



# **Ammonia and Nitrate Removal in Refinery Wastewater using Aerobic Treatment Systems**

by

AIMRAN BIN ABD HAMID

8729

Disertation

Bachelor of Engineering (Hons)  
(Civil Engineering)

JULY 2010

Supervisor : AP Dr Shamsul Rahman Kutty

Universiti Teknologi Petronas

Bandar Seri Iskandar

31750 Tronoh

Perak Darul Ridzuan

# CERTIFICATION OF APPROVAL

## **Ammonia and Nitrate Removal in Refinery Wastewater using Aerobic Treatment Systems**


By

Aimran Bin Abd Hamid

A project dissertation submitted to the  
Chemical Engineering Programme

Universiti Teknologi PETRONAS  
in partial fulfilment of the requirement for the  
BACHELOR OF ENGINEERING (Hons)  
(CIVIL ENGINEERING)

Approved by,

  
(AP Dr Shamsul Rahman Kutty)

UNIVERSITI TEKNOLOGI PETRONAS  
TRONOH, PERAK  
July 2010

## CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.



---

AIMRAN BIN ABD HAMID



## ABSTRACT

Refinery wastewater produces high content of Ammonia which could be dangerous to the environment in many ways. Currently, the treatment system applied does not reduce the ammonia content to the Department of Environment limit which is 5 mg/L. In this thesis, the objective of the project is to study the feasibility of applying Aerobic Treatment System to refinery wastewater treatment system and to degrade the ammonia content.

The project comprises of two phases. The first phase focuses on the degradation of ammonia and nitrate in refinery wastewater using the Sequencing Batch Reactor treatment system. In this phase experiment were conducted by using 4 setup of SBR reactors consisting on different wastewater and the control of air into the reactors. The second phase was conducted after the objective of the first phase is fulfilled which was to study the feasibility of using aerobic treatment system in degrading the ammonia in refinery wastewater. The second phase uses Activated Sludge treatment system which uses activated sludge that is constantly pumped with air. Two reactors were run which one reactor acting as a control reactor while the other was used to degrade the refinery wastewater.

The result from the first phase shows that Ammonia and Nitrate from Petroleum Wastewater can be degraded using the Aerobic Treatment System. Out of the 4 reactors tested, aerobic system performed better than the anaerobic system. The aerobic system managed to degrade the ammonia up to 98% removal. The results of the second phase indicate the aerobic treatment system particularly in this case activated sludge treatment system can be used to degrade ammonia and oxidize it to nitrate. Addition of 10% refinery wastewater was conducted and the system is able to cope with the system well with 95% reduction of ammonia content. However, the other reading like Phosphorus content indicates the system is not fully stabilizes and needs more testing. The system also needed to have more refinery wastewater added to the system.

This topic may actually give a better understanding on the effectiveness of Aerobic Treatment system in degrading Ammonia content in Petroleum wastewater and the overall process of Nitrification. The system can be a viable alternative in terms of high strength wastewater treatment system in the near future.

## ACKNOWLEDGEMENTS

*In the name of Allah the Most Gracious and the Most Merciful*

Praise to Him the Almighty that in His will and given strength, had I managed to complete this two semesters Final Year Project titled Ammonia and Nitrate Removal in Refinery Wastewater using Aerobic Treatment System.

A lot has transpired during this period of time and I am indebt to so many who had made this project an illuminating and enriching venture. My deepest gratitude goes to my supervisors, Associate Proffesor Dr Shamsul Rahman whose continuous support, enlightening supervision and countless hours spent in sharing their insightful understanding, profound knowledge and valuable experiences throughout the project accomplishment.

Greatest appreciation goes to the Final Year Project committee, UTP Civil Engineering Department lecturers and laboratory technicians, Mr Khairul Anwaar whom I used to work with, for their help and kind support. Many thanks also go to Mr Megat and Mr Gasim for helping me during the procedures in the lab.

Last but not least, a special recognition to the oral presentation panel examiners, for their affirmative critics and kind evaluation on the assessment of the project. May the Almighty bless them all.

*Aimran Bin Abd Hamid*

*July 2010*

# TABLE OF CONTENTS

|                   |   |    |
|-------------------|---|----|
| <b>ABSTRACT</b>   |   | i  |
| <b>CHAPTER 1:</b> | <b>INTRODUCTION</b>   | 1  |
|                   | 1.1 Background of Study   | 1  |
|                   | 1.2 Problem Statement   | 2  |
|                   | 1.2.1 Problem Identification  | 2  |
|                   | 1.2.2 Significant of the Project  | 3  |
|                   | 1.3 Objectives  | 3  |
|                   | 1.4 Scope of Study  | 4  |
| <b>CHAPTER 2:</b> | <b>LITERATURE REVIEW</b>  | 5  |
|                   | 2.1 Petroleum Refinery Wastewater   | 5  |
|                   | 2.2 Sequencing Batch Reactor  | 6  |
|                   | 2.3 Design Criteria of SBR  | 6  |
|                   | 2.4 Nitrogen and Phosphorus Removal   | 7  |
|                   | 2.5 Parameters of conducting Biological<br>Treatment System                             | 8  |
|                   | 2.6 Treatability Study  | 8  |
|                   | 2.7 Acclimatization Period  | 9  |
|                   | 2.8 Stoichiometry of Biological Nitrification   | 9  |
|                   | 2.9 Nitrogenous and Phosphorus Matters<br>Removal from the Domestic Wastewater          | 10 |
|                   | 2.10 Biological Component of Activated Sludge<br>System                                 | 11 |
|                   | 2.11 Oxidation of Nitrate to Ammonia in<br>Anaerobic System (Annamox)                   | 13 |
|                   | 2.12 Identification of Trace Organics in Treated<br>Lubricating Oil Refinery Wastewater | 14 |



|                       |                                   |        |
|-----------------------|-----------------------------------|--------|
| <b>CHAPTER 3:</b>     | <b>METHODOLOGY</b>                | 15     |
| 3.1                   | Overall Methodology               | 15     |
| 3.1.1                 | Reading and Info Gathering        | 17     |
| 3.1.2                 | Phase 1-Experimental Methodology  | 17     |
| 3.1.3                 | Phase 1-Testing Parameters        | 20     |
| 3.1.4                 | Phase 1-Result Analysis           | 21     |
| 3.1.5                 | Phase 2 – Experimental Procedure  | 21     |
| 3.1.6                 | Phase 2- Refinery Wastewater Feed | 23     |
| <br><b>CHAPTER 4:</b> | <br><b>RESULT AND DISCUSSION</b>  | <br>24 |
| 4.1                   | Phase 1 Results                   | 24     |
| 4.1.1                 | Phase 1A Results                  | 24     |
| 4.1.2                 | Conclusion from Phase 1A          | 27     |
| 4.1.3                 | Phase 1B Results                  | 28     |
| 4.1.4                 | Conclusion from Phase 1B          | 32     |
| 4.2                   | Phase 2 Results                   | 33     |
| 4.2.1                 | Phase 2A Results                  | 33     |
| 4.2.2                 | Conclusion from Phase 2A          | 35     |
| 4.2.3                 | Phase 2B Results                  | 36     |
| 4.2.4                 | Conclusion from Phase 2B          | 41     |
| <br><b>CHAPTER 5:</b> | <br><b>CONCLUSION</b>             | <br>42 |
| <br><b>CHAPTER 6:</b> | <br><b>ECONOMIC BENEFITS</b>      | <br>43 |
| <br><b>REFERENCES</b> |                                   | <br>45 |



## LIST OF FIGURE

|             |  |    |
|-------------|--|----|
| Figure 2.1  | : Nitrobacter SP   | 12 |
| Figure 3.1  | : Schematic Diagram of Methodology of Study                    | 16 |
| Figure 3.2  | : Overview of Reactors   | 18 |
| Figure 3.3  | : Schematic Diagram of Reactors                                | 19 |
| Figure 3.4  | : Experimental Procedure for Phase 1                           | 20 |
| Figure 3.5  | : Phase 2 Reactor Setup  | 21 |
| Figure 3.6  | : Schematic Diagram of Phase 2 Reactor                         | 22 |
| Figure 4.1  | : Plot of mg/L of Ammonia vs Hours of Degradation for 24 Hours | 24 |
| Figure 4.2  | : Plot of mg/L of Nitrate vs Hours of Degradation for 24 Hours | 25 |
| Figure 4.3  | : Plot of mg/L of MLSS vs Hours of Degradation for 24 Hours    | 26 |
| Figure 4.4  | : Plot of mg/L of MLVSS vs Hours of Degradation for 24 Hours   | 27 |
| Figure 4.5  | : Plot of mg/L of Ammonia vs Hours of Degradation for 7 Hours  | 28 |
| Figure 4.6  | : Plot of mg/L of Nitrate vs Hours of Degradation for 7 Hours  | 29 |
| Figure 4.7  | : Plot of mg/L of MLSS vs Hours of Degradation for 7 Hours     | 30 |
| Figure 4.8  | : Plot of mg/L of MLVSS vs Hours of Degradation for 7 Hours    | 30 |
| Figure 4.9  | : Plot of mg of Ammonia per mg of MLVSS vs Degradation Hours   | 31 |
| Figure 4.10 | : Plot of mg/L of Ammonia vs Sampling Day                      | 33 |
| Figure 4.11 | : Plot of mg/L of Nitrate vs Sampling Day                      | 34 |
| Figure 4.12 | : Plot of mg/L of Phosphorus vs Sampling Day                   | 35 |
| Figure 4.13 | : Plot of mg/L of Ammonia vs Sampling Day                      | 36 |
| Figure 4.14 | : Plot of mg/L of Nitrate vs Sampling Day                      | 37 |

|             |  |    |
|-------------|--|----|
| Figure 4.15 | : Plot of mg/L of Phosphorus vs Sampling Day                     | 38 |
| Figure 4.16 | : Plot of mg of Ammonia per mg of MLVSS vs Sampling Day          | 39 |
| Figure 4.17 | : Plot of mg/L of Theoretical and Actual Nitrate vs Sampling Day | 40 |

### LIST OF TABLE

|           |  |    |
|-----------|--|----|
| Table 2.1 | : SBR Treatment Parameter                          | 8  |
| Table 3.1 | : Differences between reactor                      | 18 |
| Table 3.2 | : Settings of Reactor                              | 19 |
| Table 6.1 | : The estimation of the operating cost of research | 44 |

### LIST OF FORMULAS

|           |   |    |
|-----------|---|----|
| Formula 1 | : $2\text{NH}_4^+ + 3\text{O}_2 \longrightarrow 2\text{NO}_2^- + 4\text{H}^+ + 2\text{H}_2\text{O}$   | 9  |
| Formula 2 | : $2\text{NO}_2 + \text{O}_2 \longrightarrow 2\text{NO}_3$  | 9  |
| Formula 3 | : $\text{NH}_4^+ + 2\text{O}_2 \longrightarrow \text{NO}_3^- + 2\text{H}^+ + \text{H}_2\text{O}$  | 9  |
| Formula 4 | : $4\text{CO}_2 + \text{HCO}_3^- + \text{NH}_4^+ + \text{H}_2\text{O} \longrightarrow \text{C}_5\text{H}_7\text{O}_2\text{N} + 5\text{O}_2$                                   | 10 |
| Formula 5 | : $\text{NH}_4^+ + 1.863\text{O}_2 + 0.098\text{CO}_2 \longrightarrow 0.00196\text{C}_5\text{H}_7\text{NO}_2 + 0.98\text{NO}_3^- + 0.0941\text{H}_2\text{O} + 1.98\text{H}^+$ | 10 |
| Formula 6 | : $\text{NH}_4^+ + \text{NO}_2^- \rightarrow \text{N}_2 + 2\text{H}_2\text{O}$  | 13 |

# **CHAPTER 1**

## **INTRODUCTION**

### **1.1 BACKGROUND OF STUDY**

The background of this study is emphasized on the reduction of ammonia in refinery wastewater using biological treatment system. The focus of the project will be aerobic treatment system. The study will go in particular depth into oxidization of ammonia to nitrate and the process that revolves around it. Ammonia is very toxic to the aquatic organisms which may include over enrichment of ammonia to sensitive aquatic ecosystems (Kurvits and Martha, 1999). The biological transformation of ammonium ions (formed when ammonia dissolves in water) to nitrate ions in soils (nitrification) and plant uptake both release acidity into the soil, contributing to soil acidification. Excess of nitrogen will also cause eutrophication where excess nutrients caused accelerated algae growth and reduce the oxygen level in water causing fish to die. Similarly, sensitive crops that are cultivated near significant sources of ammonia may be damaged by over-fertilization caused by ammonia deposition (van der Eaden, 1998).



## **1.2 PROBLEM STATEMENT**

### **1.2.1 Problem Identification**

Wastewater treatment has been an integral part of civilization since 20<sup>th</sup> century. Now, we are dealing with variety types of wastewater comprising of more and more dangerous chemicals and heavy metals. Some industrial facilities generate ordinary domestic sewage that can be treated by municipal facilities. Industries that generate wastewater with high concentrations of conventional pollutants (e.g. oil and grease), toxic pollutants (e.g. heavy metals, volatile organic compounds) or other nonconventional pollutants such as ammonia, need specialized treatment systems. Some of these facilities can install a pre-treatment system to remove the toxic components, and then send the partially-treated wastewater to the municipal system. Industries generating large volumes of wastewater typically operate their own complete on-site treatment systems

Oil accounts for a large percentage of the world's energy consumption, ranging from a low of 32% for Europe and Asia, up to a high of 53% for the Middle East (International Energy Annual 2004). Other geographic regions' consumption patterns are as follows: South and Central America (44%), Africa (41%), and North America (40%). The world consumes 30 billion barrels (4.8 km<sup>3</sup>) of oil per year, with developed nations being the largest consumers. The United States consumed 24% of the oil produced in 2004. The production, distribution, refining, and retailing of petroleum taken as a whole represent the world's largest industry in terms of dollar value (International Energy Annual 2004).

From this, petroleum industry being the world's largest industry generates a lot of wastewater from refinery process. As it produces plenty of wastewater, it also produces a lot of ammonia which will harm the environment. Simple analysis was done to determine the amount of ammonia in the refinery wastewater taken from a local refinery treatment plant shows that the ammonia content is up to 48 mg/L of the refinery wastewater. Recent Department of Environment publication of (Environmental Quality (Sewage and Industrial Effluent, 2009) Regulations 1974) indicates that the ammonia and nitrate effluent limit for Standard A and Standard B is 5.0 mg/L respectively. This shows that the refinery wastewater needed to be



treated up to 5.0 mg/L of ammonia content before it can be released to nearby stream.

Ammonia plays an important part in transporting acidic pollutants by the formation of relatively stable particles of ammonium sulphate and ammonium nitrate. Although ammonia is an alkaline gas, it contributes to acidification of soil through nitrification, and by combining with acid gases to form ammonium sulphate and nitrate aerosol particles, that then deposit from the atmosphere faster than either the acid gas or ammonia would deposit separately (National Research Council, 2002). As an alternative to these solutions, petroleum wastewater treatment plant can also use Sequencing Batch Reactors to degrade the high strength wastewater. However, there is less information known about the effectiveness of Sequencing Batch Reactor to degrade the high strength wastewater.

### **1.2.2 Significant of the Project**

The purpose of this research is to study the effectiveness of aerobic treatment system in dealing with the toxicity of refinery wastewater and the capability to degrade the ammonia-nitrogen content using the aerobic treatment system. Should the system is capable of degrading 100% concentration of the refinery wastewater and reduce the ammonia content to 99% removal, the system can be modeled in a large scale and be used as an alternative to other methods in degrading refinery wastewater.

## **1.3 OBJECTIVES OF STUDY**

- **Phase 1** - To study the feasibility of applying aerobic treatment system to refinery wastewater treatment system using SBR
- **Phase 2** - Once the first objective is fulfilled, the second part of the research is to study the degradation of ammonia and nitrate from the refinery wastewater using the aerobic treatment system.

## 1.4 SCOPE OF STUDY

The project will cover aerobic treatment system and the degradation of ammonia-nitrogen from refinery wastewater. The first scope of the project is to determine the feasibility of using an aerobic treatment system to treat refinery wastewater. The second scope is to do comprehensive analysis on the degradation of ammonia-nitrogen in the refinery wastewater. The question to ask is the system capable of degrading the ammonia in the toxic environment.

