph 4.9, the inlet phosphorus concentration was varied from approximately 30 mg/L to 10 mg/L throughout the CUBEN's operation period. Initially, there were considerable differences between the inlet and outlet phosphorus concentrations. During the first month of the unit operation, overall phosphorus removal efficiency was as high as 60%. However, the P removal efficiency was reduced to approximately 12% in the last month of continuous operation. Many parameters were changed to improve the P removal efficiency for instance COD concentration, type of COD mixture (various ratios of propanoic, butyric and acetic acid), frequency of inoculation (from twice to three times per week). Nevertheless, the operational period of CUBEN was not sufficient to observe the effect of all those parametrical changes. At least six months of continuous operation was needed to establish a stable phosphorus removal process and achieve high phosphorus removal efficiency to reach the target value of 0.1 mg/L.

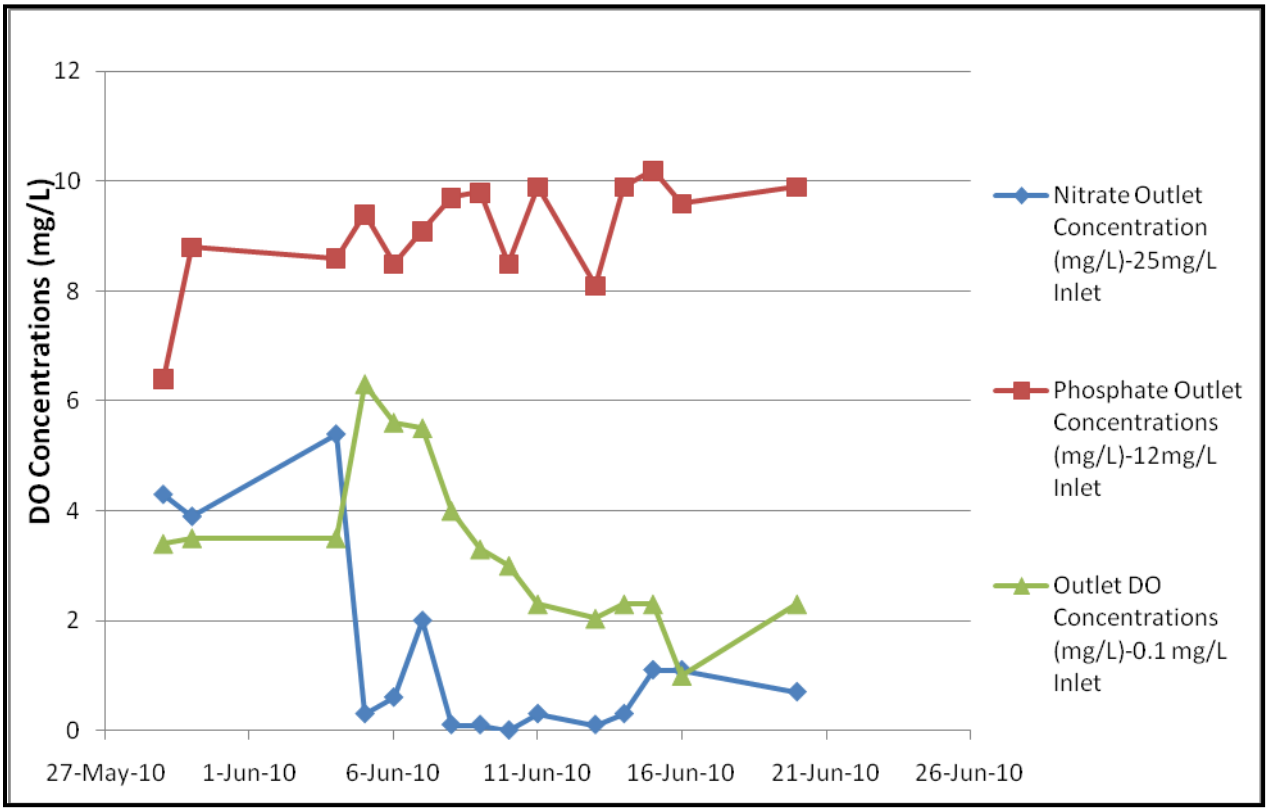


Graph 4.9: Inlet vs. Outlet Phosphorus Concentration in CUBEN

4.5 Interactions between Phosphorus and Nitrate Removal Processes

Based on the results obtained from the Anoxic, Anaerobic and Aerobic stages, there was a close relationship between denitrification and biological phosphorus removal processes. First of all, denitrification is not limited to anoxic conditions provided in the second stage of the CUBEN. Further, denitrification occurred in the Anaerobic as well as Aerobic stage and resulted in high nitrate removal efficiency. Denitrification similarly happened in the last two stages along with the phosphorus removal process. This correlation can be either due to the presence of denitrifying PAOs or solely denitrifying bacteria (non-PAOs) which coexist with PAOs in the same environment. The similarity between the two species (normal heterotrophic bacteria and PAOs) depends on many factors including inlet nitrate and phosphorus concentrations, sequence of reactor stages and biomass transport between the Anoxic, Anaerobic and Aerobic stages (Hu, Zhi-Rrong, 2002). Since, hydroxyl-Pac media is used in the Anoxic stage of the CUBEN, the possibility of biomass transport to the later stages is minimal. Therefore, it can be suggested that the occurrence of denitrification process in the Anaerobic and Aerobic stages of CUBEN could be due to the high nitrate loading of 25 mg/L in the influent. The results indicates that the inlet nitrate concentration exceeded the denitrification potential of denitrifying organisms in the Anoxic stage. That is the reason for further denitrification in the Anaerobic and Aerobic stages of CUBEN. Denitrification reached steady state at the end of the experimental period, and more denitrification happened in the Anoxic stage than in later stages. The Denitrification potential of ordinary denitrifying bacteria is significantly higher than that of DPAOs which results in considerable nitrate removal in the Anoxic stage. The remaining nitrates were removed by either DPAOs or denitrifying bacteria in the final stages of CUBEN which resulted in 98-100% denitrification efficiency.

Another reason for possible interaction between phosphorus and nitrate removal processes is the slow phosphorus removal process and consequently low P removal rates. The presence of denitrifying PAOs in the Anoxic and Anaerobic stages can slow down the overall phosphorus removal process. DPAOs simultaneously remove nitrate and release phosphorus into the liquid phase under Anoxic condition. They are excellent denitrifiers in the Anoxic and Anaerobic stages but in the Aerobic stage they act slower than normal PAOs in utilizing their internal PHAs and uptaking phosphorus.



Graph 4.10: Nitrate, Phosphate and DO Outlet Concentrations

Graph 4.10 illustrates the interaction between outlet nitrate, phosphorus and dissolved oxygen concentrations associated with their inlet concentrations of 25, 12 and 0.1 mg/L respectively. As it was mentioned earlier and it also can be observed from the above graph, oxygen concentration in all stages of CUBEN has direct effect on denitrification and phosphorus removal processes. The inlet concentration of 0.1 mg/L was sufficiently low to obtain excellent denitrfication in the Anoxic stage. In terms of the phosphorus removal process, the Anaerobic stage was well protected against DO and nitrates with inlet concentrations of 0.1mg/L for DO and less than 1.0mg/L concentration of Nitrate. However, in the Aerobic stage, as DO concentration was lowered to optimum value of 2.5-3.5 mg/L, the outlet phosphorus concentration did not show much improvement. This contradicting result was due to the instability of the overall phosphorus removal process. In order to meet CUBEN's design objectives, the unit must have been in operation for longer period of time to create a stable and large population of PAOs.

4.6 Fluorescent In Situ Hybridization (FISH) Analysis

Microbial analysis of biological phosphorus removal is of crucial importance for enhancement of this process. Various studies on EBPR have shown that the process fails intermittently since the PAO culture is still under broad investigations and they have not been explicitly identified. One of the techniques used to identify and quantify microorganisms specially the PAO community is Fluorescent in Situ Hybridization (FISH) analysis using rRNA targeted oligonucleotide probes. Several samples from the Anaerobic and Aerobic stages of CUBEN were collected during the commissioning of the unit to identify the type and number of PAOs inside the bioreactor. The results obtained using FISH analysis can be used to understand the degree of biological phosphorus removal performance in CUBEN. Before implementing the FISH analysis, the only means to understand the process was to measure the inlet and outlet phosphorus concentrations and compare the results. Although, this technique was useful to estimate the performance of the process, it was not sufficient to comprehend exactly what was happening inside the bioreactor.

According to the FISH protocol, anaerobic and aerobic samples of bacterial culture in CUBEN needed to be fixed. Fixation process stops physiological activity of the bacteria and preserve the proteins and nucleic acids in their original state. As well, fixating the samples allows the diffusion of the labeled probes into the cells. The type of fixation used in this experiment was ethanol-fixation which is used for gram-positive bacteria (many PAOs belong to this group). Upon completion of ethanol-fixation, the samples were stored at -20 ^{o}C prior to hybridization process. The fixed samples could be kept for approximately two months however, in this experiment the samples were hybridized only few days after fixation (Hausner, Martina; 2009).