The main points as the scope of study consist of the following:

- a) **Phase 1** – In Phase 1, we need to know is the aerobic treatment system suitable to treat the refinery wastewater. The study from the phase 1 is primarily done to compare the treatability studies in aerobic treatment system and anaerobic treatment system. This is the basic comparison to determine which system is more suitable to treat the refinery wastewater. Although there are many biological treatment systems available, to do better comparison between the two systems, the sequencing batch reactor is chosen as all the rest of the parameters are the same except for the exposure of air. Aerobic treatment system is given air while the anaerobic treatment system is not given any air.
- b) **Ammonia and Nitrogen Parameters** – For the Phase 1, to compare aerobic treatment system and the anaerobic treatment system of the SBR, ammonia and nitrogen parameters are tested. This will be an important indication on the determination of the suitable system.
- c) **Phase 2** – From the result of the Phase 1, further experimentation and depth will be covered in Phase 2. In Phase 2, the testing parameters will be the same which is ammonia and nitrogen. However, the penetration of the subject will be deeper which includes nitrification kinetics. In Phase 2, based on the Phase 1 results, aerobic treatment system is chosen as it is capable of degrading ammonia-nitrogen. For this system, the activated sludge treatment system is chosen.

## CHAPTER 2

### LITERATURE REVIEW

#### 2.1 Petroleum Refinery Wastewater

The refinery effluent is a major source of contaminated wastewater and a source of hydrocarbons as oil under carry to the extent that emulsions are not completely broken. Oil under carry can be the single largest source of oil losses to the wastewater treatment system. Reduction in the amount of oil in the under carry not only reduces sewer loadings but also recovers valuable raw material that would otherwise be lost. Rates vary with the water content of the crude oil and the degree of difficulty in desalting the crude, but a representative rate would be around 2-2.5 gallons of wastewater per barrel of crude oil feed to the unit (Greg Johnson, 2005). Desalter water contains salt, sludge, rust, clay, and varying amounts of emulsified oil (oil under carry). Depending on the crude oil source, it may or may not contain significant levels of hydrogen sulfide, ammonia, and phenolic compounds (Greg Johnson, 2005). Relatively high levels of suspended and dissolved solids are usually observed. Wastewater from petroleum refinery has the characteristics of high concentration of aliphatic and aromatic petroleum hydrocarbons, which could lead to heavy pollution on the surface of soil and rivers, (Yong Sun et al, 2008). The traditional treatments of refinery wastewater are based on the physicochemical, mechanical methods and further biological treatment in the integrated activated sludge treatment unit. Several solutions are proposed in this regard, including the use of coagulants and coagulation enhanced by centrifugation, ultra filtration and or sorption on organo-minerals. However, these techniques were not suitable to treat heavily contaminated water, as chemical oxygen demand concentration over 2000 mg/L, (Yong Sun et al, 2008). So, there is still a need for advanced techniques to remove non-biodegradable, high concentration organic substance of petroleum refinery wastewater.



## 2.2 Sequencing Batch Reactor (SBR)

The sequencing batch reactor (SBR) process utilizes a fill-and-draw reactor with complete mixing during the batch reactor step (after filling) and where the subsequent steps of aeration and clarification occur in the same tank. All SBR systems have five steps in common, which are carried out in sequence as follows: (1) fill, (2) react (aeration), (3) settle (sedimentation/clarification), (4) draw (decant), and (5) idle (Metcalf and Eddy 2004). For continuous flow applications, at least two SBR tanks must be provided so that one tank receives flow while the other completes its treatment cycle. Several process modifications have been made in the times associated with each step to achieve nitrogen and phosphorus removal (Metcalf and Eddy 2004).

## 2.3 Design Criteria of SBR

For any wastewater treatment plant design, the first step is to determine the anticipated influent characteristics of the wastewater and the effluent requirements for the proposed system. These influent parameters typically include design flow, maximum daily flow  $BOD_5$ , TSS, pH, alkalinity, wastewater temperature, total Kjeldahl nitrogen (TKN), ammonia-nitrogen ( $NH_3 - N$ ), and total phosphorus (TP) (Toprak, 2005). For industrial and domestic wastewater, other site specific parameters may also be required. The state regulatory agency should be contacted to determine the effluent requirements of the proposed plant. These effluent discharge parameters will be dictated by the state in the National Pollutant Discharge Elimination System (NPDES) permit. The parameters typically permitted for municipal systems are flowrate,  $BOD_5$ , TSS, and Fecal Coliform. In addition, many states are moving toward requiring nutrient removal. Therefore, total nitrogen (TN), TKN,  $NH_3 - N$ , or TP may also be required. It is imperative to establish effluent requirements because they will impact the operating sequence of the SBR. For example, if there is a nutrient requirement and  $NH_3 - N$  or TKN is required, then nitrification will be necessary (Toprak 2005).



If there is a TN limit, then nitrification and denitrification will be necessary. Once the influent and effluent characteristics of the system are determined, the engineer will typically consult SBR manufacturers for a recommended design (Toprak 2005). Based on these parameters, and other site specific parameters such as temperature, key design parameters are selected for the system. An example of these parameters for a wastewater system loading is listed in table given below.

| Parameter                             | Municipal     | Industrial    |
|---------------------------------------|---------------|---------------|
| F / M (kg BOD / kg MLSS.day)          | 0.15 - 0.40   | 0.15 - 0.60   |
| Treatment cycle duration (hr)         | 4             | 4 - 24        |
| Typically low water level MLSS (mg/L) | 2,000 - 2,500 | 2,000 - 4,000 |
| Hydraulic retention time (hr)         | 6 - 14        | Varies        |

**Table 2.1**

## **2.4 Nitrogen and Phosphorus Removal using Sequencing Batch Reactor**

A study has been made by J.Y. Hu et al (2005) on usage of Sequencing Batch Reactor system for nitrogen and phosphorus removal from municipal wastewater. From the journal, the study is made by comparing the efficiency of sequencing batch biofilm reactor (SBBR) with conventional SBR system. The SBBR system uses attached suspended growth SBR which is a process of coupling suspended activated sludge and attached growth process into a single system. From the results, the study has demonstrated that the attached growth suspended SBR and the conventional SBR were efficient for treating municipal sewage in terms of nitrogen, phosphorus and organic carbon removal (J.Y. Hu et al 2005). The Taguchi Method, which is the base for the design of the SBR system, is a design of experiment method originally practiced in quality engineering, could be successfully applied for optimizing the treatment performance. This experimental design method is useful for obtaining a rough selection of optimal controlling factors and levels but one must realize that the

basis of comparison (removal efficiency, effluent concentration, mass removed) will affect the ranking obtained.

## **2.5 Parameters of conducting Biological Treatment System or Chemical Treatment System**

Domestic wastewater is economical to be degraded using biological treatment system such as Activated Sludge system or even Sequencing Batch Reactor. For industrial wastewater, further studies needed to be done to determine the feasibility of applying the biological treatment system. In normal condition, the industrial wastewater will use physical-chemical treatment process which included the usage of clarifiers, filtration and carbon adsorption system combining with the small requirement and high efficiency of the system, (Wang, 1978)The usage of physical-chemical treatment system will only be feasible in terms of economy when the wastewater flow is more than 10 mgd (Wang et al, 1975). For wastewater flow less than 10 mgd, the more economical biological treatment system is more preferred. However, according to Wang et al, treatability studies needed to be conducted to determine whether the industrial wastewater which in this project involves Petroleum wastewater is non-toxic and biodegradable. A treatability study here refers to biodegradability of the wastewater or the combination of industrial wastewater and domestic wastewater.

## **2.6 Treatability Study**

The best example of conducting treatability studies (Wang et al, 1975) is by measuring the oxygen uptake rate (Beer et al, 1975). The steps involving in the measurement of the oxygen uptake rate include placing a measured quantity of wastewater in the biological reactor. The step is then followed by adding acclimatized activated sludge solids. Next, the reactor which contains activated sludge solids and required wastewater is aerated for 20 minutes while maintaining a constant temperature and mixing rate. The next step involves closing the air supply and measuring the rate or oxygen uptake with time which is then followed with the reading of dissolved oxygen (DO) within the reactor.



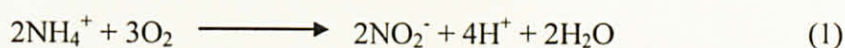
## 2.7 Acclimatization Period

However, before measuring the oxygen uptake rate, it is vital to have proper acclimatized activated sludge solids. This will be an integral part of the project. Activated sludge can be taken from an activated sludge treatment system which is available in the conventional sewage treatment plant. The range of the concentration of the sludge can be measured by determining the MLVSS. The MLVSS range should be 1000 - 3000 mg/l (Wang, 1978). Four parts of municipal wastewater and one part of industrial waste should serve as the initial feed solution (Wang et al). The ratio can be decreased over the period of one to two weeks with by the end of the process, the feeding solution will be 100% industrial wastewater. Progress of the acclimatization process can be monitored by measuring the oxygen uptake rate, MLVSS/hr, daily COD and BOD removal (Wang et al, 1975) When both oxygen uptake rate and BOD/COD removal rate ration approach constant, the sludge is well acclimatized and the project can be proceeded (Wang, 1978)

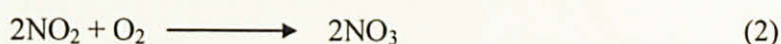
## 2.8 Stoichiometry of Biological Nitrification

According to Metcalf and Eddy (2004), the energy-yielding oxidation from ammonia to nitrate is as follows:

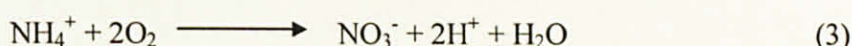
Nitroso Bacteria:



Nitro – Bacteria:



Total oxidation reaction:



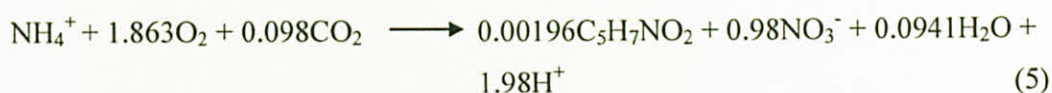
Based on the equation above, the total oxidation reaction, the oxygen required for complete oxidation of ammonia is 4.57 g O<sub>2</sub>/g N oxidized with 3.43 g O<sub>2</sub>/g used for nitrate production and 1.14 g O<sub>2</sub>/g NO<sub>2</sub> oxidized.

When synthesis is considered, the amount of oxygen required is less than 4.57 g O<sub>2</sub>/g N. In addition to oxidation, oxygen is obtained from fixation of carbon dioxide and nitrogen into cell mass. (Metcalf and Eddy, 2004) Along with obtaining energy, a portion of the ammonium ion is assimilated into cell tissue. The biomass synthesis reaction can be represented as follows:



The chemical formula C<sub>5</sub>H<sub>7</sub>O<sub>2</sub>N is used to represent the synthesized bacterial cells.

Using half reactions, it can be used to create an equation for the overall nitrification reaction.



From the above equation, it will be noted that for each g of ammonia nitrogen (as N) converted, 4.25 g of O<sub>2</sub> are utilized, 0.16 g of new cells are formed, 7.07 g of alkalinity as CaCO<sub>3</sub> are removed, and 0.08 g of inorganic carbon are utilized in the formation of new cells.

## 2.9 Nitrogenous and Phosphorus Matters Removal from the Domestic Wastewater by an Activated Sludge Reactor of Nitrification-Denitrification Type

Nitrification phenomenon is naturally taking place in the reactor by using dissolved oxygen due to the presence of aerobic and autotrophic bacteria while starting the nitrosomonas bacteria catalyzes oxidation process in order to change nitrogenous matters from ammonium to nitrite form and pursuit the nitrobacteracts to complete total oxidation of nitrogen matter which changes nitrite to nitrate. (Fulazzaky, 2002)

Assuming the chemical composition of nitrifiant bacteria is considerable inform of C<sub>5</sub>H<sub>7</sub>O<sub>2</sub>N. Therefore, utilizing one gram of ammonium needs 4.2 grams of oxygen to synthesize a new biomass which yields 0.13 grams of bacteria's cells (Fulazzaky 1998).



During the period of nitrification, it seems that the oxidation of ammonium reduces the amount of alkalinity in the water wherein one gram of ammonium consumes 8.6 grams of alkalinity. Indeed, in the case of inadequate bicarbonate in a domestic wastewater, pH decreases. The biological process meanwhile produces the effluent with riches of nitrate identifying the passing of the effluent standards. After the nitrification process is considerable stable identifying that the concentration of nitrate in the effluent is constant. The elimination of nitrogenous pollutants may be continued to change the reactor from aerobic to anaerobic condition so that, the nitrification process starts due to the presence of certain chemi-organotrope bacteria in the activated sludge which able to replace DO as sources of oxygen with the oxygen of nitrate and yielding free nitrogen as the final step of denitrification process (Fulazzaky, 2002). It is energetically remarked that the denitrification process is more effective than nitrification and, as a consequence, during the denitrification phase the growth rate of bacteria is more important so that this period is short.

## **2.10 The Biological Component of the Activated Sludge System**

The biological component of the activated sludge system is comprised of microorganisms. The composition of these microorganisms is 70 to 90 percent organic matter and 10 to 30 percent organic matter. Cell makeup depends on both the chemical composition of the wastewater and the specific characteristics of the organisms in the biological community. (Water Environment Association, 1987) Bacteria, fungi, protozoa, and rotifers constitute the biological component, or biological mass, of activated sludge. In addition, some metazoa, such as nematode worms, may be present. However, the constant agitation in the aeration tanks and sludge recirculation are deterrents to the growth of higher organisms. The species of microorganism that dominates a system depends on environmental conditions, process design, the mode of plant operation, and the characteristics of the secondary influent wastewater (Water Environment Association, 1987). The microorganisms that are of greatest numerical importance in activated sludge are bacteria, which occur as microscopic individuals from one micron in size to visible aggregations or colonies of individuals. Some bacteria are strict aerobes (they can only live in the presence of oxygen), whereas others are anaerobes (they are active only in the

absence of oxygen). The preponderance of bacteria living in activated sludge are facultative—able to live in either the presence or absence of oxygen, an important factor in the survival of activated sludge when dissolved oxygen concentrations are low or perhaps approaching depletion. While both heterotrophic and autotrophic bacteria reside in activated sludge, the former predominate. Heterotrophic bacteria obtain energy from carbonaceous organic matter in influent wastewater for the synthesis of new cells. At the same time, they release energy via the conversion of organic matter into compounds such as carbon dioxide and water. Important genera of heterotrophic bacteria include *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Citromonas*, *Flavobacterium*, *Pseudomonas*, and *Zoogloea*. (Jenkins, et al., 1993) Autotrophic bacteria in activated sludge reduce oxidized carbon compounds such as carbon dioxide for cell growth. These bacteria obtain their energy by oxidizing ammonia nitrogen to nitrate nitrogen in a two-stage conversion process known as nitrification. Due to the fact that very little energy is derived from these oxidation reactions, and because energy is required to convert carbon dioxide to cellular carbon, nitrifying bacteria represent a small percentage of the total population of microorganisms in activated sludge. In addition, autotrophic nitrifying bacteria have a slower rate of reproduction than heterotrophic, carbon-removing bacteria. Two genera of bacteria are responsible for the conversion of ammonia to nitrate in activated sludge, *Nitrobacter* and *Nitrosomonas*. (Water Environment Society, 1987)

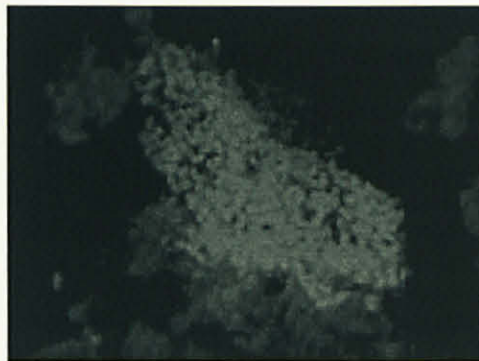


Figure 2.1 – *Nitrobacter* SP



Nitrification generally occurs when the time that the sludge stays in the system (called the mean cell residence time, or MCRT) is increased. A longer mean cell residence time, therefore, allows an adequate population of nitrifying bacteria to be built up. However, because the oxygen demand for complete nitrification is high, both the necessary oxygen supply and power requirements for the system will be increased. Moreover, optimum pH for the growth of nitrifying bacteria is in the 8 to 9 range, with pH levels below 7 causing a substantial reduction in nitrification activity. In the process of converting ammonia to nitrate, mineral acidity is produced. In instances when insufficient alkalinity exists, the pH in the system will drop, potentially inhibiting nitrification. Finally, though nitrification occurs over a wide range of temperatures, a reduction in temperature produces a slower rate of reaction. (Water Environment Society, 1987). Some activated sludge systems have been designed specifically to promote the higher growth rate of bacteria that remove carbon from influent wastewater, and adding chemicals may suppress nitrification. Other systems are operated to achieve nitrification in the second stage of a two-stage activated-sludge system due to the longer mean cell residence time (MCRT) necessary for nitrification. Still other systems are designed to promote nitrification. (Water Environment Society, 1987)

## **2.11 – Oxidation of Ammonia to Nitrate in Anaerobic System (Anammox Reaction)**

In this biological process, nitrite and ammonium are converted directly into dinitrogen gas. This process contributes up to 50% of the dinitrogen gas produced in the oceans. It is thus a major sink for fixed nitrogen and so limits oceanic primary productivity. (B.Kartal et al, 2010) The overall catabolic reaction is:



The bacteria that perform the anammox process belong to the bacterial phylum Planctomycetes, of which *Planctomyces* and *Pirellula* are the best known genera.



Currently four genera of anammox bacteria have been (provisionally) defined: *Brocadia*, *Kuenenia*, *Anammoxoglobus*, *Jettenia* (all fresh water species), and *Scalindua* (Arrigo, 2005). The anammox bacteria are characterized by several striking properties: they all possess one anammoxosome, (B.Kartal et al, 2010) a membrane bound compartment inside the cytoplasm which is the locus of anammox catabolism. Further, the membranes of these bacteria mainly consist of ladderane lipids so far unique in Biology. Of special interest is the turnover of hydrazine (normally used as a high-energy rocket fuel and poisonous to most living organisms) as an intermediate. A final striking feature of the organism is the extremely slow growth rate. The doubling time is nearly two weeks. The anammox process was originally found to occur only from 20°C to 43°C ( Strous et al, 1999) but more recently, anammox has been observed at temperatures from 36°C to 52°C in hot springs (Jaeschke et al. 2009) and 60°C to 85°C at hydrothermal vents located along the Mid-Atlantic Ridge (Byrne et al, 2008)

## **2.12 – Identification of Trace Organics in a Treated Lubricating Oil Refinery Wastewater**

The COD of untreated mineral oil refinery wastewaters is mainly caused by aliphatic and aromatic hydrocarbons, phenols and to a less degree by heterocycles. Therefore, in many countries, it is not allowed to discharge these wastewaters without pre-treatment to rivers, lakes or to the sea or to sewers. Most of the hydrocarbons can be eliminated by a physio-chemical treatment stage as e.g. parallel plate interceptors, flocculation and/or floatation. Refinery wastewaters prepared in this way can be satisfactory purified by one or more aerobic biological stages (Tows, et al, 1994)

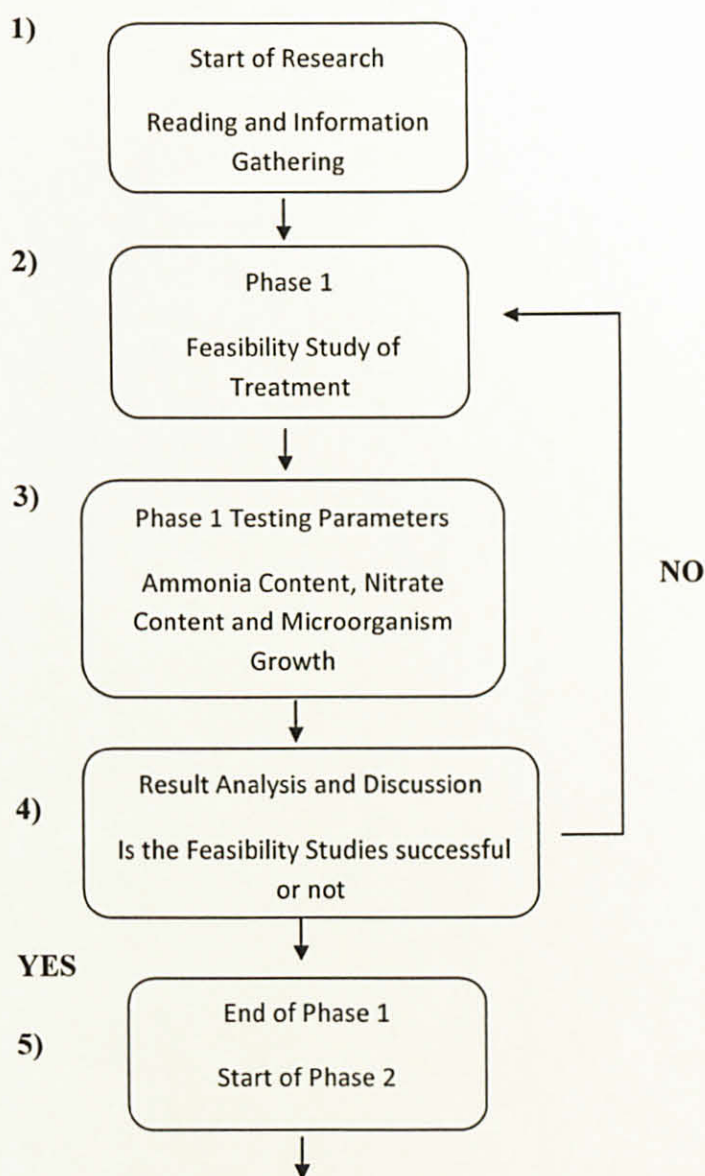
# CHAPTER 3

## METHODOLOGY

### 3.1 OVERALL METHODOLOGY

For this Final Year Project, research methodology comprises of two parts. The first part is the Phase One of the project which will be conducted in Semester 1 and the Phase Two is will be conducted on the second semester.

The overall methodology for the whole project is as follow:



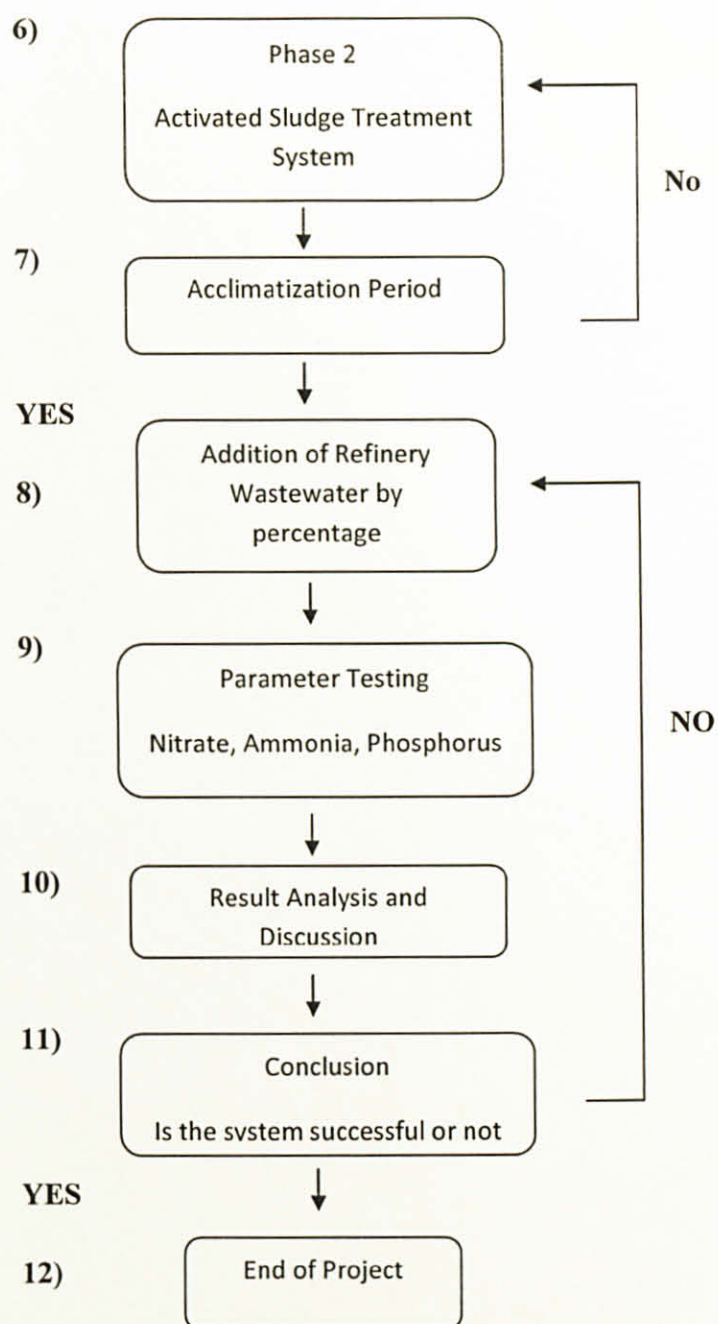


Figure 3.1 – Schematic Diagram of Methodology of Study



### **3.1.1 Reading and Information Gathering**

Information gathering is very crucial for the research. It is the first part of the project where as much information is searched to assist the author for the research purpose and also thesis writing. The information gathering is also important to prepare for the laboratory experimentation.

Few methods are used to get the vital information for the project. They are:

- a) Interview with Project Supervisor, Phd Student Research partner, other lecturers and also Lab Technician.
- b) Information from related Journals and Papers from previous students and lecturers at the Information Research Center (IRC).
- c) Extraction from books related with the topic such as the Wastewater Textbook
- d) Lab Manuals
- e) Internet, books, articles and journals.

### **3.1.2 Phase 1 – Experimental Methodology**

Phase 1 is the Feasibility or Treatability Studies on the Aerobic treatment system on the Refinery wastewater.

In this phase, we will conduct 2 types of system. The treatment system comprises of:

- a) Aerobic treatment system
- b) Anaerobic treatment system

The sample of wastewater that will be used for this experiment is the Petroleum Refinery Wastewater from the Kerteh Petroleum processing refinery. There will be four reactors running to test different setting for the wastewater. Each explained in table below

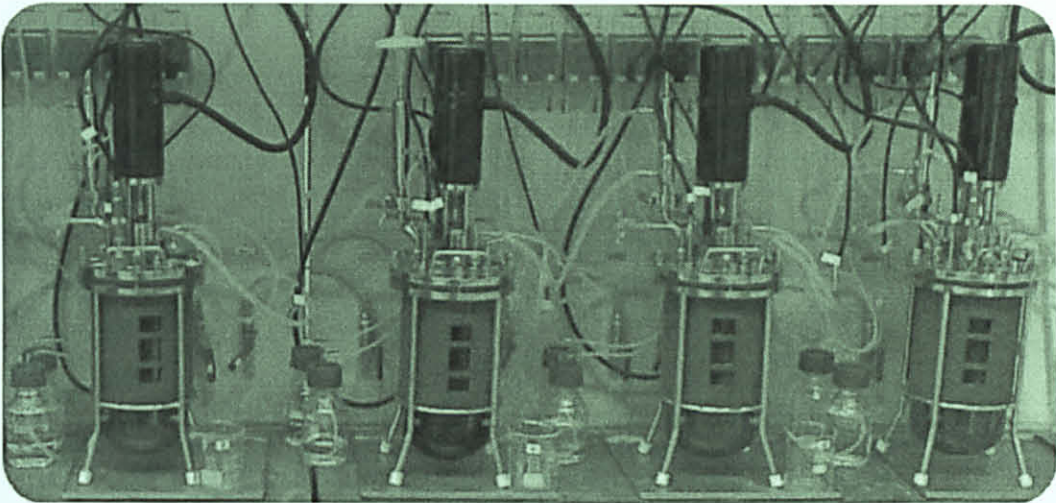


Figure 3.2 – Overview of the Reactors

| Reactor 1                | Reactor 2                  | Reactor 3   | Reactor 4                |
|--------------------------|----------------------------|---|--------------------------|
| Aerobic treatment system | Anaerobic treatment system | Effluent Reactor 2 treated using Aerobic treatment system | Aerobic treatment system |

Table 3.1 – Differences between Reactors

| Settings            | Reactor 1                  | Reactor 2                  | Reactor 3                  | Reactor 4                   |
|---------------------|----------------------------|----------------------------|----------------------------|-----------------------------|
| Influent wastewater | Raw Refinery               | Raw Refinery               | Effluent Reactor 2         | Refinery mixed with 50% STP |
| System              | Aerobic                    | Anaerobic                  | Aerobic                    | Aerobic                     |
| Cycle               | 24 Hours                   | 24 Hours                   | 24 Hours                   | 24 Hours                    |
| Volume              | 2 L Sludge, 1 L wastewater | 2 L Sludge, 1 L wastewater | 2 L Sludge, 1 L wastewater | 2 L Sludge, 1 L wastewater  |

|                        |                        |                        |                        |                        |
|------------------------|------------------------|------------------------|------------------------|------------------------|
| <b>Aeration Time</b>   | 22 hours               | 22 hours               | 22 hours               | 22 hours               |
| <b>Settling Time</b>   | 1 hour                 | 1 hour                 | 1 hour                 | 1 hour                 |
| <b>Fill and Decant</b> | 0.5 hours respectively | 0.5 hours respectively | 0.5 hours respectively | 0.5 hours respectively |

Table 3.2 – Setting of Reactor

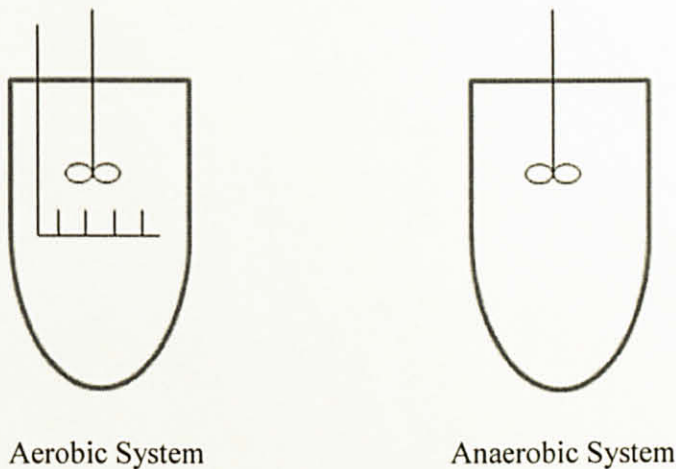


Figure 3.3 – Schematic Diagram of the reactors

All the system above is run as a Sequencing Batch Reactor (SBR) system. All the parameters are the same except for the presence of air in the aerobic treatment system and no air in the anaerobic treatment system. As the system is a SBR system, there will be (1) fill, (2) react (aeration), (3) settle (sedimentation/clarification), (4) draw (decant), and (5) idle. The SBR system is set to be running 22 hours of aeration time or react time, 1 hour for settling process, 0.5 hours for filling and decanting respectively making the whole SBR cycle to be 24 hours. The volume of each reactor is 3.0 Litres where 1 Litre is Acclimatized sludge, and another 2 Litres is the wastewater. All the reactors are set to run according to treatment system based on the table above. The aerobic treatment systems will be equipped with air supply mechanism to continuously provide air to the reactors. The anaerobic treatment



system will be sealed and prevented from making any contact with air. All the four reactors are equipped with magnetic stirrer to continuously stir the wastewater preventing it to settle. For reactor 3, the refinery wastewater needed to be degraded firsthand using the anaerobic treatment system before the wastewater is degraded with aerobic treatment system. For reactor 4, the refinery wastewater needed to be diluted with addition of 50% sewage treatment plant wastewater. For initial testing, the reactors were run for a period of 24 hours. Samples were taken from each reactor for every 6 hours interval. Few testing were conducted which included Ammonia content testing, nitrate content testing, Mixed Liquor Suspended Solid Testing (MLSS) and Mixed Liquor Volatile Suspended Solid Testing (MLVSS).