The fixed samples were first added to the hybridization slide and were allowed to dry for 10 minutes in the hybridization oven set at 46 ^{o}C. Then the hybridization buffer was added to each sample which consist of the following chemicals: NaCl, Tris/HCl, SDS, Formamide, Sterile $_{dd}H_2O$ (double distilled water). Finally, the fluorescent labeled probes were added to each sample on the hybridization slide which was then placed in moist chamber and kept in the hybridization oven at 46 ^{o}C for 1.5 hours. After 1.5 hours the slide was removed from the hybridization oven and was placed into a washing buffer consisting NaCl, EDTA, Tris/HCl, SDS, Formamide, Sterile $_{dd}H_2O$. Afterwards, the hybridization slide was kept in the hybridization oven set at 48 ^{o}C

for 20 minutes. Prior to Microscopic analysis, the slide which consists the hybridized anaerobic and aerobic samples was air dried, then embedded with Citifluor. The slide was finally covered in an aluminum foil ready for microscopic examination (Hausner, 2009). A Confocal Laser Scanning Microscope (CLSM) was used to obtain high resolution optical images of the hybridized samples. The microscopic analysis of the hybridized samples could identify and quantify the PAOs and GAOs (PAO competitors) population in each sample.

During the preparation of the hybridized slides explained above, four types of probes were used to identify the bacterial community in both anaerobic and aerobic samples. The probes used were labelled as EUB338, PAO651, PAO846 and GAOQ431. The PAO651 and PAO846 probes could illuminate *Candidatus Accumulibacter Phosphatis*, a type of PAO which belongs to the subclass of β-proteobacteria. The Probe GAOQ431 could target only GAO bacteria and the EUB338 probe could target the rest of the β-proteobacteria. During the experiment, EUB338 probes were first used along with PAO651 then probe PAO846 and finally GAOQ431for both anaerobic and aerobic samples. Microscopic analysis conducted on the samples could clearly identify the presence of PAOs, more specifically *Candidatus Accumulibacter Phosphatis* bacteria in both stages of CUBEN. Also, an insignificant number of GAOs was identified by GAOQ431 probe.

Various ratios of PAOs to overall β-proteobacteria in both anaerobic and aerobic stages were calculated using the microscopic area occupied by PAO, GAO and β-proteobacterial probes. These ratios could provide semi-quantification of the type of bacteria especially PAOs present in the bioreactor. In the anaerobic stage, there were ratios (PAOs: Overall β-proteobacteria) of 1:3 and 1:7 using probes PAO651/EUB338 and PAO846/EUB338 respectively. In the aerobic sample the ratios of PAOs to overall β-proteobacteria was found to be 1:5 for PAO651/EUB338 and no biomass was found in the sample with PAO846/EUB338. This might have been due to errors in preparation of the hybridized cells. Also, a ratio of 1:250 was found using probes GAOQ431/EUB338 for the anaerobic sample and inconclusive results were obtained for GAO identification of the aerobic sample.

The microscopic pictures below show the presence of PAOs in the Anaerobic stage which were targeted by red florescent probes. The blue section includes other non-PAO microorganisms in the liquid water.

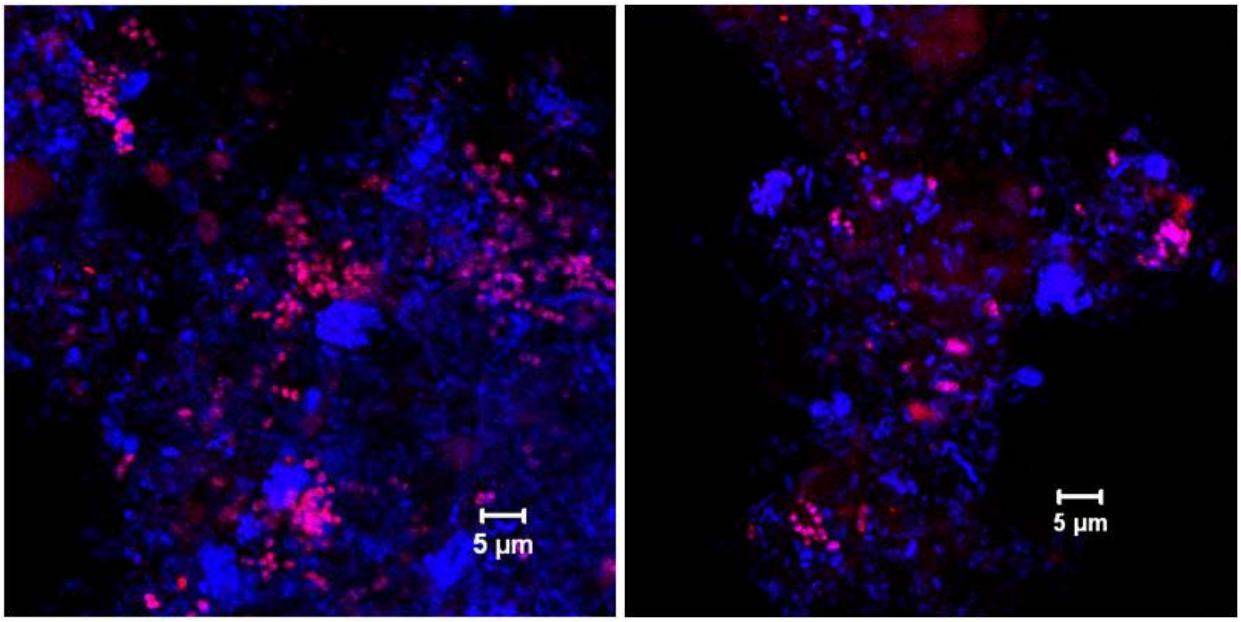


Figure 4.2: Microscopic Images of the Aerobic and Anaerobic Samples Using CLSM

The above images were taken using probes EUB338 (blue) and PAO651 (red). It is important to mention that not all red and blue spots on the above images are PAOs and general bacteria. There is a high possibility of having hybridized particles or compounds. Since PAO communities usually form clusters therefore, they can be distinguished from the individual red spots illuminating the non-PAO entities.

CONCLUSIONS AND RECOMMENDATIONS

5.0 CONCLUSIONS AND RECOMMENDATIONS

The presence of excessive nutrients, such as nitrates and phosphates in wastewater released into lakes and rivers, is the source of major environmental problems domestically and internationally. The excess of nutrients in water is responsible for two phenomena: *eutrophication*, which is the extravagant growth of algae and degradation of aquatic life, and *hypoxia* (oxygen depletion) that reduces the quality of receiving water and the sustainability of reuse. The economic losses of $3 billion annually, loss of recreational capacity, tourist repulsion, and aquatic and human toxicity have imposed increasingly strict nutrient discharge limits set and regulated by Canadian government and other industrialized countries. This issue obliges many professionals in the water and wastewater field to propose, design and operate more efficient nutrient removal systems and processes.

5.1 Conclusions

The objective of this thesis was to construct, operate and demonstrate the viability of a biological nutrient removal reactor which is cost effective and innovative in both structure and efficiency. A new designed bioreactor (CUBEN) is filed as a US patent pending. It requires a much smaller footprint, lower pumping costs, and has higher removal efficiency than existing conventional systems.

The Compact Upright Bioreactor for the Elimination of Nutrients (CUBEN) consists of four stages.

- The Deaeration stage where physical removal of dissolved oxygen takes place under vacuum. The DO concentration in the effluent of this stage was less than 0.1 mg/L.
- Anoxic stage where the anoxic conditions (high nitrate concentration and no DO concentration) promote the enrichment of denitrifying bacteria to accomplish denitrification. The effluent concentration of nitrate from this stage approached less that 0.5 mg/L during the experimental period
- The Anaerobic stage where phosphorus accumulating organisms (PAOs) are used to uptake acetates from water/wastewater and form polyhydroxyalkanoates (PHAs) inside their cells. In this stage, PAOs release orthophosphates into the surrounding liquid as a result of breakages of internal polyphosphate bonds to obtain energy, and

- The Aerobic stage where PAOs enriched with PHAs are exposed to oxygen concentration of 2.5-3.5 mg/L. In this stage PAOs utilize reserved PHAs for cellular growth, reconstruction and reproduction. They also have the unique capability to uptake orthophosphates from the water/wastewater and form intracellular polyphosphates. Thus, removing phosphorus from the liquid phase

CUBEN is designed in compact vertical alignment. Its procurement and construction took one year and the bioreactor was commissioned for two months using synthetic wastewater, similar to a secondary treatment effluent with 25 mg/L of inlet nitrate concentration and inlet phosphorus concentration of 10-30mg/L. The unit was inoculated with sludge taken from actual wastewater treatment plants and was regularly seeded to enhance and maintain the bacterial communities inside the bioreactor. The deaeration stage located at the top section of the column was under continuous vacuum throughout the experimental study. This stage can efficiently remove dissolved oxygen concentration to zero mg/L. The high performance of the Deaeration stage resulted in excellent denitrification in the Anoxic stage with a removal efficiency of 98-100%.