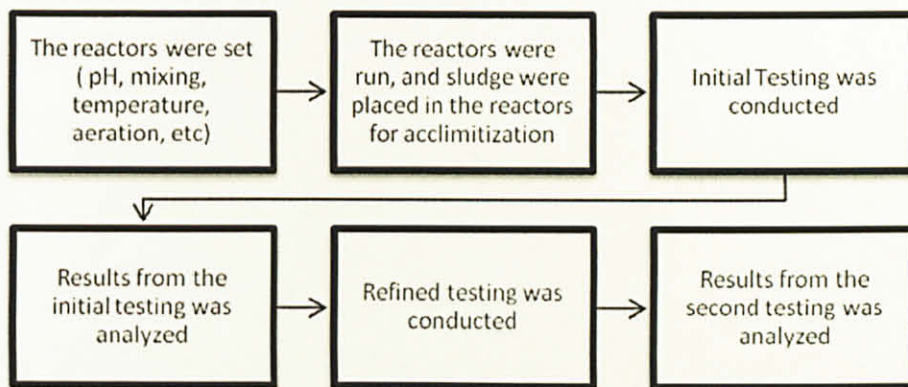


Figure 3.4 – Experimental procedures for Phase 1

### 3.1.3 Phase 1 – Parameters for Testing

The parameters that will be tested for the Phase 1 project are:

- a) Nitrate content test
- b) Ammonia content test
- c) MLSS and MLVSS tests

### **3.14 Phase 1 – Result Analysis and Discussion**

Based on the results from doing the testing in section above, the results were analyzed between the 4 reactors. Comparisons were made in terms of microorganism growth, ammonia reduction rate and the nitrate content from each effluent of the reactor.

### **3.15 Phase 2 – Activated Sludge Treatment System**

#### **3.15.1 – Experimental Procedure**

There are 2 stages of the activated sludge experimentation

- a) Setting up the system and acclimatization period
- b) Petroleum Wastewater Feeding process and Parameter monitoring



Figure 3.5 - Reactor setup

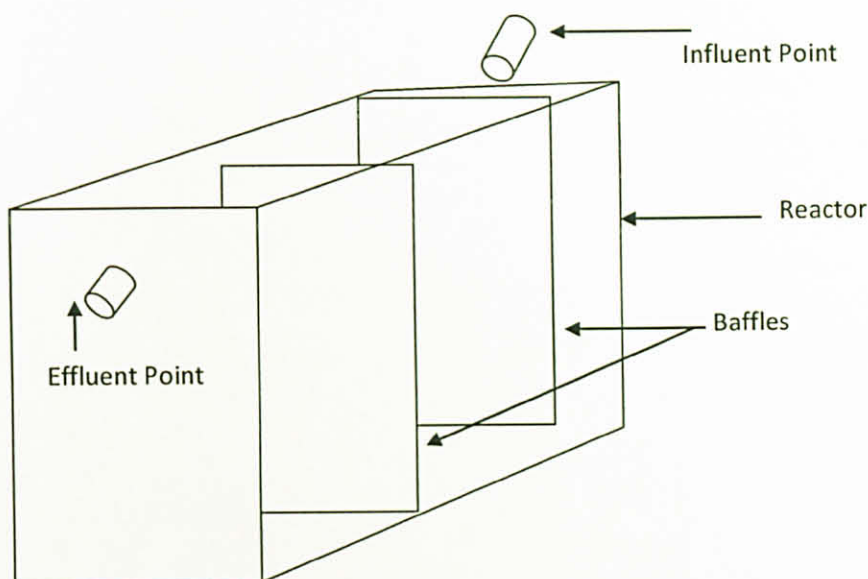


Figure 3.6 – Schematic Diagram of Reactor

For the first stage, the setup of the reactor involves 2 activated sludge reactors. Both of the reactors were filled with domestic sludge taken from the UTP Sewage Treatment Plant. To acclimatize the bacteria in the sludge, the food or the feeding process involved the wastewater from the clarifier of the UTP Sewage Treatment System. The reactors were sealed on top with continuous flow of air is pumped into the sludge. The intake rate of the wastewater is standardized throughout the acclimatization period. The wastewater is also continuously mixed using a standard mixer. This is to avoid settling process. The acclimatization period will be continued until the parameters such as COD, MLVSS and BOD is stable. (Wang et al). Both of the reactors is set to run on the same setting during the acclimatization process. The settings of the Activated Sludge are as follows:

- a) Sludge Age – 60 Days
- b) MLVSS required – 3000 mg/L
- c) Wastewater flow rate – 1 ml/min



d) Hydraulic Retention Time – 2 days

At the end of the acclimatization process is completed, the nitrification kinetics will be calculated based on the data gathered over the period.

### **3.16 Phase 2B – Refinery Wastewater Feeding**

For the second stage of the project, after the acclimatization process had been achieved, the feeding process of refinery wastewater will be conducted. The feeding of the wastewater will only be conducted to one of the reactors (Reactor A). Reactor B will maintain the same system as reference to the performance of reactor A. For the earlier stage, the refinery wastewater feeding will involved the mixture of refinery wastewater with the conventional STP wastewater. The first step will be feeding 10% of the refinery wastewater with 90% of conventional STP wastewater. This is to avoid shocking condition of the micro-organism. Testing will then be conducted including the identification of the nitrification kinetics. Based on the result, addition of the refinery will be increased to 20% of the total wastewater intake. The process will repeat itself until 100% of the wastewater flow consists of the Refinery Wastewater. The testing of both stages of the experiment includes:

- a) Nitrate content testing
- b) Ammonia content testing
- c) Phosphorus content testing

## CHAPTER 4

### RESULTS AND DISCUSSION

#### 4.1 PHASE 1 RESULT

##### 4.1.1 PHASE 1A – Initial Testing on 24 Hours

The experiments were conducted two times throughout the semester. For the first time, the focus of the experiment is to study the degradation of ammonia and nitrate in a 24 hours cycle. The cycle mentioned here is referring to the SBR cycle which starts from filling to decanting. By conducting this experiment, it is identified at which point the full degradation occurs. Below are the plotted graphs from the first experiment.

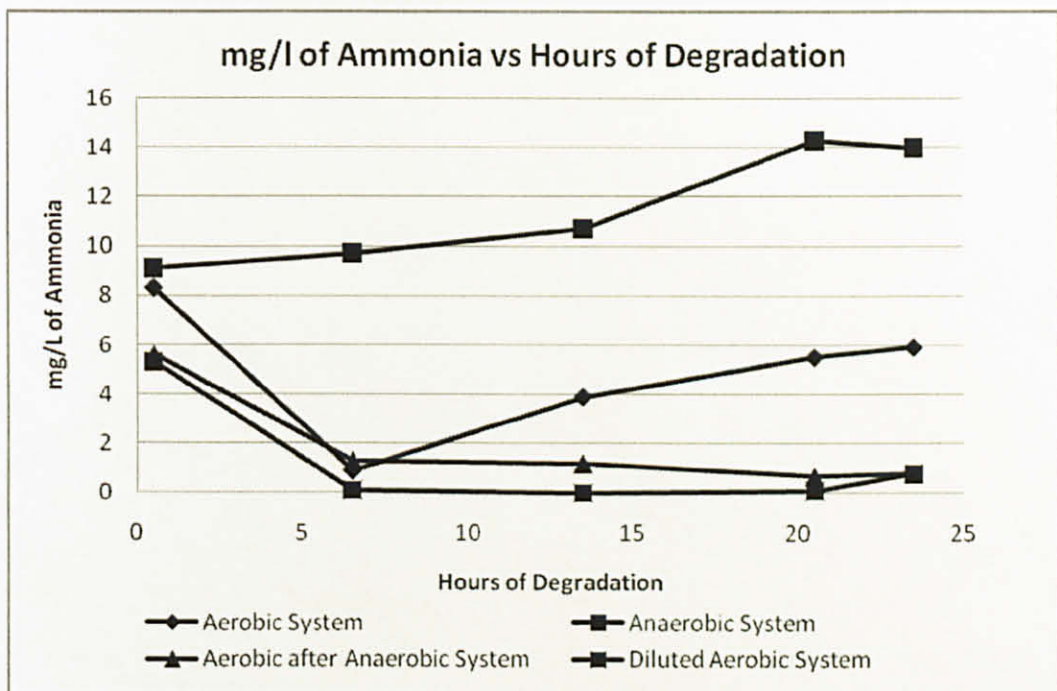


Figure 4.1 – Plot of mg/L of Ammonia vs Hours of degradation for 24 hours cycle

For ammonia content, the anaerobic aeration does not show any decrease of ammonia content. On the contrary, the ammonia content increases steadily over the

period of 24 hours. For the rest 3 reactors, the result shows that the ammonia is degraded by the first 6 hours instead of the whole 24 hours. Referring to the plots of degradation of ammonia and nitrate, it is seen that the reactor 3, aerobic after anaerobic aeration has managed to degrade all the ammonia inside the wastewater. It is known that when ammonia is degraded, the nitrate content in the water will increase.

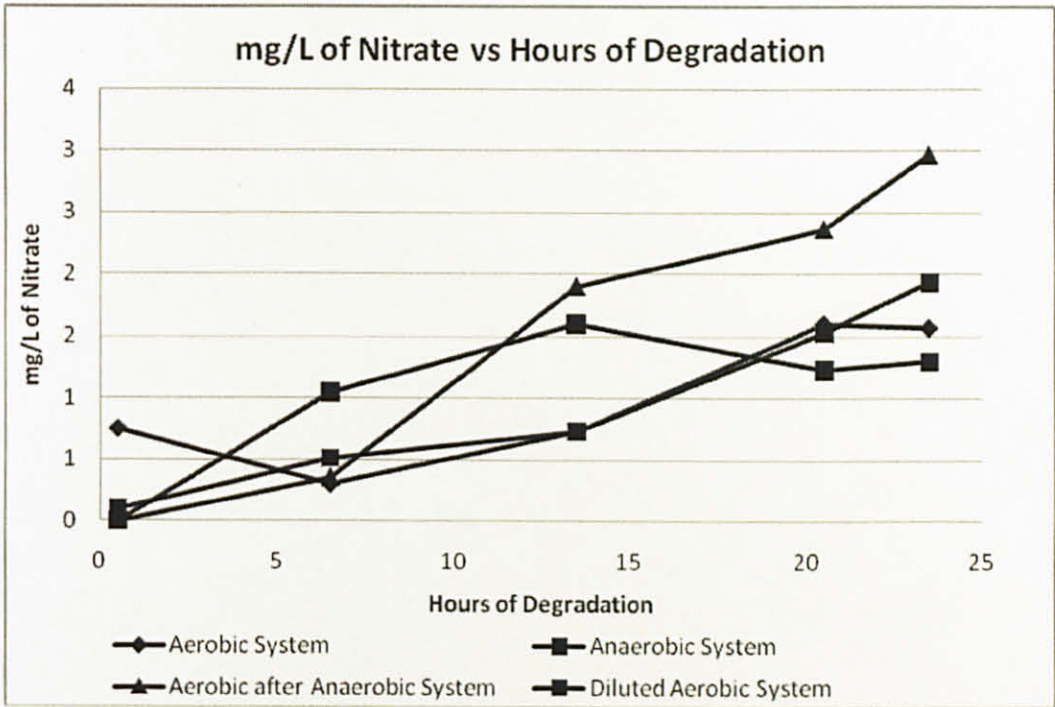
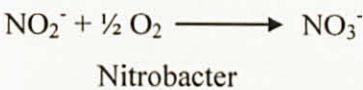
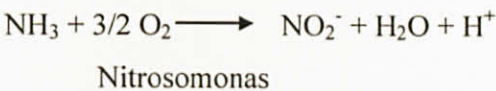


Figure 4.2 – Plot of mg/L of Nitrate vs Hours of degradation for 24 hours cycle

The overall nitrate content in the wastewater increases from the start point of the experiment to the end part of the experiment. Although at some point the level of nitrate drops, the overall content is much higher than the original nitrate content



For the third reactor, the aerobic after anaerobic aeration, the nitrate content was much higher than the rest of the reactors. The first reactor, aerobic aeration process, provided the least increase in the nitrate content. From the nitrate plot above, it is seen that for Reactor 3, aerobic aeration after anaerobic process, records the highest increase in nitrate content over the time period of 24 hours. From the nitrate plot above, it is seen that for reactor 3, aerobic aeration after anaerobic process, records the highest increase in nitrate content over the time period of 24 hours.

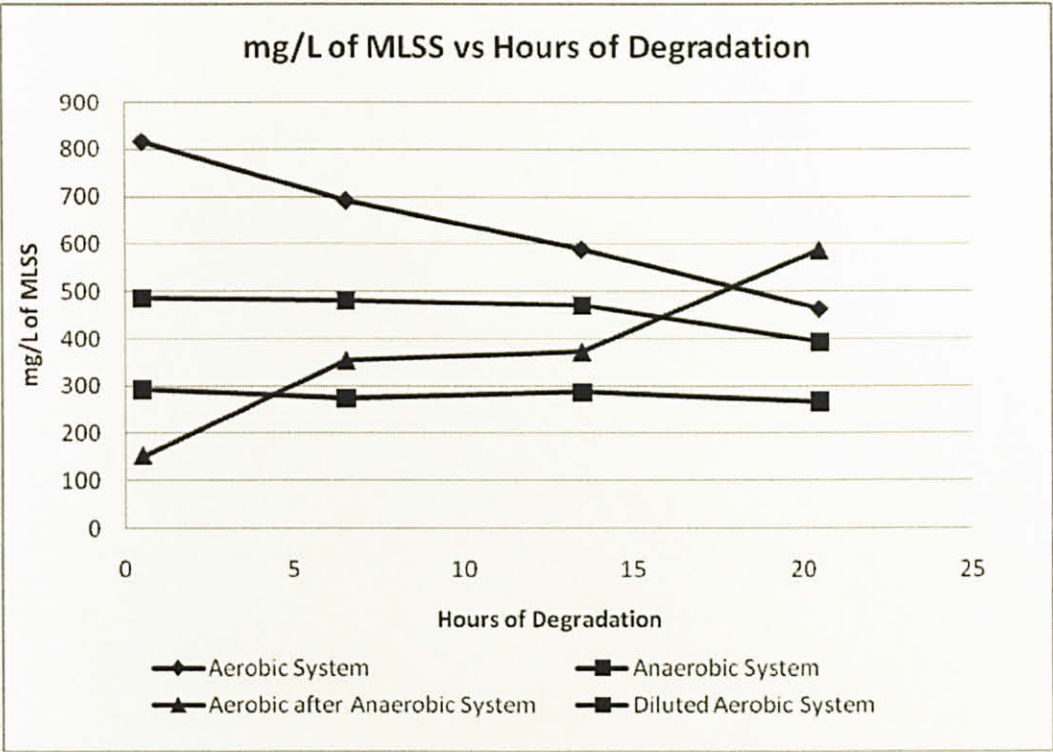


Figure 4.3 – Plot of mg/L of MLSS vs Hours of degradation for 24 hours cycle

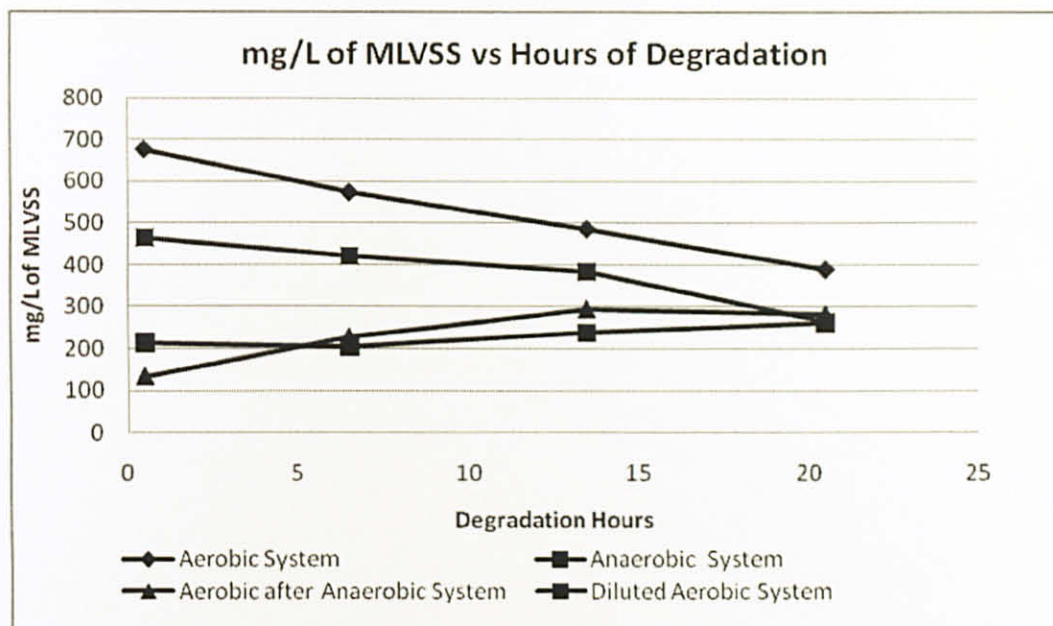


Figure 4.4 – Plot of mg/L of MLVSS vs Hours of Degradation for 24 hours cycle

To get a better understanding on the degradation of ammonia and nitrate, a better view on the MLSS and the MLVSS content is required. From the MLVSS plot, it is seen that the bacteria decreases in all reactors except the aerobic after anaerobic reactor. From the graph, it is seen that the bacteria in reactor 3 steadily growing until the decanting process where all the bacteria is filtered out of the system. From this observation, the bacteria in the rest reactors were dying from the experiment start time until the completed 24 hours. This is most probably due to the toxic content in the wastewater itself are not suitable for the growth of the bacteria

However, in the case of aerobic process after anaerobic reaction, the bacteria are able to grow. This is most likely the bacteria have acclimatized with the toxic condition of the wastewater.