The biological phosphorus removal process could not meet the target value of 0.1mg/L. The enrichment of PAOs after bioreactor's inoculation was a long term process and was not fully accomplished during the commissioning of the unit. In general, biological phosphorus removal process and, in particular, PAOs responsible for phosphorus removal are hypersensitive organisms and require long term operation to reach steady state which is estimated to be between 40-100 days. Many of the environmental parameters such as inlet phosphorus, pH, COD and DO concentration were changed during the commissioning period to optimize the overall phosphorus removal process. However, CUBEN needed at least six months or more to have stable and efficient biological phosphorus removal.

5.2 Recommendations and Future Directions

The design of CUBEN as an innovative and efficient bioreactor provides many future research opportunities. Every stage of the unit from the Deaeration to the Aerobic stages along with all the physical and biological processes taking place continuously inside the bioreactor is a source of further investigation, expansion, improvement, process modeling and simulation.

Based on the operational experience and experimental results, I make the following recommendations:

- One of the areas in both biochemical and chemical engineering that needs to be deeply investigated is the biofilm formation of bacterial community. The Anoxic stage of CUBEN provides an opportunity for further investigation of the biofilm structure of denitrifying bacteria. The mathematical modeling of biofilm on the surface of the hydeoxyl-pac media in the Anoxic stage can be used to estimate, predict and control the rate and efficiency of denitrification process.
- In addition, the samples from Anoxic, Anaerobic and Aerobic stages can be used to analyze and test the accuracy of the already proposed models such as Comeau/Wentzel model , Mino model and many other models postulated so far for both phenomena including denitrification and phosphorus removal (i.e. anaerobic acetate uptake, anaerobic phosphorus release and aerobic phosphorus uptake). Further work on the designed unit (CUBEN) can be done to establish and test new mathematical models which can provide quantitative and qualitative results. In deriving and using quantitative models, one has to determine all the required parameters, make necessary assumptions and find solution with numerical procedures. Alternatively, the new driven model can be used to simulate different environmental conditions by varying related parameters (Wuertz, Stefan, 2003).
- One of the problems found during the commissioning period of the unit was lack of proper mixing in the Anaerobic stage. Due to the vertical configuration of the bioreactor and its stacked staging, the best option for proper mixing would be static mixer.
- Also, utilizing an efficient separation method and technology is crucial for increasing the efficiency of biological processes most importantly phosphorus removal process. In this study, a ceramic microfiltration membrane was used to separate the sludge from effluent. However, the ceramic membrane was too small to handle CUBEN's flowrate and the amount of sludge

in its effluent. Therefore, the membrane fouled frequently and it was only used for a short time. There are many parameters and constrains associated with this new bioreactor design (i.e. complete mixing in the Anaerobic stage and more suitable membrane filtration unit) which need to be improved and enhanced and consequently bring about future research opportunities in this interesting field.

- Finally, there is a need for development of algorithms leading to a process control which relate ORP to phosphorus removal process

6.0 REFERENCES

Alvarez-Cuenca, Manuel., (September 1979), *Oxygen transfer in bubble columns and three-phase fluidized bed*, Ph.D. Dissertation, Department of Chemical Engineering, The University of Western Ontario, London, Canada

Alvarez-Cuenca, Manuel., and Nerenberg, M. A., (January 1981), *Oxygen Transfer in Bubble Columns working at Large Gas and Liquid Flowrates*. American Institute of Chemical Engineers (AIchE) Journal, Vol. 27, Issue 1, Pages: 66-73

Alvarez-Cuenca, Manuel., and Reza, Maryam., (August 2010). *Compact Upright Bioreactor for the Elimination of Nutrients (CUBEN)*, US Patent Filed, Serial No. 12/852,068

Baetens, Danielle (2000-2001), *Enhanced Biological Phosphorus Removal: Modeling and Experimental Design*, Ph.D Thesis. Department of Technical Chemistry and Environmental Engineering and the Department of Applied Mathematics, Biometrics and Process Control (BIOMATH), Ghent University, Belgium

Beer, M., Kong., Y.H., Seviour, R.J., (2004). Ar*e some putative glycogen accumulating organisms (GAO) in anaerobic: aerobic activated sludge systems members of the a-Proteobacteria?* Microbiology Vol. 150, Pages: 2267–2275.

Brown, E. William., and Hankins, W. Doug., (May 2009), *Converting & Capturing Phosphorus;* Water and Wastes Digest

Chang, W. C., Ouyang, C. F., Chiang, W. L. and Hou, C. W., (June 1997), *Sludge Pre-Recycle Control of Dynamic Enhanced Biological Phosphorus Removal System: An Application of On-Line Fuzzy Controller*, National Central University, Taiwan

Dabert, Patrick., Delgenès, Jean-Philippe., and Godon, Jean-Jacques., (2005), *Monitoring the impact of bioaugmentation on the start up of biological phosphorus removal in a laboratory scale activated sludge ecosystem*, Environmental Biotechnology, Applied Microbiology and Biotechnology Vol. 66, Number 5, pages: 575-588

Dabkowski, Bob., (2006), *Applying oxidation reduction potential sensors in biological nutrient removal systems,* (Hach Wastewater Specialist). HACH COMPANY World Headquarters, USA Determination of Treatment Requirements for Municipal and Private Sewage Treatment Works Discharging to Surface Waters, Ministry of Environment (MOE), Canada

Dodds, Walter., Bouska, Wesw., Eitzmann, Jeffrey L., Pilger, Tyler J., Pitts, Kristen L., Riley, Alyssa J., Schloesser, Joshua T., and Thornbrugh, Darren J., (October 2008), *Eutrophication of U.S. Freshwaters: Analysis of Potential Economic Damages*, Division of Biology, Kansas State University, Manhattan, Kansas 66506

Dynaflow Engineering, Inc, Middlesex, NJ, USA; Retrieved on June 24, 2010 from http://www.dynafloweng.com/gearpumps.html

Filipe, Carlos D. M., Gaigger, Glen T., and Grady Jr., C. P. Leslie., (January 2001), *Stoichiometry and Kinetics of Acetate Uptake under Anaerobic Conditions by an Enriched Culture of Phosphorus-Accumulating Organisms at Different pHs*; Department of Environmental Engineering and Science, L. G. Rich Environmental Research Laboratory, Clemson Research Park, Clemson University, Clemson, South Carolina, USA.

Grady, Leslie C.P., Daigger, Glen T., Lim, Henry C. (1999), *Biological Wastewater Treatment*, Second Edition. PG. 49-51, 105, 106, 218, 490, 502, 547

Haandel, van Adrianus., and Lubbe, Jorden van der., (September 2007), *Handbook Biological Wastewater Treatment Design and Optimization of Activated Sludge Systems*, Quist Publishing – Leidschendam-The Netherlands

Hausner, Martina., (October 2009), *Protocol slide-based fluorescent in situe hybridization (FISH) of prokaryotes.* 3rd Edition, Ryerson University, Toronto, Canada

Headworks® BIO, IFAS, Retrieved on July 4, 2010 from http://www.headworksusa.com/products/ifas.cfm

Hu, Zhi-Rong., Wentzel, M.C., and Ekama, G.A., (2002), *Anoxic growth of phosphate-accumulating organisms (PAOs) in biological nutrient removal activated sludge systems*. Department of Civil Engineering, Water Research Group, University of Cape Town, South Africa; Water Research, Vol. 36, Issue 19, Pages: 4927-4937

Industry Canada (March 2003), Retrieved on December 4th, 2008 from http://www.ic.gc.ca/epic/site/ea-ae.nsf/en/ea02178e.html

Janssen P. M. J., Meinema, K., and Van der Roest, H. F., (2002). *Biological Phosphorus Removal: Manual for Design and Operation*. IWA Publishing; London UK PP. 34-35

Jeyanayagam, Sam., (January 2005), *True Confessions of Biological Nutrient Removal Process*, Florida Water Resources Journal

Jules N. Pretty., Mason, F. Chistopher., Nedwell, B. David., Hine, E. Rachel., Leaf, Simon., and Dils, Rachael., (2003). *Environmental Costs of Freshwater Eutrophication in England and Wales*, Centre for Environment and Society and Department of Biological Sciences, University of Essex, Colchester, U.K. and the Environment Agency, Wallingford, Evenlode House, Howberry Park, Wallingford, U.K