#### 4.1.2 CONCLUSION FROM THE PHASE 1A TESTING

- 1) The best reactor that degrades the ammonia content as well as best inhibitor for the bacteria is the Reactor 3, aerobic aeration after anaerobic aeration.
- 2) The degradation time for the ammonia is only for the first 6 hours.
- 3) Further testing and experimentation will be conducted on the first 6 hours of the experiment.

**4.1.3 PHASE 1B – EXPERIMENT ON THE FIRST 7 HOURS OF  
DEGRADATION FOR THE SBR TREATMENT SYSTEM  
ON THE HIGH STRENGTH WASTEWATER**

From the first initial experiment on the 24 hours cycle of the SBR treatment, it is found out that the most of the ammonia is completely degraded after the first 6 hours of the experiment. Thus a second experiment focusing on the degradation of ammonia for the first 8 hours of the experiment is conducted using the same methodology for the first experiment. The only difference in this experiment is that the sample is taken for every 1 hour 30 minutes instead of every 6 hours. The experiment is ended after 6 samples are taken which is on the 7<sup>th</sup> hour of the experiment. Below are the plots from the experiment.

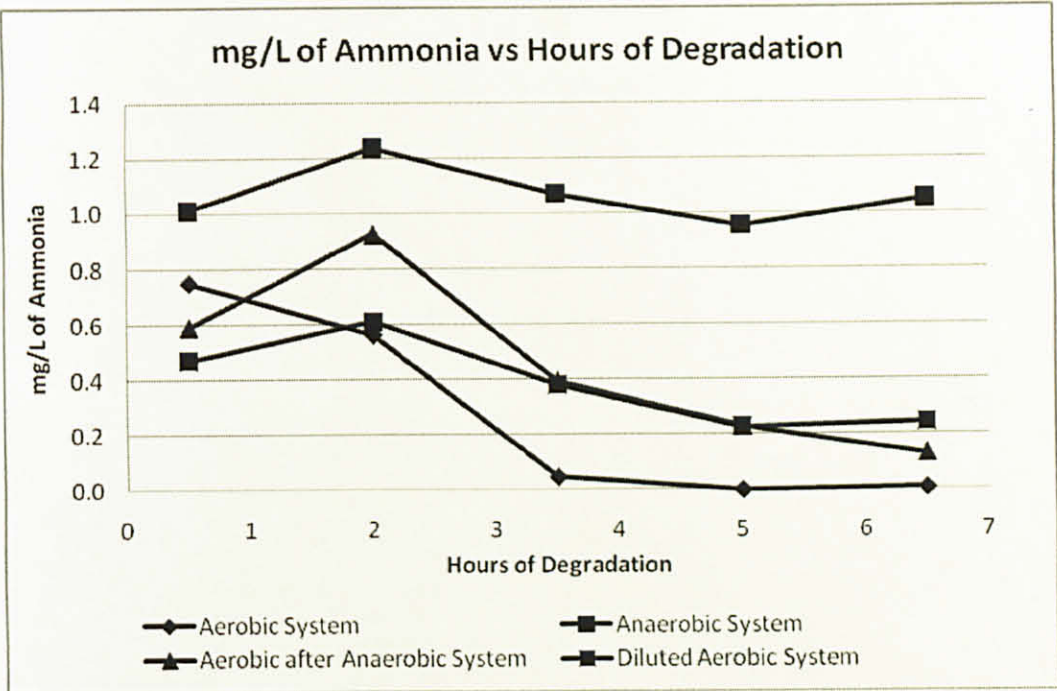


Figure 4.5 – Plot of mg/L of Ammonia vs Hours of Degradation for 7 hours cycle



For ammonia content, the anaerobic aeration reactor is the reactor that degrades the least ammonia. For the rest 3 reactors, the result shows that the ammonia is degraded by the first 4 hours instead of the whole 24 hours. Again, the results indicate the anaerobic system does not oxidize ammonia. The results shows it is consistent with the 24 hours testing conducted before. As explained in the literature review, the annamox condition allows the oxidization of Ammonia to Nitrate in anaerobic condition. In this situation, there is no annamox process ongoing as the specific bacteria needed for the annamox condition does not present.

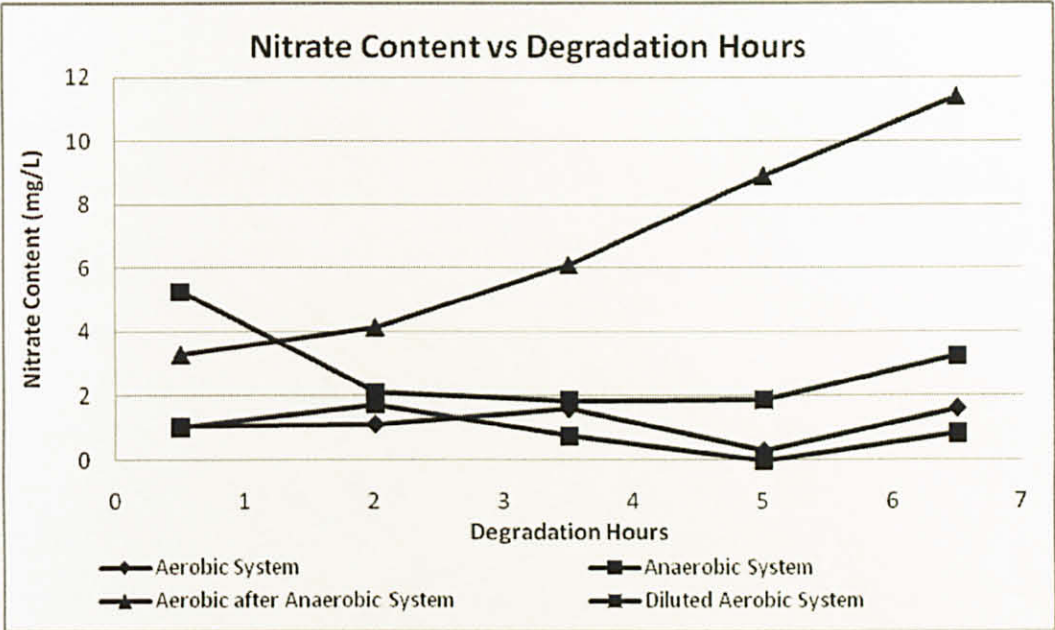


Figure 4.6 – Plot of mg/L of Nitrate vs Hours of Degradation for 7 hours cycle

The nitrate content for three reactors was more or less stable and only increases or drops ever slightly. For the third reactor, the aerobic after anaerobic reaction, the nitrate content was much higher than the rest of the reactors. The nitrate content kept increasing steadily from start of the experiment until the end of 7 hours.

The second reactor, anaerobic aeration process and the fourth reactor, the diluted aerobic system reactor, provided the least increase in the nitrate content while the, the nitrate content increases for the first reactor, aerobic and the third reactor aerobic after anaerobic reactor.

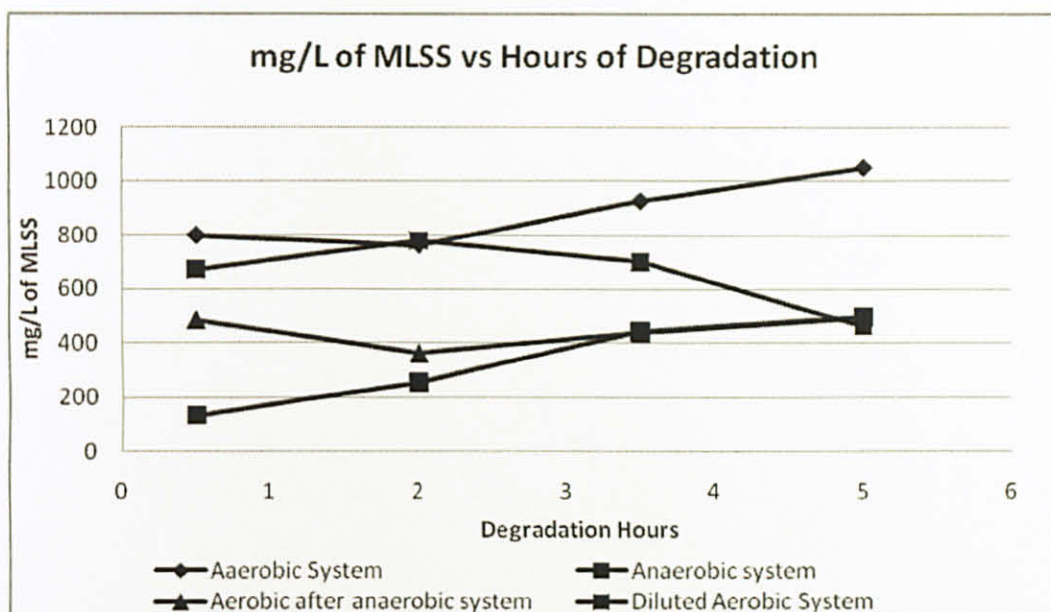


Figure 4.7 – Plot of mg/L of MLSS vs Hours of Degradation for 7 hours cycle

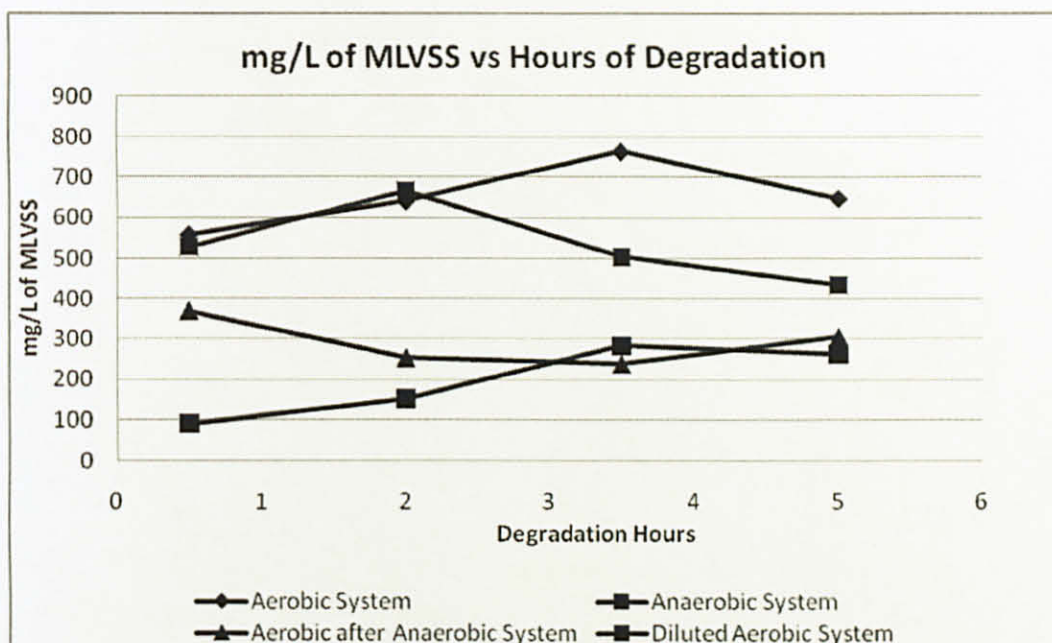


Figure 4.8 – Plot of mg/L of MLVSS vs Hours of Degradation for 7 hours cycle

To get a better understanding on the degradation of ammonia and nitrate, a better view on the MLSS and the MLVSS content is required. From the MLVSS plot, it is seen that the bacteria decreases in all reactors from the graph, it is seen that the

bacteria in reactor 3 which is the aerobic after the anaerobic aeration is the reactor that lost the least amount of bacteria. The drop in bacteria content in all 4 reactors suggests that the bacteria are not coping well with the toxic content of the wastewater. In the last experimentation, the reactor 3 was able to sustain the growth of the bacteria. However, this is not the case in this experiment. The possible reason for this is that the batch of bacteria used in this experiment is not acclimatizing enough to adapt to the toxic environment of the wastewater. However, in the reactor 3, the aerobic after anaerobic aeration, shows the least amount of bacteria lost and this still suggest that the reactor 3 is still the best reactor to be used to degrade this type of wastewater. The most increase in terms of nitrate content as well as the ability to degrade the ammonia also supports this discussion.

From the explanation above, it can be concluded that all the reactors except the reactor 3 which is the aerobic after anaerobic aeration are not efficient in degrading ammonia

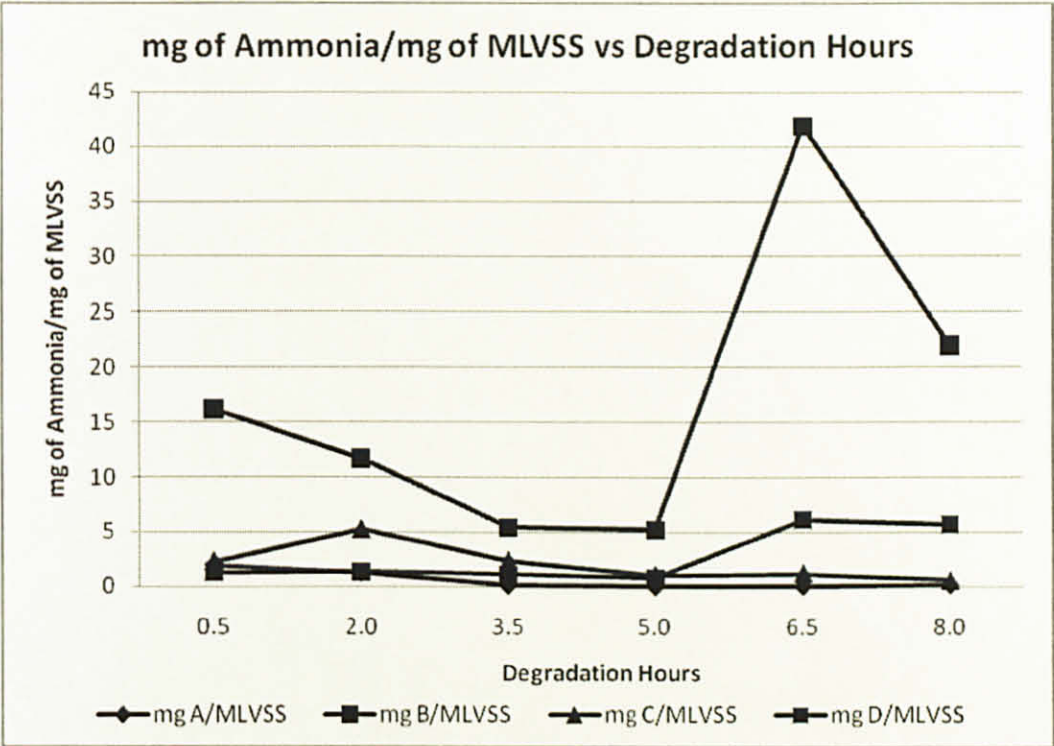


Figure 4.9 – Plot of mg of Ammonia per mg of MLVSS vs Hours of Degradation



Based on all the results obtained, a graph on mg of ammonia per mg of MLVSS for the testing (Figure 4.9) was plotted. From the plot it is obvious that for 3 types of reactors, Type 1, 3, and 4 managed to degrade the ammonia while maintaining the bacteria content. Only the reactor that conduct the anaerobic process did not managed to do it because the ration of ammonia over MLVSS is very high. This means that the ammonia content is high while the MLVSS content is low. However from the discussion above and from the 24 hours cycle testing conducted by the author, it is proven that the best reactor and consistent in degrading the ammonia will be the reactor 3, the aerobic after anaerobic process.