Kortstee1, G. J. J., Appeldoorn, K. J., Bonting1, C. F. C., Niel van, E. W. J., Veen van, H. W., (December 1999), *Recent Developments in the Biochemistry and Ecology of Enhanced Biological Phosphorus Removal*, Laboratory of Microbiology, Department of Biomolecular Sciences, Wageningen University, Netherlands

Kuba, T., Murnleitner, E., van Loosdrecht, M. C. M., and Heijnen, J.J., (1996) *A metabolic model for the biological phosphorus removal by denitrifying organisms*. Biotechnology and Bioengineering, Vol. 52, Issue.6, Pages: 685-695

Landman, M.J., Heuvel van den M. D., (2003), *An Improved System for the Control of Dissolved Oxygen in fresh water Aquaria*, N Z Forest Research Institute Limited, New Zealand

Mahendraker, Venkatram., Mavinic, S. Donald., and Hall, J. Kenneth., (January 2005), *The Impact of Influent Nutrient Ratios and Biochemical Reactions on Oxygen Transfer in an EBPR Process: A Theoretical Explanation.* Wiley Periodicals, Inc. pages 22-42, Canada

Mahendraker, Venkatram., Mavinic, S. Donald., and Hall, J. Kenneth., (May 2005), *Comparison of Oxygen Transfer Parameters Determined from the Steady State Oxygen Uptake Rate and the Non-Steady-State Changing Power Level Method*, Journal of Environmental Engineering, Pages 692-701, Canada

Metcalf & Eddy (2003) *Wastewater Engineering: Treatment and Reuse*, Metcalf & Eddy Inc., 4th edition. Pages: 616, 617, 810, 811

Mino, T., Vanloosdrechy M. C., and Heijnen, J. J., (1998) *Microbiology and biochemistry of the enhanced biological phosphorus removal process*; Department of Urban and Environmental Engineering, the University of Tokyo, Japan & Biochemical Engineering, Delft University of Technology, Netherlands

Mulkerrins, D., Dobson, A. D. W., and Colleran, E., (2003), *Parameters affecting biological phosphate removal from wastewater*, Department of Microbiology, University College Cork, Cork, Ireland and Department of Microbiology, National University of Ireland, Galway, Ireland

Murnleitner, E., Kuba, T., van Loosdrecht, M. C. M., and Heijnen, J. J., (June 1997) *An integrated metabolic model for the aerobic and denitrifying biological phosphorus removal*. Department of Biochemical Engineering, Delft University of Technology, Netherlands, Biotechnology and Bioengineering Vol. 54, Issue 5, Pages: 434-450

Nancy MacDonald (August 24, 2009), *Canada's Sickest Lake*; MACLEN's Magazine
National Sewage Report Card III, Grading the sewage treatment of 22 Canadian Cities, September 2004

National Survey of Wastewater Treatment Plants (June 2001), Submitted to: Canadian Water and Wastewater Association (CWWA) and Environment Canada, National Pollution Release Inventory (NPRI) Ottawa, Ontario

Oehmen, Adrian., Lemos C. Paulo., Carvalho, Gilda., Yuan, Zhiguo., Keller, Jurg., Blackall, L. Linda., Reis A.M. Maria., (June 2007), *Advances in Enhanced Biological Phosphorus Removal: From Micro to Macro Scale*. Water Research Journal Vol. 41, Issue 11, Pages: 2271-2300

Oehmen, Adrian., Zeng, J. Raymond., Yuan, Zhiguo., and Keller, Jurg., (January 2005), *Anaerobic Metabolism of Propionate by Poly-Phosphate Accumulating Organisms in Enhanced Biological Phosphorus Removal System,* The University of Queensland, Australia

Oldham K. William., and Rabinowitz, Barry., (2002), *Development of Biological Nutrient Removal Technology in Western Canada,* Journal of Environmental Engineering and Science, NRC, Canada

Prominent® Manufacturer website, Retrieved on June 24, 2010 from, http://www.prominent.com/desktopdefault.aspx/tabid-11/

Protecting Our Future, The City of Calgary Wastewater (# 37), P.O. Box 2100, Stn M, Calgary, AB T2P 2M5, Canada. Website: www.calgary.ca/wastewater

Randall, C.W., Stansel, H.D. and Barnard, J.L. Design of activated sludge biological nutrient removal plants. *Design and retrofit of wastewater treatment plants for biological nutrient removal,* Technomic Publishing Co. Inc., 125-126 (1992)

Romansky J, Heider., and Wiesmann, M. U., (1997). *Kinetics of anaerobic orthophosphate release and substrate uptake in enhanced biological phosphorus removal from wastewater*. Water Research Vol. 31, No. 12 pages 3137–3145.

Schici, Rich., (May 2009), *Influence of biofilm Modification on Nutrient Removal within Sewers*, Water and Wastes Digest

Scheer, Holger., and Seyfried, Carl F., (1996), *Enhanced Biological Phosphate Removal: Modelling and Design in Theory and Practice*, University of Hannover, Germany. Water Science and Technology, Vol. 34, Issue. 1-2, Pages: 57-66

Seviour, Robert & Nielsen, H (2010), *Microbial ecology of activated sludge*. IWA Publishing, London, UK

Shuler, Michael L., and Kargi, Fikret., (2002), *Bioprocess Engineering: Basic Concepts*, Second Edition, Publisher: Prentice Hall, ISBN 0130819086

Selman, M., Greenhalgh, S., Diaz, R. and Sugg, Z., (March 2008), *Eutrophication and Hypoxia in Coastal Areas: A Global Assessment of the State of Knowledge;* World Resources Institute, WRI Policy Note, Washington DC

Smolders, G.J.F., van der Meij, J., van Loosdrecht, M.C.M. and Heijnen, J.J., (1995), *A structured metabolic model for the anaerobic and aerobic stoichiometry and kinetics of the biological phosphorus removal process*; Biotechnology & Bioengineering Vol. 47, 277-287

United Nations and Environment Program (2009), *Planning and Management of Lakes and Reservoir: An Integrated Approach to Eutrophication*. Retrieved January 16, 2009 from http://www.unep.or.jp/ietc/Publications/TechPublications/TechPub-11/5-3-3a.asp

Wentzel, M.C., Ekama, G.A., and Loewenthal, R.E. (1989) *Enhanced polyphosphate organism cultures in activated sludge systems-Part II: Experimental behavior*. Chemical and Civil Engineering Departments, University of Cape Town, South Africa, Water. SA Vol. 15, pages: 71-88

Wentzel, M.C., Lötter, L.H., Ekama, G.A., Loewenthal, R.E. and Marais, G. V. R., (1991) *Evaluation of biochemical models for biological excess phosphorus removal*. Water Science and Technology WSTED4, Vol. 23, No. 4/6, Pages: 567-576

Wiesmann, Udo., Choi, In Su., and Dombrowski, Eva-Maria., (2007), *Fundamental of Biological Wastewater Treatment*, pages 188-265. Wiley-VCH Publishing

Wong, M.T., Tan, F.M., Ng, W.J., Liu, W.T., (2004). *Identification and occurrence of tetrad-forming Alphaproteobacteria in anaerobic–aerobic activated sludge processes*
Environmental Science and Engineering Program and Department of Civil Engineering, National University of Singapore Microbiology Journale Vol.150, pages: 3741–3748.

Wuertz, Stefan., Paul, L. Bishop., and Wilderer, A. Peter., (2003), *Biofilm in Wastewater Treatment; An Interdisciplinary Approach*, Published by IWA, London, UK

Zeng, R.J., Yuan, Z.G., Keller, J., (2003), *Enrichment of denitrifying glycogen-accumulating organisms in anaerobic/anoxic activated sludge system*. Biotechnology and Bioengineering Journal 81 (4), 397–404.

7.0 GLOSSARY

Acetyl-CoA: It is an important molecule in cellular metabolism, used in many biochemical reactions. Its main use is to convey the carbon atoms within the acetyl group to the citric acid cycle (TCA) to be oxidized for energy production.

BNR: Biological Phosphorus Removal

BOD_5: Biological Oxygen Demand

COD: Chemical Oxygen Demand

DO: Dissolved Oxygen

CLSM: Confocal Laser Scanning Microscopy

EBPR: Enhanced Biological Phosphorus Removal Process

ED Pathway: The Entner–Doudoroff pathway describes an alternate series of reactions that catabolize glucose to pyruvate using a set of enzymes different from those used in either glycolysis or the pentose phosphate pathway. There are a few bacteria that substitute classic glycolysis with the ED Pathway including Pseudomonas (a type of PAO)

EMP Pathway: Embden-Meyerhof pathway consists of a sequence of reactions in which glucose is degraded to pyruvate; the six-carbon stage converts glucose to fructose-1,6-bisphosphate, and the three-carbon stage produces ATP while changing glyceraldehyde-3-phosphate to pyruvate.

Fumerate: is an intermediate compound in TCA cycle and is formed by oxidation of succinate

Gram-negative: Gram-negative bacteria are those bacteria that do not retain crystal violet dye and stain pink when undergoing the Gram staining process

Gram-positive: Gram-positive bacteria are those that are stained dark blue or violet by Gram staining

HRT: Hydraulic Residence Time

IFAS: Integrated Fixed-Film Activated Sludge is a process typically used as a retrofit solution for small scale bioreactors or conventional activated sludge systems that are beyond facility expansion

MLR: Mixed Liquor Recycle

NADH and **$FADH_2$**: are principally used to drive the processes of oxidative phosphorylation, which are responsible for converting the reducing potential of NADH and $FADH_2$ to the high energy phosphate in ATP.

Neisser Positive Stain: Neisser staining is used to identify and locate polyphosphate granules in cells. Neisser Positive bacteria stained light purple

PAO: Phosphorus Accumulating Organism

PHA: Poly-hydroxyalkanoates are linear polyesters which are produced naturally by certain types of microorganisms such as PAOs and can be stored inside their cells as a source of energy. More than 150 monomers can be combined within the family of PHA to form polymers with different physical and chemical properties including PHBs and PHVs. PHAs can be either thermoplastic or elastomeric polymers with wide range of boiling points (40-180 ^{o}C).

PHB: Poly-hydroxybutyrate is a thermoplastic polymer that is synthesized inside PAO cells in the presence of the enzymes including β-ketothiolase, acetoacetyl-CoA reductase and poly-hydroxyl synthase

PHV: Poly-hydroxyvalerate belongs to the polyester class of PHA. PHVs are produced by PAOs and are reserved inside their cells. PHVs after PHBs are the most common type of PHA that are synthesized by PAOs.

PHB Staining: The poly-ß-hydroxybutyrate stain is used to stain granules present within the confines PAO cells

Pyruvic acid: (CH3COCOOH) is an organic acid. It is also a ketone, as well as being the simplest alpha-keto acid. The carboxylate (COOH) ion (anion) of pyruvic acid, CH3COCOO-, is known as pyruvate, and is a key intersection in several metabolic pathways

TCA Cycle: Tri-carboxylic Cycle, also known as Krebs cycle is a series of enzyme-catalyzed chemical reactions which is important for all the living organisms that use oxygen as part of their cellular respiration

TN: Total Nitrogen including nitrate (NO_3^-), nitrite (NO_2^-), organic nitrogen and ammonia

TKN: Total Kjeldahl Nitrogen (It is a test performed in laboratory analysis that is made up of both organic nitrogen and ammonia).

TSS: Total Suspended Solids

TP: Total Phosphorus

Succinate: It is also known as butanedioic acid which plays an important role in TCA cycle

SRT: Solid Residence Time

VFA: Volatile Fatty Acids

WWTP: Wastewater Treatment Plant

8.0 APPENDICES

APPENDIX A. Equipment Specifications

GALA Prominent Metering Pump

Quantity Two (2) Prominent Metering Pumps

- Model: GALA0220PCE260UD112000

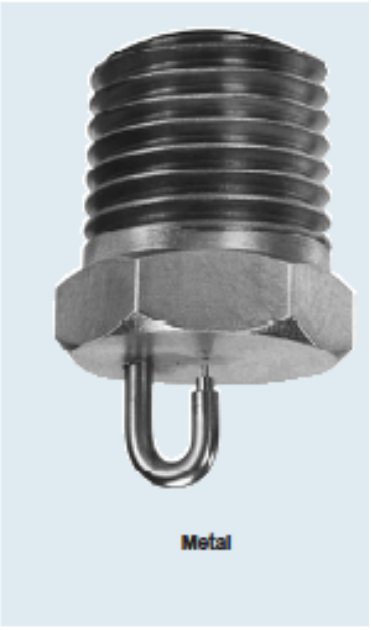
• rated 19 L/hr at maximum backpressure 29 psi

- Model: GALA0413PCE260UD112000
-
- rated 12.3 L/hr at maximum backpressure 58 psi
- Control Variants: manual + external 1:1 with analog control
- Fault Relay, drops out
- Remote On/Off capability
- Electrical Connection: 115 VAC, 60 Hz
- Digital stroke frequency adjustment from 0-180 spm
- PVC liquid end with EPDM seals
- Liquid end version: with bleed valve
- Includes foot valve, injection valve and 2m control cable
- UL and CSA approved

Misting Nozzle

PJ

Smallest Physical Size

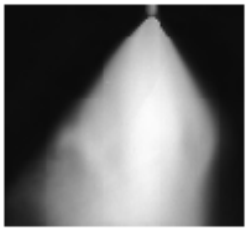


Metal

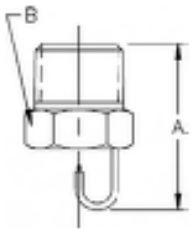
DESIGN FEATURES
- High energy efficiency
- One-piece, compact construction
- No whirl vanes or internal parts
- 1/8" or 1/4" male connection
- 100-mesh screen, 10 micron paper filter or polypropylene filter optional

SPRAY CHARACTERISTICS
- Finest fog of any direct pressure nozzle
- Produces high percentage of droplets under 50 microns

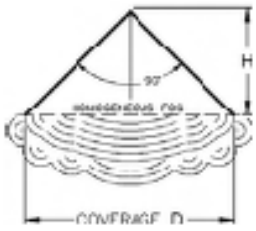
Spray pattern: Cone-shaped Fog
Spray angle: 90°. For best 90° pattern operate nozzle at or above 60 psi
Flow rates: 0.013 to 1.4 gpm



Fog



Male



Fog Pattern

PJ with polypropylene filter

Dimensions are approximate. Check with BETE for critical dimension applications.

PJ Flow Rates and Dimensions
Impingement, 90° Spray Angle, 1/8" or 1/4" Pipe Sizes

Male Pipe Size	Nozzle Number	K Factor	GALLONS PER MINUTE @ PSI 30 PSI	40 PSI	50 PSI	60 PSI	80 PSI	100 PSI	200 PSI	400 PSI	Approx. Orifice Dia. (in.)	Approx. Coverage (inches) D	Approx. Spray Height H (in.)	Pipe Size	Approx. Dim. (in.) A	B	Wt. (oz.) Metal
	PJ6	0.00095			0.006	0.007	0.008	0.010	0.013	0.019	0.006	10	5				
	PJ8	0.00180			0.013	0.014	0.016	0.018	0.025	0.036	0.008	10	5				
1/8	PJ10	0.00269		0.017	0.019	0.021	0.024	0.027	0.038	0.054	0.010	10	5	1/8	0.75	0.44	
	PJ12	0.00384		0.023	0.026	0.028	0.033	0.036	0.051	0.073	0.012	10	5				
	PJ15	0.00585	0.032	0.037	0.041	0.045	0.052	0.059	0.083	0.117	0.015	10	5				0.25
OR	PJ20	0.0106	0.058	0.067	0.075	0.082	0.095	0.11	0.15	0.21	0.020	12	6				
	PJ24	0.0158	0.087	0.10	0.11	0.12	0.14	0.16	0.22	0.32	0.024	18	8				
	PJ28	0.0206	0.11	0.13	0.15	0.16	0.18	0.21	0.29	0.41	0.028	18	9	1/4	0.97	0.56	
1/4	PJ32	0.0285	0.16	0.18	0.20	0.22	0.25	0.28	0.40	0.57	0.032	22	11				
	PJ40	0.0443	0.24	0.28	0.31	0.34	0.40	0.44	0.63	0.89	0.040	24	12				

Flow Rate (GPM) = $K\sqrt{PSI}$

Standard Materials: Brass, 303 Stainless Steel and 316 Stainless Steel.

ASHCROFT

Duralife® Pressure Gauge Type 1009, ASME B 40.1 Grade 1A (±1% of span)

DESIGNED FOR SAFETY AND LONGER LIFE

- *5-year limited warranty*
- *Patented PowerFlex™ movement isolates movement from shock and vibration for longer life*
- *All stainless, all-welded construction for long life*
- *ASME Grade 1A, 1% accuracy full scale*
- *True Zero™ pointer indication – no stop pin to mask false zero reading – ensures safety and process control*

The following Table is *not* for conversion purposes.