#### **4.1.4 CONCLUSION FROM THE PHASE 1B TESTING**

From the testing conducted, it can be concluded that the aerobic treatment system can be used to degrade the Petroleum wastewater. To further improve the experimentation, below is the result from the activated sludge treatment system.

The results from above suggest that aerobic treatment process can be used to degrade ammonia-nitrogen in the refinery wastewater.

With the end of Phase 1 Testing, the setup and the testing for Phase 2 can begin

4.2 PHASE 2 RESULTS

4.2.1 PHASE 2A - EXPERIMENT ON ACCLAMATIZATION PERIOD OF ACTIVATED SLUDGE TREATMENT SYSTEM

The Phase 2A concentrates on getting the sludge to acclimatize with the environment in the reactor. This is important as it will ensure the wastewater will be able to cope with the toxic condition of the refinery wastewater later on. There are 2 reactors, reactor A and B and all the samples are tested with nitrate, ammonia and phosphorus parameter. Below is the result of the testing.

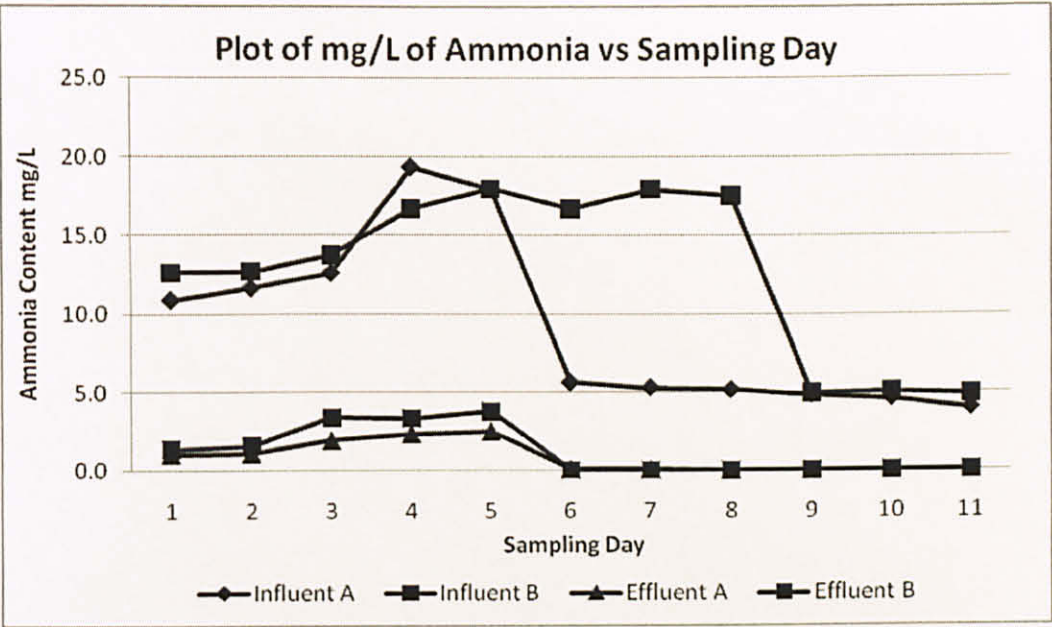


Figure 4.11 – Plot of mg/L of Ammonia vs Sampling Day

The ammonia content shows considerable drop from the influent to effluent. This supports the previous result that was carried out in the different reactor. Ammonia was converted to nitrate. That is why the amount of ammonia decreases after the influent passed through the activated sludge treatment system. Towards the end of the acclimatization period, from Sampling Day 7 to sampling day 11, the degradation rate of ammonia is consistent. The system is capable to achieve 98% removal of ammonia. This show the bacteria are comfortable with the environment and acclimatization has been achieved.

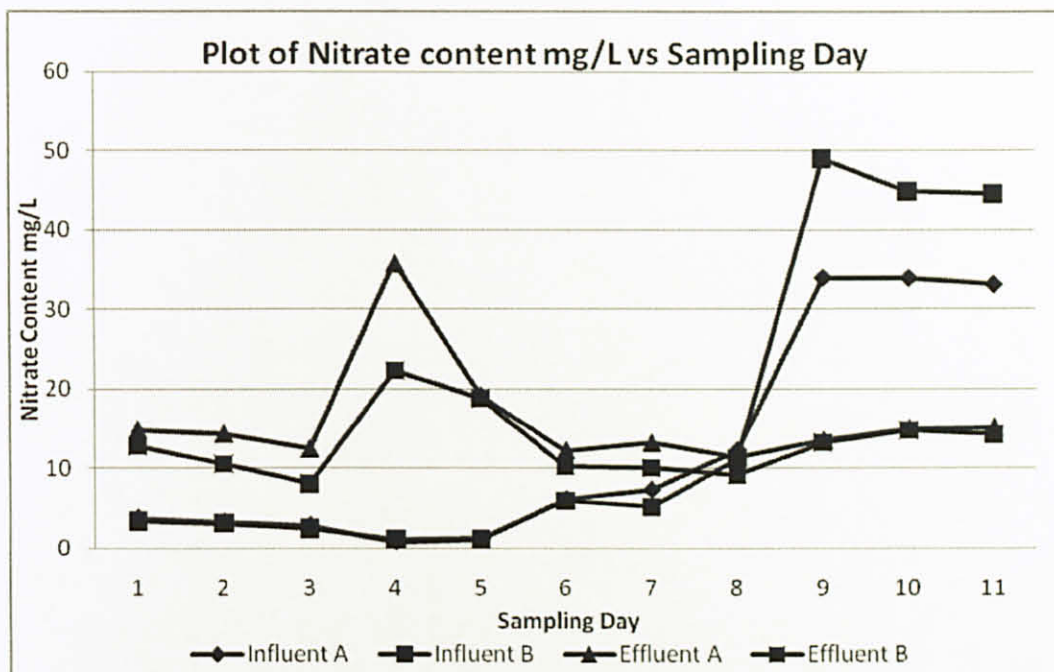


Figure 4.10 – Plot of mg/L of Nitrate vs Sampling Day

From the nitrate graph, it shows that the nitrate content increases from influent to effluent. The ammonia content shows considerable drop from the influent to effluent. This supports the previous result that was carried out in the different reactor. Ammonia was converted to nitrate. That is why the amount of ammonia decreases after the influent passed through the activated sludge treatment system. The effluent for both reactors became more stable in terms of nitrate content where both reactors producing around 15 mg/L of nitrate.



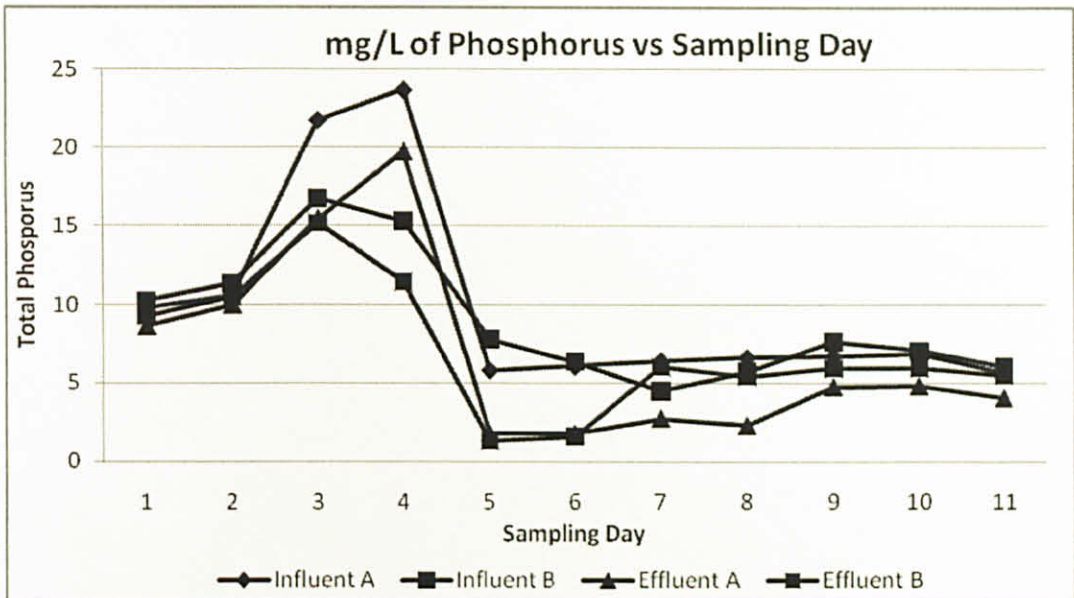


Figure 4.12 – Plot of mg/L of Phosphorus content vs Sampling Day

From the Phosphorus point of view, the activated sludge managed to degrade some of the phosphorus content. However, the degradation rate is not encouraging. Although this is still the acclimatization period, the bacteria are already starting to degrade the phosphorus. Towards the end of the acclimatization period, the amount of Phosphorus being degraded is quite consistent from Sampling Day 8 to 11. As the degradation is consistent, it shows that the bacteria are already settled with the environment and ready for more challenges.

#### 4.2.2 CONCLUSION FROM PHASE 2A TESTING

The results show that initially the degradation rate is inconsistent and amount of Nitrate, Ammonia and Phosphorus in the effluent are inconsistent. On later stages of the acclimatization period, the bacteria are able to cope better with the environment and produced better results. This shows that the acclimatization period is over and the addition of 10 % refinery wastewater can begin.

4.2.3 PHASE 2B - EXPERIMENT ON DEGRADATION OF REFINERY  
WASTEWATER USING ACTIVATED SLUDGE  
TREATMENT SYSTEM

After the acclimatization process is achieved, the real experiment can begin. The Phase 2B concentrates on the degradation of Refinery wastewater using the acclimatized Activated Sludge treatment system. The first stage of the Phase 2B will see the addition of 10% refinery wastewater and 90% STP Wastewater. This is important as addition of full 100% refinery wastewater will shock the bacteria in the system and causing them to die. There are 2 reactors, Reactor A and B and all the samples are tested with nitrate, ammonia and phosphorus parameter. Below is the result of the testing. Only reactor B will be added with refinery wastewater while reactor A will continue to degrade 100% STP wastewater.

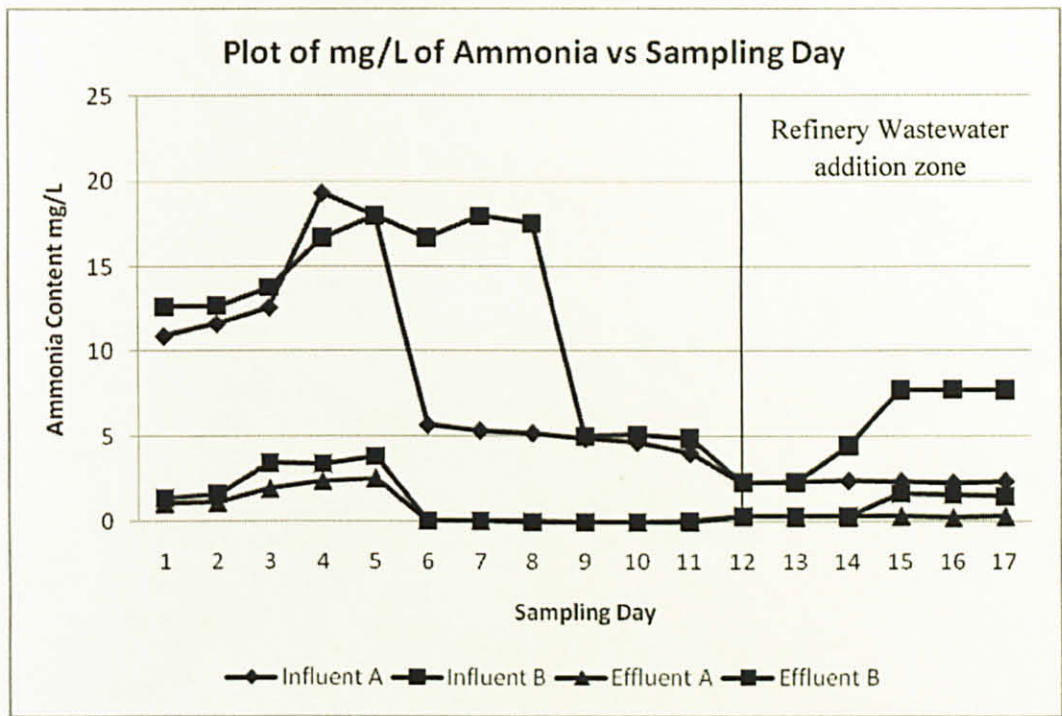


Figure 4.13 – Plot of mg/L of Ammonia vs Sampling Day

Figure 4.13 above shows the result of ammonia degradation. Notice the horizontal bar changes the value from 1-11 to 12 to 17. Sampling day 1-11 is the results from the acclimatization stage while 12 to 17 are the results of addition 10% refinery wastewater in the system. The addition of 10% refinery wastewater caused the ammonia amount to increases in the influent B. The increment continues in Influent B until sampling day 15. From sampling day 15 to 17, the amount of ammonia begins to stabilize at around 8 mg/L. The results are quite consistent with the Effluent B line. Initially from sampling day 12 to 15, the ammonia content in effluent B increases. Later from sampling day 15 to 17, the ammonia content stabilizes. Results from Effluent B line shows the ammonia is degraded after gone through the system. From around 8.0 mg/L of ammonia, the level decreases to around 1.0 mg/L of ammonia after treated using the system. This shows that within 4 sampling days, the system is capable of reducing ammonia to around 80%.

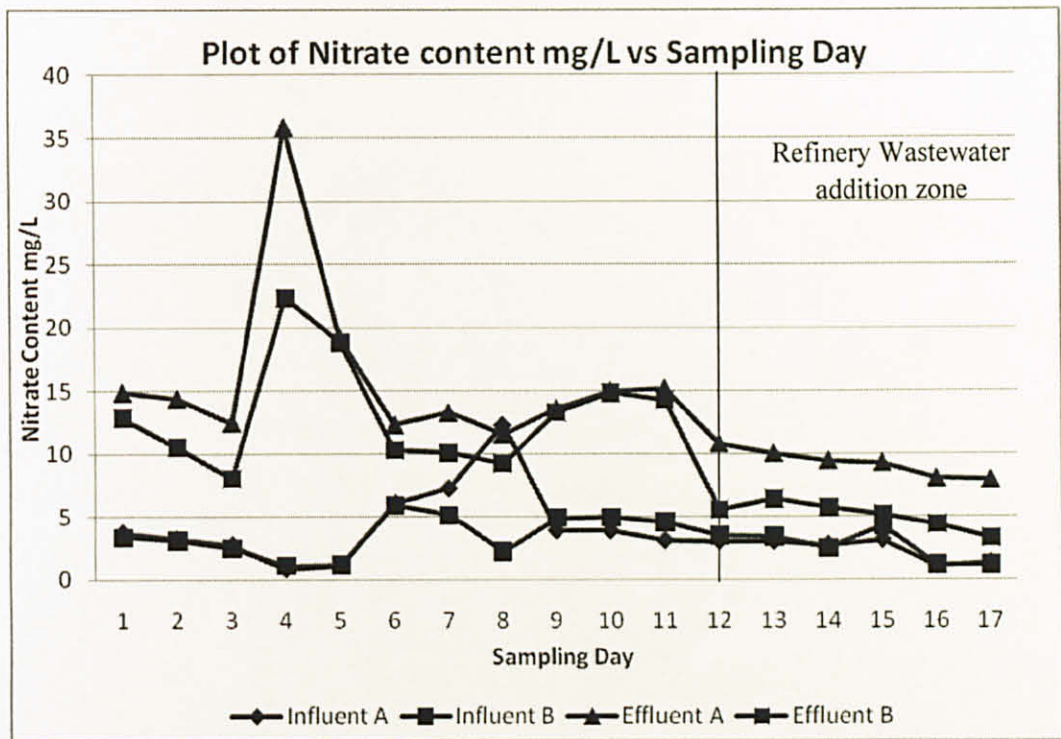


Figure 4.14 – Plot of mg/L of Nitrate vs Sampling Day



Figure 4.14 above shows the amount of nitrate in the influent and effluent in the system after the addition of 10% Refinery wastewater. Notice the horizontal bar changes the value from 1-11 to sampling day 12 to 17. Sampling day 1-11 is the results from the acclimatization stage while 12 to 17 are the results of addition 10% refinery wastewater in the system. The results shows that the 10% refinery wastewater in influent B, show minimal impact to the increment of nitrate. The results are quite consistent with the acclimatization period before the addition of the refinery wastewater. However, the amount of nitrate increases after being treated. This is consistent with any results conducted before. The ammonia content is oxidized to nitrate. This is why the level of Nitrate increases after the treatment process. However, compared to effluent A, effluent B show less increment in the Nitrate content. This might be due to the toxicity of the refinery wastewater affecting the bacteria activity in the system.

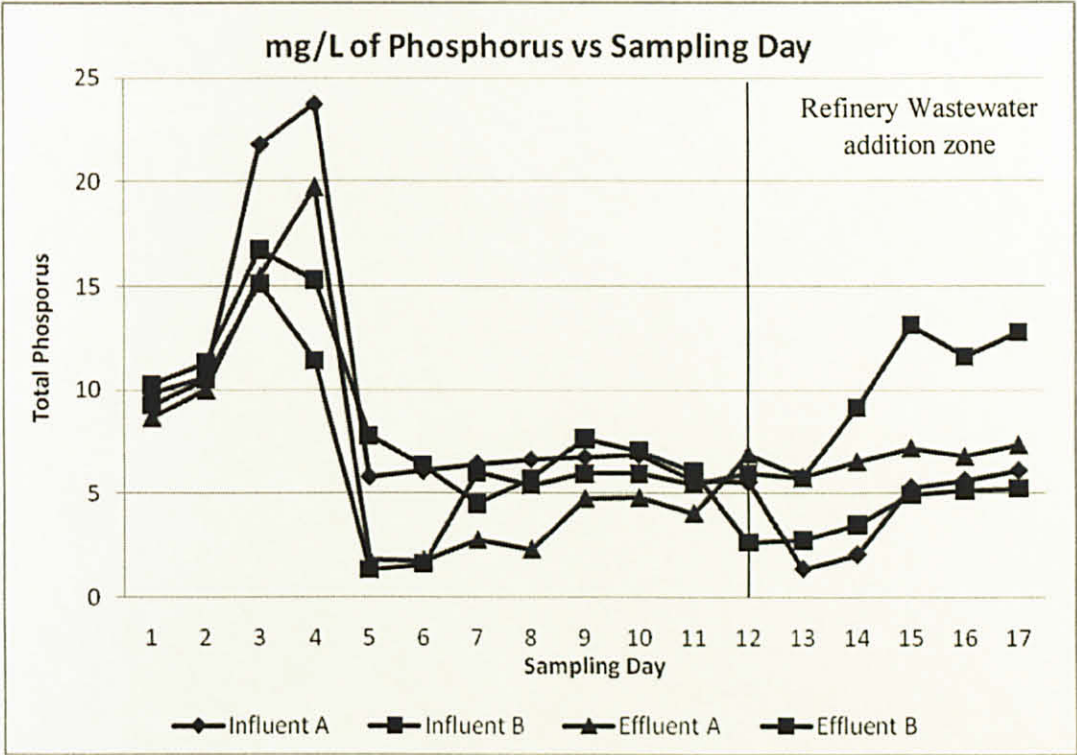


Figure 4.15 – Plot of mg/L of Phosphorus vs Sampling Day

Figure 4.15 above shows the amount of Phosphorus in the influent and effluent in the system after the addition of 10% refinery wastewater. Notice the horizontal bar changes the value from 1-11 to 12 to 17. Sampling day 1-11 is the results from the acclimatization stage while sampling day 12 to 17 is the results of addition 10% refinery wastewater in the system. The result shows that the level of phosphorus in influent B is higher than influent A from sampling day 12 to 14. From sampling day 14 to 17, the amount of Phosphorus in both type of influents begin to stabilize. For the effluent, inclusive of refinery wastewater shows increase of Phosphorus content in the Effluent B. The amount of Phosphorus in Effluent B is significantly higher than Effluent A. This shows the addition of refinery wastewater to the effluent affects the condition of the bacteria in the system. As this is initial addition of refinery wastewater to the system, further testing needed to be conducted to determine the effect or refinery wastewater to Phosphorus content.

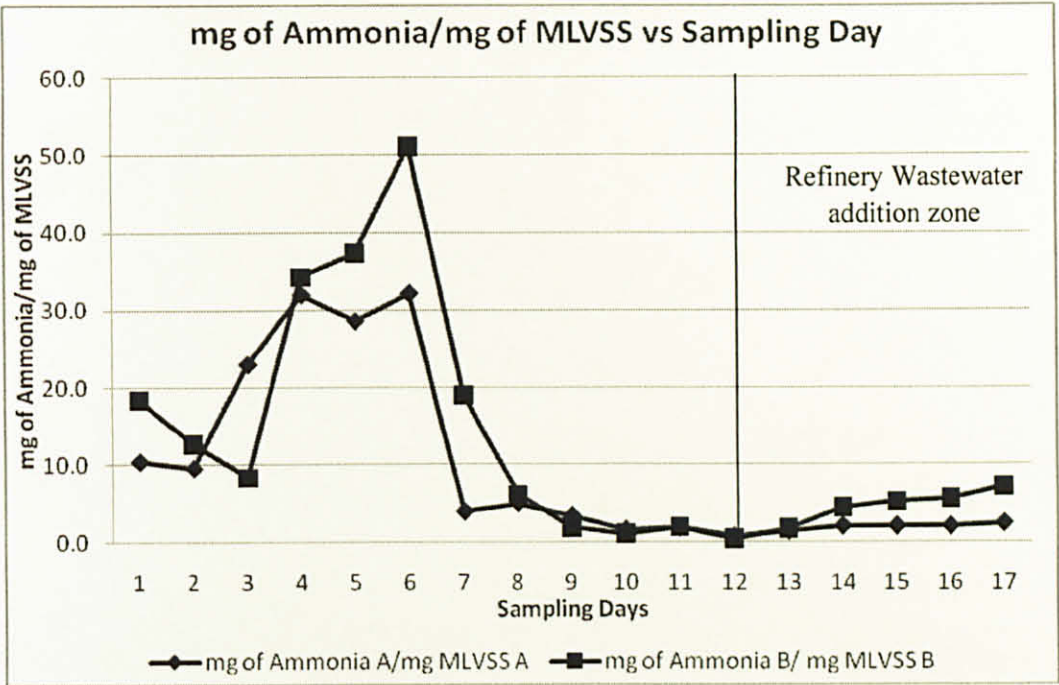


Figure 4.16 – Plot of mg of Ammonia/mg of MLVSS vs Sampling Day

Figure 4.16 above is the plot of mg of ammonia over mg of MLVSS for every sampling day. The purpose of plotting this graph is to show the relationship between ammonia content and amount of bacteria in the reactor.

As shown above, in the earlier stages of experiment during the acclimatization phase, the reading of ammonia/MLVSS is inconsistent. This is due to bacteria did not acclimatize and the MLVSS reading are inconsistent. Furthermore, the reading of ammonia in plot 4.13 shows the ammonia reading is inconsistent for the earlier stages of acclimatization phase. Towards the end of the acclimatization phase, the readings begin to stabilize from sampling day 8 to 12. Plus the reading drops for both reactors from around 30 to 5. This is due to the amount of ammonia managed to be degraded to around 90%. As the value of ammonia decreases, the ratio of ammonia over MLVSS will also decrease. After the addition of refinery wastewater in the system, the reading increases in reactor B which the refinery wastewater was added. The amount of ammonia increases while the amount of MLVSS decreases due to the toxicity of the refinery wastewater. The reading shows the effect of addition of the refinery wastewater affects the content of ammonia as well as decreases the amount of bacteria in the reactor. While the reactor B shows increase in the amount of Ammonia/MLVSS, reactor A which did not contain any refinery wastewater does not show any increase in the points.

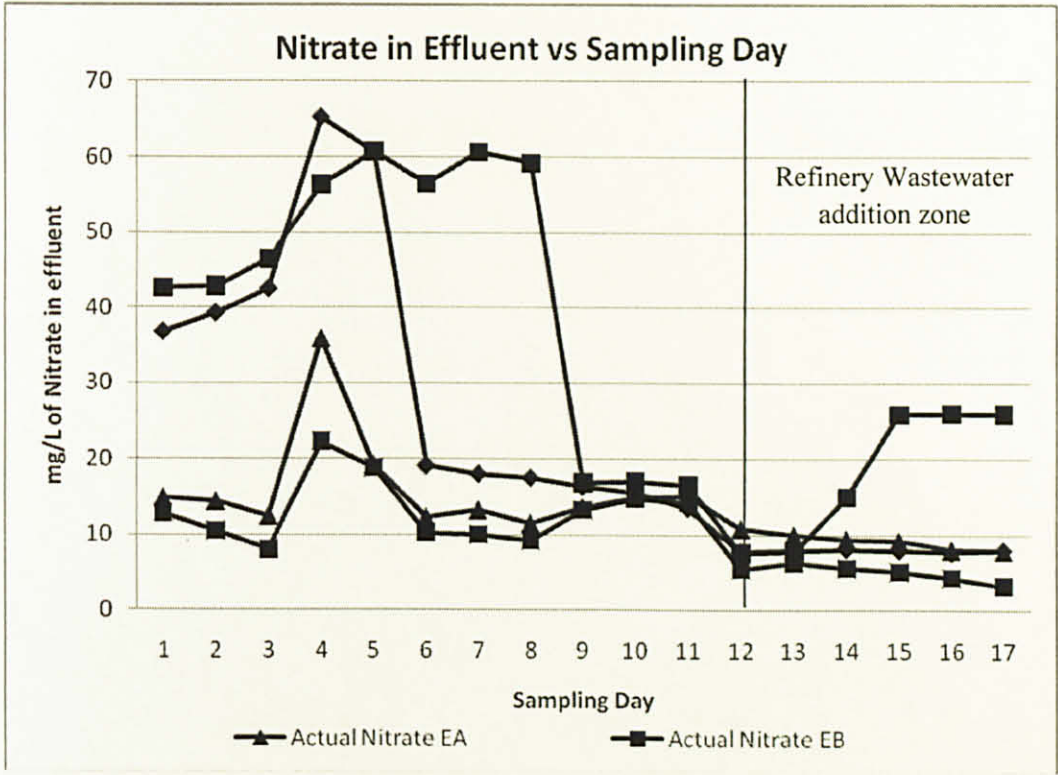


Figure 4.17 – Plot of mg/L of Theoretical and Actual Nitrate vs Sampling Day



Figure 4.17 shows the differences between theoretical values of nitrate produced in the nitrification process compared to the actual value of nitrate produced. The theoretical value of nitrate is calculated by using the stoichiometry of ammonia oxidized to nitrate. It is assumed that all ammonia is converted to nitrate and no ammonia is used up by the bacteria during the process when calculating the theoretical value. From the results, it is shown that the theoretical value for both reactors are more than the actual value of nitrate produced. This happened consistently during the acclimatization phase and the addition of refinery wastewater. In sampling day 12 to 17 where addition of refinery wastewater to reactor B shows the amount of theoretical nitrate to be around 25 mg/L but the actual nitrate produced is around 5 mg/L only. This could be the nitrate is used up by the bacteria in the reactor in the process of degrading the refinery wastewater. As the theoretical nitrate produced only assumed direct conversion of ammonia to nitrate, this might be the reason why the actual nitrate produced is much lower than the theoretical value.

#### **4.2.4 CONCLUSION FROM PHASE 2B TESTING**

The results show that initially the degradation rate is inconsistent and amount of nitrate, ammonia and phosphorus in the effluent are inconsistent. On later stages of the refinery wastewater addition, the results improved and became more consistent. However, since it is the first stage of addition of refinery wastewater, further testing should be conducted to give more consistent results.

## **CHAPTER 5**

### **CONCLUSION**

As a conclusion, the problem statement has been identified. From the problem statement, clear objectives of the study were able to be drafted. Following a proper and well drafted methodology, results from the experiment was able to be determined. As a conclusion, it can be classified that the aerobic system can be used to degrade the ammonia content from the petroleum wastewater for the Phase One testing. Results indicate the Anaerobic System does not able to degrade the Ammonia while the Aerobic System achieved 95% removal. From the result of the experiment as well, it can be concluded that the best reactor that can be used to degrade the ammonia content will be the aerobic aeration after anaerobic aeration reactor. From the conclusive results of Phase 1, the testing for Phase 2 was conducted on Activated Sludge Treatment system. The results for Phase 2 show that the activated sludge treatment system can be used to degrade Ammonia when the addition of refinery is 10%. The percentage removal is up to 98% for the ammonia content. However, more percentage of refinery wastewater needed to be added before ultimate conclusion can be made on the system. The result shows that further testing must be conducted to have conclusive result.

## **CHAPTER 6**

### **ECONOMIC BENEFITS**

This project was conducted in conjunction with a PhD and Master's Degree research program. This enables the research to be entitled to receive some amount of funding from the research grant available for the PhD research program.

However, the cost of the research for this project is not substantial as the cost is only for the testing done for the research and the cost for maintaining the reactor.

For testing conducted, there will be only cost for using the chemicals during the experiments such as Ammonia content testing and Nitrate content testing. However, most of the chemicals and equipments used for the testing are already available in the Environmental Lab in UTP. So, the money will only be used for buying chemicals that are not available in the lab such as Nitra-Ver 5 reagent powder used in the Nitrate content testing. The Nitra-Ver 5 reagent powder package costs RM 160.00. There is no other cost for testing experiments conducted. However, below is the estimation of the total cost for conducting the experiments should all the items needed to be bought. The cost is estimated to be RM 400.00 for chemicals used for the testing and RM 700.00 for the tools used for the testing such as Pipette and Sample Cell.

The refinery wastewater was collected 2 times during the research period. The transportation cost includes the fuel, road toll and driver allowance which are estimated around RM 300.00 per trip. As the sample was collected 2 times, the cost for transportation is RM 600.00

For the running of the reactor, there is no actual cost that has to be supported by the researcher. The reactor itself is a common aerobic tank already used in previous researches and available before the start of the research. However, the reactor is estimated to cost RM 300.00 each. As there are 2 reactors involved, the cost is around RM 600.00. For the running of the reactor, it needs continuous supply of air.



The air is pumped into the reactor using the aerator available at the lab. It uses electrical supply. The electricity usage for air and continuous mixing is estimated to be around RM 1.00 per day. The estimated cost for running the reactor is estimated to be RM 240.00 for the whole research time.

Below is the sum of all the possible cost involved in the research. Some of the cost are not relevant as it is already consumed by the university like the equipments and items used. The cost is only an estimate as it is difficult to determine the exact cost for the overall operations.

| ITEMS                          | COST       |
|--------------------------------|------------|
| Chemicals used for experiments | RM 400.00  |
| Tools used for testing         | RM 700.00  |
| Transportation of Sample       | RM 600.00  |
| Reactor and maintenance        | RM 840.00  |
| TOTAL                          | RM 2540.00 |

Table 6.1 –The estimation of the operating cost for the research

The cost is only estimated to the current date. As the research is still ongoing, the cost for the research will be more.