STANDARD RANGES [3][4][5]

Pressure psi	kg/cm² - bar	kPa
0/15	0/1	0/100
0/30	0/1.6	0/160
0/60	0/2.5	0/250
0/100	0/4	0/400
0/160	0/6	0/600
0/200	0/10	0/1000
0/300	0/16	0/1600
0/400	0/25	0/2500
0/600	0/40	0/4000
0/800	0/60	0/6000
0/1000	0/100	0/10,000
0/1500	0/160	0/16,000
0/2000	0/250	0/25,000
0/3000	0/400	0/40,000
0/4000	0/600	0/60,000
0/5000	0/1000	0/100,000
0/6000		
0/7500		
0/10,000		
0/15,000		
Vacuum		
30 in./0 in.Hg	–1/0	–100/0
Compound		
30 in.Hg/15 psi	–1/0/1.5	–100/0/150
30 in.Hg /30 psi	–1/0/3	–100/0/300
30 in.Hg /60 psi	–1/0/5	–100/0/500
30 in.Hg /100 psi	–1/0/9	–100/0/900
30 in.Hg /150 psi	–1/0/15	–100/0/1500
30 in.Hg /300 psi	–1/0/24	–100/0/2400

- *New PLUS!™ Performance Option:*
  - *Liquid-filled performance in a dry gauge*
  - *Fights vibration and pulsations without liquid-fill headaches*
  - *See pages 6-7 for details*
  - *Order as option XLL*

OTHER FEATURES:

Available in 2½" and 3½" dial sizes, Duralife® pressure gauges are liquid fillable and field convertible for panel mounting. Both zero and span adjustments are standard.

The gauge is available dry, liquid-filled weatherproof or hermetically sealed and *now* with *PLUS!™* performance option. A five year limited warranty is standard with the Duralife® 1009.

BOURDON SYSTEM SELECTION[1]

Ordering Code	Bourdon Tube & Tip Material[2][3]	Socket Material	Tube Type	Range Selection Limits (psi)	NPT Conn.[6]
AW	316 stainless steel	Bronze	C-Tube	Vac/600	¼
AW	316 stainless steel	Bronze	Helical	1000	¼
SW	316 stainless steel	316 stainless steel	C-Tube	Vac/600	¼ & ½
SW	316 stainless steel	316 stainless steel	Helical	800/15,000	¼ & ½

(1) For selection of the correct Bourdon system material, see the media application table on page 243.
(2) ¼ NPT available 316" lower SW system only.
(3) Type 1009 gauges may be ordered with metric single-scale dial: kPa,bar or kg/cm².
(4) Dual-scale dials will be supplied with standard metric inner scale and equivalent psi outer scale or with standard psi inner scale and equivalent metric outer scale–please specify.
(5) Special logos and scales available upon request.
(6) ¼" JIS, BSP or DIN threads available on SW systems.

TO ORDER THIS 1009 DURALIFE PRESSURE GAUGE:

Select:	3S	1009	SW	(L)	02L	XXX	1000#
1. Dial size–2½", 3½"							
2. Case type–1009							
3. Tube and socket material							
4. Liquid filled (glycerin), leave blank if dry							
5. Connection size–⅛ (01), ¼ (02) ½ (04)							
6. Connection location–Lower (L), Back (B)							
7. Optional Features–see page 176							
8. Standard pressure range–1000 psi							

Accessories: see pages 233-238

Consult factory for guidance in product selection
Phone (203) 385-0217, Fax (203) 385-0602 or
visit our web site at www.ashcroft.com

GLASS TUBE FLOWMETERS

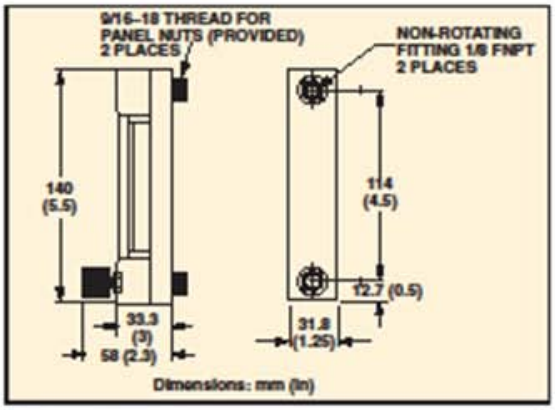
E-Z TUBE CHANGE WITH MAGNIFYING FRONT SHIELD

- Standard Millimeter Scales with Flow Curves
- E-Z Change Design Allows Quick Interchangeability of Tube Assemblies
- Glass Tube Allows Compatibility with Most Gases and Fluids
- Rotating Lens Allows 180° View with Magnification

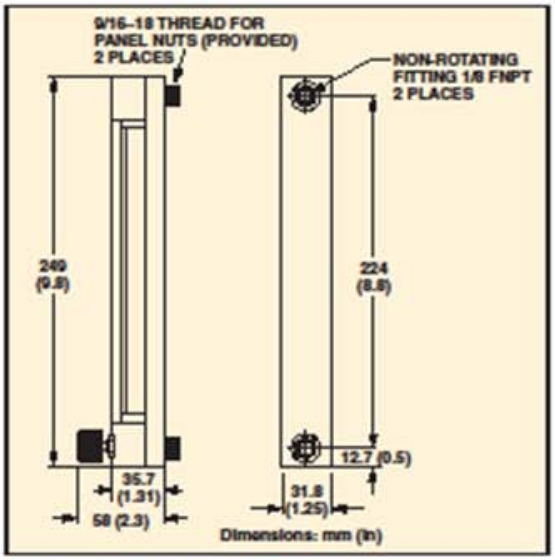
FL-5000 Series Starts at

$125

1 YEAR WARRANTY · MADE IN USA · RoHS



65 mm (2.5") FLOWMETER						
Millimeters	140.0	33.3	58.0	31.8	114.0	12.7
Inches	5.5	1.4	2.3	1.3	4.5	0.5



150 mm (5.9") FLOWMETER						
Millimeters	245.0	35.7	56.0	31.8	224.0	12.7
Inches	9.8	1.31	2.3	1.25	8.8	0.5

FL-5411G Series flowmeter, $234, basic model, shown actual size.

FL-5511G-NV Series flowmeter, aluminum, $136, basic model, shown smaller than actual size

SPECIFICATIONS

DISPLAY:
3 1/2 digit LED, 1/2" high digits

MEASURING RANGES:
D.O.: 0-20.00 ppm and 0-100% saturation, switch selectable
Temperature: 0-40°C (32 to 104°F)

POWER REQUIREMENTS:
98-132 Vac, 50/60 Hz (less than 10 VA)
Optional: 196-264 Vac, 50/60 Hz

AMBIENT CONDITIONS:
-30 to 50°C (-22 to 122°F)
0 to 90% R.H. non-condensing

ANALOG OUTPUTS:
Non-isolated 0-1 mA, 100 ohms maximum load
Non-isolated 0-5 Vdc, 1000 ohms minimum load
Isolated 4-20 mA, 800 ohms maximum load
The isolated output is isolated from the input, ground line power and all other outputs
Range Expand: The 4-20 mA analog output can be made to represent any segment of the measuring scale. Minimum segment is 10% of full scale.
Output Hold: The analog outputs are automatically placed on hold during calibration or other setup operations.
Temperature Output: The 0-5 Vdc output can be programmed to follow either the process temperature or dissolved oxygen.

TEMPERATURE COMPENSATION:
Automatic 0 to 100°C (32 to 212°F)

ALTITUDE COMPENSATION:
Automatic 0 to 40°C (32 to 104°F)

DIAGNOSTICS:
When system error is indicated, call STATUS to obtain condition code. On-board simulated input to aid in troubleshooting to obtain condition code.

MEMBRANE ALARM:
A relay activates if membrane is perforated.

TEST:
Display value and analog outputs can be set manually to any value for testing and diagnostic purposes. This feature allows the outputs to be tested independently of process value.

SAFETY & SECURITY:
Non-volatile memory (EEPROM)
Passcode protected if selected
Watch-dog timer monitors microprocessor
Instrument automatically returns to on-line operation if accidentally left in menu mode
(This feature may be held disabled if desired)

ACCURACY: % Saturation: ± 1%
ppm: ± 0.1
Temp: ± 0.2°C

STABILITY: 0.1% of span per 24 hrs., non-cumulative

RESOLUTION: % Saturation: 0.1
ppm: 0.01
Temp: 0.1°C

REPEATABILITY: 0.1% of span or better

RESPONSE TIME: 20 seconds to 90% of value upon step change at 20°C

ENCLOSURE: NEMA 4X fiberglass reinforced polyester enclosure with four 1/2" conduit holes and mounting feet for surface mount

MOUNTING CONFIGURATIONS:
Standard is surface mount
Optional panel mount hardware. Part No. DOCN600-PM
Optional pipe mount hardware. Part No. DOCN600-PIPE

NET WEIGHT: 3 1/2 lbs. (1.6 kg)

DR 2700 (Spectrophotometer)

Specifications*

Operating Mode
Transmittance (%), Absorbance, and Concentration

Source Lamp
Tungsten

Pre-Installed Programs
More than 130

Available User Programs
10

Data Storage
200 points

Export Capability
.csv (comma-separated values) file format

Wavelength Range
400 to 900 nm

Wavelength Accuracy
±1.5 nm

Wavelength Resolution
1 nm

Spectral Bandwidth
5 nm

Wavelength Calibration
Internal, automatic at power-on, visual feedback

Wavelength Selection
Automatic; based on selected method in all modes except stored methods

Enclosure Rating
IP41 with lid closed
IP42 with Protective Cover

Operating Temperature
10 to 40°C (50 to 104°F)

Operating Humidity
80% relative humidity, non-condensing, maximum

Storage Requirements
Temperature: -25 to 60°C (-13 to 140°F)
Humidity: 80% relative humidity, non-condensing, maximum

Power Requirements
Line: 100 to 240 V; 47/63 Hz; automatic changeover
Battery: Lithium-Ion 11 V/4400 mAh

Interface
USB 1.1 (10 ft. (3 m) cable, maximum)

Languages
English, French, German, Italian, Spanish, Portuguese, Korean, Japanese, Chinese, Czech, Danish, Dutch, Hungarian, Polish, Romanian, Russian, Slovak, Swedish, and Turkish

Connections
USB Master 1x
USB Slave 1x

Sample Cell Compatibility
1-inch square
1-inch round
1-cm square
1x5-cm
13-mm round
16-mm round
Multipath 1-inch/1-cm
Pour-Thru™ with 1-in. path length

Accessories
Included:
- 1-in. square matched glass sample cells
- Cell adapters for 1-inch round/AccuVac cells, 1x1-cm cells, and multi-path 1-inch/1-cm cells
- Universal power supply, 100 to 240V, 47/63Hz, with plug adapters for EU, GB, US, China
- Protective cover for storing adapters

Optional:
- Hach Pour-Thru cell
- External USB keyboard
- DataTrans™ Software

Dimensions
216 x 132 x 330 mm (8.5 x 5.2 x13.0 in.)

Weight
Without battery: 4.0 kg (8.8 lbs.)
With battery: 4.3 kg (9.5 lbs.)

**Specifications subject to change without notice.*

Engineering Specifications

1. The spectrophotometer instrument shall be a multiwavelength spectrophotometer designed for laboratory or field analysis of multiple analytes.
2. The instrument shall be capable of measuring the following substances or characteristics: alachlor; aluminum; arsenic; atrazine; barium; benzotriazole; boron; bromine; cadmium; chloride; chlorine dioxide; chlorine; chromium; cobalt; color; copper; cyanide; cyanuric acid; dissolved oxygen; fluoride; formaldehyde; hardness; hydrazine; iodine; iron; lead; manganese; mercury; molybdenum/molybdate; monochloramines; nickel; nitrogen (as ammonia, nitrate, nitrite, total nitrogen); chemical oxygen demand; oxygen scavengers; ozone; polychlorinated biphenyls; phenols; phosphonates; phosphorus; potassium; quaternary ammonium compounds; selenium; silica; silver; sulfate; sulfide; surfactants; suspended solids; tannin and lignin; total organic carbon; tolyltriazole; total petroleum hydrocarbons; trihalomethanes; toxicity; volatile acids; and zinc.
3. The following tests shall conform to USEPA-approved methods: arsenic; chlorine (free); chlorine (total); chlorine dioxide; chromium (hexavalent); copper; fluoride; iron (total); lead; manganese; nickel; nitrogen (nitrite); chemical oxygen demand; phenols; phosphorus (reactive); phosphorus (total); sulfate; sulfide; and zinc.
4. The wavelength range of the instrument shall be 400 to 900 nm with accuracy of ±1.5 nm, resolution of 1 nm, and maximum bandwidth of 5 nm.
5. The instrument, depending on the test selection, shall automatically select the wavelength.
6. Readout modes shall include transmittance, absorbance, and concentration.
7. The interface of the instrument shall be graphical with touch screen.
8. The instrument shall provide graphical display and be capable of printing test results.
9. The instrument shall be equipped with storage capacity for 200 data points (date, time, results, sample ID, user ID) and 10 user-defined calibrations.
10. Information stored in the instrument shall be capable of being downloaded in standard report format.
11. The instrument shall be capable of accepting 1-in. (25.4-mm) round cells/vials, 1-in. square cells, 13-mm round cells, 16-mm round cells, 1x5-cm cells, and Pour-Thru cells with 1-in. path.
12. Power requirement shall be line voltage or optional rechargeable battery.
13. The instrument shall be warranted for one full year against defects in materials and workmanship.
14. The instrument shall be model DR 2700 Spectrophotometer, manufactured by Hach Company.

Equipment Type	Part Number	Description	Company
pH Electrode	PHE-7352-1	• Submersion pH Electrode CPVC, Max. temp. 65.5°C (150°F) • Designed for mounting in tanks, flumes and etc.	OMEGA
ORP Electrode	PHE-7352-15-	Submersion ORP Electrode CPVC, Max. temp. 65.5°C (150°F)	OMEGA
Temperature (Thermocouple)	TJ36-CPSS-116U-6	Copper-constantan with sheath diameter of 1.59mm or (1/16 inches)	OMEGA
General purpose pressure sensor	PX302-015GV	• Rugged all stainless steel construction • Integral Strain Relief for cable • High sensitivity 10 mV/V output • Cable and subminiature available • NEMA 3 enclosure • NIST	OMEGA
Differential Pressure Sensor	PX273-020DI	• Accuracy: +-0.75% • Output: 4-20mA	OMEGA
Preamplifier	PHTX-21		OMEGA

APPENDIX B. Synthetic Wastewater Preparation

CH_3COOH (The amount of Acetic Acid was varied during the commissioning period in order to find optimum amount for denitrifiers and PAOs) Amount of acetic acid in mass: 200mg/Lwater=? mg ace acid/126L water=25200mg or **25.2 g** 1049.10 g ac. Acid/1L=25.2g/?L= 0.024L or **24 ml** Amount of COD produced by Acetic Acid:
1.07gacet.Ac/1g COD=25.2g/?COD= 23.55g COD

CH_3OH ((The amount of Methanol was varied during the commissioning period in order to find optimum amount for denitrifiers and PAOs)**)**
Amount of Methanol in mass: 200mg/LCOD*1g methanol/1.5g COD=133.3 mg/L of methanol
133.3mg/L of methanol*126L water=**16.8 g methanol**
At 20 ^{O}C: 791.30g of methanol/1 L of methanol = 16.8g*1L/791.30 g = **21.2 ml**

KNO_3 (Potassium Nitrate)
20mgNO_3 /L*101.1g/mol KNO_3/62g/mol of NO_3 =32.6 mg/L of KNO_3
For 126L of water: 32.6 mg KNO_3/L/1L of water= ? mg/126 L of water = **4.109 g of KNO_3**

KH_2PO_4 (Potassium Phosphate Monobasic)
10mgP /L*136.07g/mol KH_2PO_4/ 30.97g/mol of P =43.94 mg/L of KH_2PO_4
For 126L of water:
43.94 mg KH_2PO_4/L/1L of water= ? mg/126 L of water = **5.535 g of KH_2PO_4**

$Na_2HPO_4.H_2O$ (Sodium phosphate Monobasic)
10mgP /L*137.99g/mol $Na_2HPO_4.H_2O$ / 30.97g/mol of P =44.56 mg/L of $Na_2HPO_4.H_2O$
For 126L of water:
44.56 mg $Na_2HPO_4.H_2O$ /L/1L of water= ? mg/126 L of water = **5.614 g of $Na_2HPO_4.H_2O$**

Na_2HPO_4 (Sodium phosphate Dibasic)
10mgP /L*141.97g/mol Na_2HPO_4 / 30.97g/mol of P =45.84 mg/L of Na_2HPO_4
For 126L of water: 45.84 mg Na_2HPO_4 /L/1L of water= ? mg/126 L of water = **5.776 g of**

Na_2HPO_4
Nitrogen, Nitrate (500 ml)
10mg/L as N 44.3mg/L NO_3
44.3mg/L NO_3/500ml=5 mg of NO_3/?ml = 56.4ml

$NaHCO_3$ (sodium bicarbonate)
Concentration required: 275.4 mg/L
275.4mg/L $NaHCO_3$*126L= 34.7g

KCl (Potassium chloride)
Concentration required: 275.4 mg/L
275.4mg/L $NaHCO_3$*126L= 34.7g

$CaCl_2$ (calcium chloride)
Concentration required: 12 mg/L
12mg/L $CaCl_2$*126L= 1.512g

$MgSO_4.7H_2O$ (Magnesium Sulphate-heptahydrate)
Concentration required: 12 mg/L
90mg/L $MgSO_4.7H_2O$ *126L= 11.340g

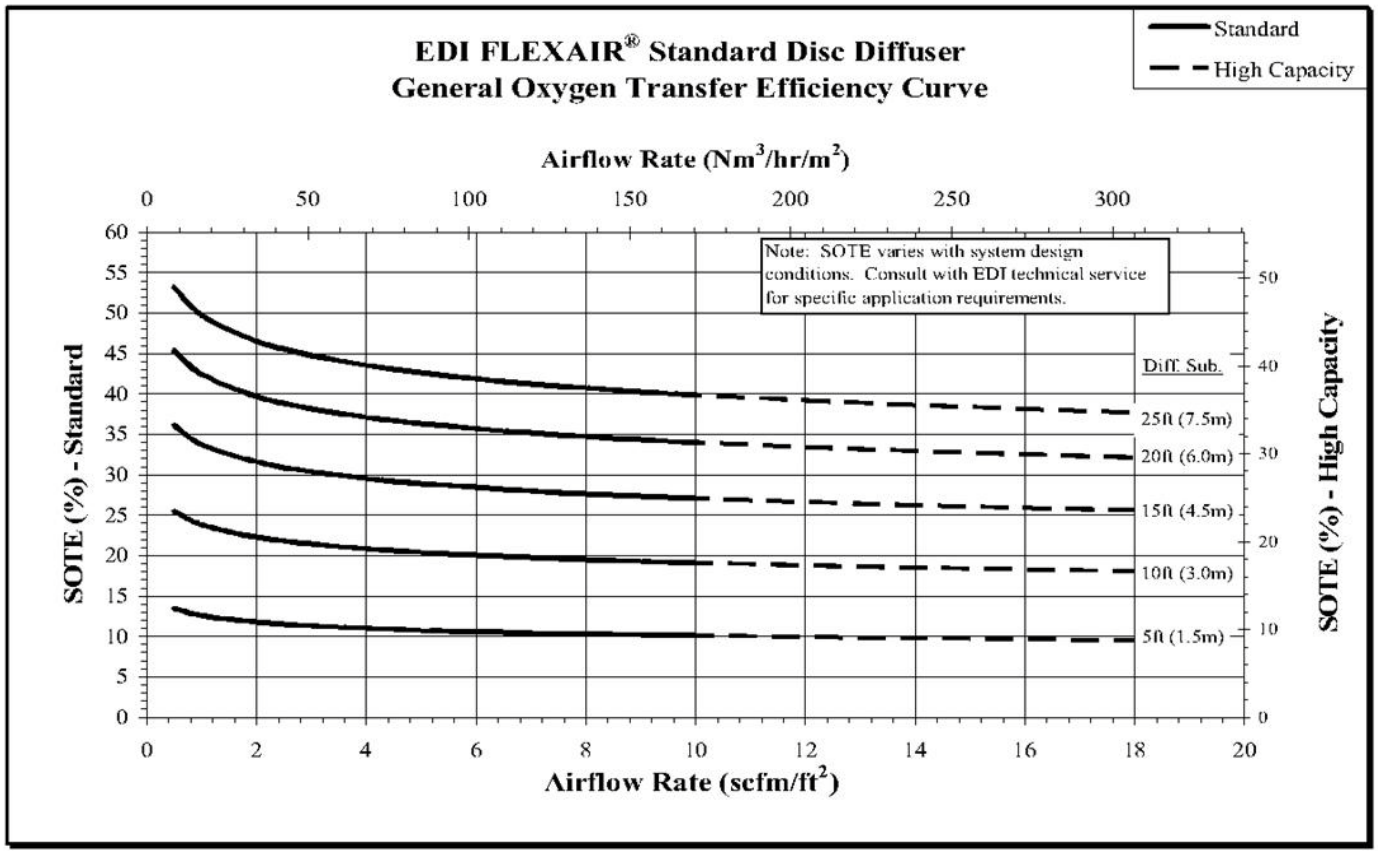
$FeCl_3$ (Iron III chloride or Ferric chloride)
Concentration required: 1.5 g/L
0.3ml of $FeCl_3$/1L of Synthetic WW*126L=37.8ml volume of $FeCl_3$
Prepare 1.5g/L or 1500mg/L of $FeCl_3$ and add only 37.8 ml of $FeCl_3$ to the feed

Na_2SO_4 (Sodium Sulphate Anhydrous)
Concentration required: 0.1 g/L
0.3ml of Na_2SO_4/1L of Synthetic WW*126L=37.8ml volume of Na_2SO_4
Prepare 0.1g/L or 100mg/L of Na_2SO_4 and add only 37.8 ml of Na_2SO_4 to the feed

$ZnCl_2$ (Zinc Chloride)
Concentration required: 0.12 g/L
0.3ml of $ZnCl_2$/1L of Synthetic WW*126L=37.8ml volume of $ZnCl_2$
Prepare 0.12g/L or 120mg/L of $ZnCl_2$ and add only 37.8 ml of $ZnCl_2$ to the feed

APPENDIX C. Performance Curves

Flexible Membrane Performance Curves



APPENDIX D. Recorded Raw Data

Date	Flowrate (L/Day)	Inlet Nitrate (Mg/L)	Outlet Nitrate (Mg/L)	Inlet Phosphorus (Mg/L)	Outlet Phosphorus (Mg/L)	Inlet COD (Mg/L)	Outlet COD (Mg/L)	Inlet DO (Mg/L)	Outlet DO (Mg/L)	pH	Inlet Temperature (^{o}C)
May 13	130	22.3	-	33.9	5.8	-	-	0.26	8.01	7.3	26
May 14	120	-	-	-	-	61	-	0.15	7.94	-	24
May 16	110	-	-	-	-	-	-	0.74	8.22	-	27
May 17	120	24.8	6.4	27	26.2	-	-	0.20	6.65	3.2	27
May 18	120	25.8	5.4	29	26	679	267	0	7.20	7.5	27
May 19	120	33.9	0.3	31	26.8	1443		0	5.89	8	27.5
May 20	120	24.1	2.8	29	26.1	275	124	-	-	-	-
May 21	120	-	-	30	27	-	-	0	0.58	-	-
May 24	120	30	14.1	20	10.8	528	325	0	1.35		
May 25	120	25.8	2.0	15.73	16.7	325	-	0	3.2	8.5	30
May 26	120	24.7	0.1	20	17.73	-	-	0.14	4.7	8.6	26.8
May 27	120	25	1.3	20	10.8	-	-	0.14	6.2	6	28
May 28	120	25	3.9	20	10.8	-	-	0.14	-	7.0	27
May 29	120	25	0.1	10.6	6.4	344	-	0.15	3.4	7.3	26.7
May 30	120	25	0.1	12	8.8	247	32	0.15	3.5	7.5	25.6

May 31	120	25	-	16.8	11.1	-	-	0.14	4	8	25
June 1	150	25	-	16.45	8.4	-	-	0.14	4	7	24.5
June 2	150	24.3	0	16.1	6.4	267	-	0.14	3.1	8.5	26.6
June 3	150	24.7	0.3	16.4	13.7	298	122.4	1.05	4.3	8.5	24.7
June 4	150	23.7	0.1	11.5	8.6	306	5	0.14	3.5	8.5	25
June 5	150	24	0.8	12	9.4	204	12	0.14	6.3	8.0	25.2
June 6	150	21	0.3	11.4	8.5	265	193	0.14	5.6	7.0	26
June 7	150	22.9	1.4	11.6	9.1	461	84.5	0.15	5.5	7.0	25.2
June 8	150	25.8	1.1	11.5	9.7	302	-	0.14	4.0	6.5	25.7
June 9	150	28.6	1.1	12.1	9.8	173	-	0.14	3.3	6.5	25.5
June 10	150	25.4	3.3	12.1	8.5	591	186	1.5	3.0	8.5	27.3
June 11	150	25	0.2	12	9.9	267	140	0.14	2.3	6.1	26.5
June 12	150	28.7	0.6	12	9.6	300	80	0.14	2.86	8.8	26.5
June 13	150	23.3	0	11.2	8.1	198	-	0.14	2.04	8.6	26
June 14	150	24	1.2	11.4	9.9	214	55	0.15	2.3	7.1	25
Jun 15	150	24.8	2.0	12.2	10.2	133	-	0.14	2.3	8.0	25
June 16	150	23.3	2.0	11.7	9.6	150	-	0.14	1.0	8.5	25.3
June 17	150	24	-	12.3	12.1	150	-	0.14	2.5	8.5	27.2
June 20	150	24	-	11.1	9.9	150	-	0.14	2.3	8.5	21
June 24	150	24	-	12	10.6	150	-	0.14	3.0	8.5	27
June 26	150	25	-	12	10.9	200	-	0.14	2.5	8.0	25

June 27	150	25	-	20.8	14	200	-	0.14	2.8	7.5	25
June 28	150	25	-	28.7	14	688	-	0.14	3.0	8.0	24
June 30	150	25	-	35.6	26.7	405	208	0.14	3.0	7.6	23
July 1	150	25	0.7	35	23.5	345	162	0.14	3.7	8.0	23.7
July 3	150	25	-	35	22.4	350	-	0.14	4.7	7.0	23
July 5	150	25	-	35	21.6	194	88	0.14	3.6	8.2	24.7