After the research is completed and the results proves that aerobic treatment system can be used as a treatment of refinery wastewater, further large scale research needed to be conducted before the reactor can be used for treatment process. This will involve more cost which is estimated to reach Millions of Ringgit. However, once all the research process is completed and final system is established, it can be used as an alternative for chemical treatment system used currently. The usage of biological treatment system perhaps need fewer operating cost compared to the conventional system and this will give edge to the organizations involved in the operation.

## REFERENCES

- Alemayehu Mekonen, Pradeep Kumar, Arvind Kumar 2001 “Use of Sequencing Batch Reactor for Biological Denitrification of High Nitrate Containing Water” in *Journal of Environmental Engineering*, ASCE
- Arrigo, R. A. 2005 “Marine microorganisms and global nutrient cycles” in *Journal of Engineering Science and Technology* (2005)
- Anna Llop, Eva Pocurull, Francesc Borrull 2008 “Evaluation of Removal of Pollutants from Petrochemical Wastewater using a Membrane Bioreactor Treatment Plant” in *Water Science and Technology Vol 37* No 4-5 pp 105-108
- B. Kartal, G.J. Kuenen and M.C.M van Loosdrecht 2010 “Sewage Treatment with Anammox” in *Science*, 2010
- Beer, C., Wang, L. K. and Bergenthal, J 1976 “Kinetics and Control Tests of Nitrification-Denitrification Process, Part II: Respiration and Control Tests”, *Institute of Environmental Sciences* 1976
- Byrne, N., Strous, M., Crepeau, V, 2008. “Presence and activity of anaerobic ammonium-oxidizing bacteria at deep-sea hydrothermal vents”

- Fulazzaky, M. A. 2002 “Carbonaceous, Nitrogenous and Phosphorus Matters removal from domestic wastewater by an activated sludge reactor of nitrification-denitrification type” in *Journal of Engineering Science and Technology* Vol. 4, No. 1 (2009) 69 – 80
- Fulazzaky, M. A. 1998 “Biological-chemical treatment of domestic wastewater – Chemical phosphorus removal with ferric iron by simultaneous precipitation in an activated sludge of discontinues anoxic-aerobic reactor” in *Journal of Engineering Science and Technology* (2002)
- Greg Johnson, 2005 “Petroleum Wastewater, Desalter Case Study”, in *Journal Environmental Technology*, 1479-487X, Volume 12, Issue 2, 1991, Pages 131 – 145
- Jaeschke, M.I. 2009 “16s rRNA gene and lipid biomarker evidence for anaerobic ammonium-oxidizing bacteria (anammox) in California and Nevada hot springs”
- Jenkins, D., Richard, M. G. and Daigger, G. T 1993 “Manual on the Causes and Control of Activated Sludge Bulking and Foaming”, 2nd ed. Boca Raton: Lewis Publishers
- Lawrence K Wang, Calvin P.C, M H Wang 1977 “Control Test and Kinetics of Activated Sludge Process” in *Water, Air, and Soil Pollution* 8 (1977) 315-351
- Metcalf and Eddy 2004 “Sequencing Batch Reactor Process” in *Wastewater Engineering Treatment and Reuse 4<sup>th</sup> Edition* page 720 – 724 McGraw Hill Production



Metcalf and Eddy 2004 "Stoichiometry of Biological Nitrification" in *Wastewater Engineering Treatment and Reuse 4<sup>th</sup> Edition* pg 612-614 McGraw Hill Production

Strous, M ., Kuenen, J.G. and Jetten, M.S. 1999 "Key Physiology of Anaerobic Ammonium Oxidation. App. Environ. Microb" in *Water, Science and Technology*, 1999

Toprak, R. 2005 "Sequencing Batch Reactor to Combat against Bulking Sludge" in *Journal of Environmental Engineering*, ASCE 2005

Water Environment Association. (1987) Activated Sludge, Manual of Practice #9

Y. Hu, S.L.Ong, W.J.Ng and W.Liu 2005 "Use of a Sequencing Batch Reactor for Nitrogen Removal from Municipal Wastewater" in *Journal of Environmental Engineering*, ASCE 2005

Yong Sun, Yaobin Zhang and Xie Quan 2008 "Treatment of petroleum refinery wastewater by microwave-assisted catalytic wet air oxidation under low temperature and low pressure" in *Journal of Separation and Purification Technology*, Vol 62, pg 565-570

## LIST OF APPENDIX

- Appendix 1 : Table on the raw sample for Ammonia content (mg/L)
- Appendix 2 : Table on the raw sample for Nitrate content (mg/L)
- Appendix 3 : Table on the raw sample for TSS and VSS Content (mg/L)
- Appendix 4 : Table on the Nitrate content testing for 24 hours cycle (mg/L)
- Appendix 5 : Table on the Ammonia content testing for 24 hours cycle (mg/L)
- Appendix 6 : Table on the Ammonia content testing for 7 hours (mg/L)
- Appendix 7 : Table on the Nitrate content testing for 7 hours (mg/L)
- Appendix 8 : Table on the mg of Ammonia over mg of MLVSS testing for 7 hours (mg/L)
- Appendix 9 : Table on Nitrate content based on Sampling Day and Type of Sample from Acclimatization to Refinery Wastewater Addition
- Appendix 10 : Table on Ammonia content based on Sampling Day and Type of Sample from Acclimatization to Refinery Wastewater Addition
- Appendix 11 : Table on Phosphorus content based on Sampling Day and Type of Sample from Acclimatization to Refinery Wastewater Addition
- Appendix 12 : Methodology for Ammonia Content Testing using Nessler Method
- Appendix 13 : Methodology for Nitrate content Testing using the Powder Pillow method
- Appendix 14 : Methodology for MLSS and MLVSS Testing
- Appendix 15 : Tools and Equipment

Appendix 1

Table on the Raw sample for Ammonia content (mg/L)

| Testing Time    | Sample Type (Result) |      |      |                 |               |      |      |               |                       |      |      |        |
|-----------------|----------------------|------|------|-----------------|---------------|------|------|---------------|-----------------------|------|------|--------|
|                 | Very High Strength   |      |      |                 | High Strength |      |      |               | Diluted High Strength |      |      |        |
|                 | A                    | B    | C    | Avg             | A             | B    | C    | Avg           | A                     | B    | C    | Avg    |
| Raw Data        | 1.76                 | 1.93 | 1.99 | 47.333          | 0.77          | 0.78 | 0.79 | 7.8           | 0.97                  | 1    | 0.98 | 9.8333 |
|                 | 1.75                 | 1.9  | 1.96 | 46.75           | 0.76          | 0.78 | 0.78 | 7.7333        | 0.97                  | 1.01 | 0.98 | 9.8667 |
|                 | 1.78                 | 1.94 | 1.99 | 47.583          | 0.77          | 0.78 | 0.78 | 7.7667        | 0.97                  | 1.02 | 0.98 | 9.9    |
| 47.222          |                      |      |      | 7.7667          |               |      |      | 9.8667        |                       |      |      |        |
| Dilution factor |                      |      |      | Dilution factor |               |      |      | Dilution Fact |                       |      |      |        |
| 1:25            |                      |      |      | 1:10            |               |      |      | 1:10          |                       |      |      |        |

Appendix 2

Table on the Raw sample for Nitrate content (mg/L)

| Testing Time | Sample Type (Result) |     |     |        |               |     |     |        |                       |     |     |        |
|--------------|----------------------|-----|-----|--------|---------------|-----|-----|--------|-----------------------|-----|-----|--------|
|              | Very High Strength   |     |     |        | High Strength |     |     |        | Diluted High Strength |     |     |        |
|              | A                    | B   | C   | Avg    | A             | B   | C   | Avg    | A                     | B   | C   | Avg    |
| Raw Data     | 1.3                  | 2.5 | 2.1 | 1.9667 | 0.3           | 0.5 | 0.9 | 0.5667 | 0.4                   | 0.1 | 0.1 | 0.2    |
|              | 1.3                  | 2.2 | 2.1 | 1.8667 | 0.3           | 0.5 | 0.7 | 0.5    | 0.5                   | 0.2 | 0.3 | 0.3333 |
|              | 1.5                  | 2.5 | 2.3 | 2.1    | 0.3           | 0.4 | 0.7 | 0.4667 | 0.6                   | 0.2 | 0.3 | 0.3667 |
| 1.9778       |                      |     |     | 0.5111 |               |     |     | 0.3    |                       |     |     |        |

Appendix 3

Table on the Raw sample for TSS and VSS Content (mg/L)

| Test Type | Sample Type |       |       |
|-----------|-------------|-------|-------|
|           | V.H.S       | H.S.  | D.H.S |
| TSS       | 55.67       | 55.67 | 86.00 |
| VSS       | 24.67       | 45.50 | 50.00 |



## Appendix 4

Table on the Nitrate content testing for 24 hours cycle (mg/L)

| Testing Time | Sample Type (Result) |     |     |        |             |     |      |        |                          |      |      |        |                   |      |     |        |
|--------------|----------------------|-----|-----|--------|-------------|-----|------|--------|--------------------------|------|------|--------|-------------------|------|-----|--------|
|              | 3 Aerobic            |     |     |        | 4 Anaerobic |     |      |        | 5 Aerobic from Anaerobic |      |      |        | 6 Diluted Aerobic |      |     |        |
|              | A                    | B   | C   | Avg    | A           | B   | C    | Avg    | A                        | B    | C    | Avg    | A                 | B    | C   | Avg    |
| 11.00 a.m.   | 0.8                  | 0.7 | 2.4 | 0.75   | -0.9        | 1.5 | -0.1 | 0.1667 | -0.1                     | -1.4 | -1.6 | -1.033 | -0.1              | -0.3 | 0.7 | 0.1    |
|              | 0.9                  | 0.6 | 2.5 | 0.75   | -1          | 1.6 | -1.2 | -0.2   | -0.1                     | -1.4 | -1.6 | -1.033 | -0.1              | -0.2 | 0.7 | 0.1333 |
|              | 0.9                  | 0.6 | 2.3 | 0.75   | -0.9        | 1.4 | -1.1 | -0.2   | -0.1                     | -1.3 | -1.6 | -1     | -0.1              | -0.3 | 0.6 | 0.0667 |
|              |                      |     |     | 0.75   |             |     |      | -0.078 |                          |      |      | -1.022 |                   |      |     | 0.1    |
| 6.00 p.m.    | 0.2                  | 0.4 | 0.4 | 0.333  | 0.8         | 1.1 | 1.2  | 1.0333 | 0.2                      | 0.3  | 0.3  | 0.2667 | 0.3               | 0.8  | 0.5 | 0.5333 |
|              | 0.1                  | 0.4 | 0.4 | 0.3    | 0.8         | 1.1 | 1.1  | 1      | 0.3                      | 0.4  | 0.3  | 0.3333 | 0.3               | 0.8  | 0.5 | 0.5333 |
|              | 0.1                  | 0.3 | 0.4 | 0.2667 | 0.9         | 1.2 | 1.2  | 1.1    | 0.4                      | 0.6  | 0.4  | 0.4667 | 0.2               | 0.7  | 0.5 | 0.4667 |
|              |                      |     |     | 0.300  |             |     |      | 1.0444 |                          |      |      | 0.3556 |                   |      |     | 0.5111 |
| 1.00 a.m.    | 0.7                  | 0.8 | 0.7 | 0.7333 | 1.6         | 1.8 | 1.4  | 1.6    | 1.4                      | 3.1  | 1    | 1.8333 | 0.5               | 0.5  | 1.2 | 0.7333 |
|              | 0.7                  | 0.8 | 0.7 | 0.7333 | 1.6         | 1.8 | 1.4  | 1.6    | 1.4                      | 3.5  | 1.2  | 2.0333 | 0.5               | 0.5  | 1.2 | 0.7333 |
|              | 0.7                  | 0.8 | 0.7 | 0.7333 | 1.6         | 1.8 | 1.4  | 1.6    | 1.4                      | 3.1  | 1    | 1.8333 | 0.5               | 0.6  | 1.1 | 0.7333 |
|              |                      |     |     | 0.7333 |             |     |      | 1.6    |                          |      |      | 1.9    |                   |      |     | 0.7333 |
| 8.00 a.m     | 1.7                  | 1.6 | 1.5 | 1.6    | 1.2         | 1.3 | 1.2  | 1.2333 | 2.6                      | 1.8  | 2.7  | 2.3667 | 1.6               | 1.5  | 1.5 | 1.5333 |
|              | 1.7                  | 1.6 | 1.5 | 1.6    | 1.2         | 1.3 | 1.2  | 1.2333 | 2.6                      | 1.8  | 2.7  | 2.3667 | 1.6               | 1.5  | 1.5 | 1.5333 |
|              | 1.7                  | 1.6 | 1.5 | 1.6    | 1.2         | 1.3 | 1.2  | 1.2333 | 2.6                      | 1.8  | 2.7  | 2.3667 | 1.6               | 1.5  | 1.5 | 1.5333 |
|              |                      |     |     | 1.6    |             |     |      | 1.2333 |                          |      |      | 2.3667 |                   |      |     | 1.5333 |
| 10.00 a.m    | 1.5                  | 1.6 | 1.6 | 1.5667 | 1.2         | 1.3 | 1.4  | 1.3    | 3.9                      | 2.4  | 2.6  | 2.9667 | 2.1               | 1.9  | 1.8 | 1.9333 |
|              | 1.5                  | 1.6 | 1.6 | 1.5667 | 1.2         | 1.3 | 1.4  | 1.3    | 3.9                      | 2.4  | 2.6  | 2.9667 | 2.1               | 1.9  | 1.8 | 1.9333 |
|              | 1.5                  | 1.6 | 1.6 | 1.5667 | 1.2         | 1.3 | 1.4  | 1.3    | 3.9                      | 2.4  | 2.6  | 2.9667 | 2.1               | 1.9  | 1.8 | 1.9333 |
|              |                      |     |     | 1.5667 |             |     |      | 1.3    |                          |      |      | 2.9667 |                   |      |     | 1.9333 |

## Appendix 5

Table on the Ammonia content testing for 24 hours cycle (mg/L)

| Testing Time | Sample Type (Result) |      |      |      |             |      |      |      |                          |      |      |      |                   |       |       |       |
|--------------|----------------------|------|------|------|-------------|------|------|------|--------------------------|------|------|------|-------------------|-------|-------|-------|
|              | 3 Aerobic            |      |      |      | 4 Anaerobic |      |      |      | 5 Aerobic from Anaerobic |      |      |      | 6 Diluted Aerobic |       |       |       |
|              | A                    | B    | C    | Avg  | A           | B    | C    | Avg  | A                        | B    | C    | Avg  | A                 | B     | C     | Avg   |
| 11.00 a.m.   | 0.83                 | 0.81 | 0.84 | 0.83 | 0.91        | 0.92 | 0.93 | 0.92 | 0.56                     | 0.56 | 0.56 | 0.56 | 0.53              | 0.54  | 0.52  | 0.53  |
|              | 0.83                 | 0.81 | 0.82 | 0.82 | 0.90        | 0.91 | 0.92 | 0.91 | 0.56                     | 0.56 | 0.56 | 0.56 | 0.53              | 0.54  | 0.52  | 0.53  |
|              | 0.84                 | 0.82 | 0.83 | 0.83 | 0.90        | 0.90 | 0.92 | 0.91 | 0.56                     | 0.56 | 0.56 | 0.56 | 0.53              | 0.54  | 0.52  | 0.53  |
|              |                      |      |      | 0.83 |             |      |      | 0.91 |                          |      |      | 0.56 |                   |       |       | 0.53  |
| 6.00 p.m.    | 0.07                 | 0.11 | 0.08 | 0.09 | 0.97        | 0.98 | 0.95 | 0.97 | 0.16                     | 0.10 | 0.13 | 0.13 | 0.01              | 0.02  | 0.01  | 0.01  |
|              | 0.07                 | 0.11 | 0.08 | 0.09 | 0.97        | 0.98 | 0.95 | 0.97 | 0.16                     | 0.10 | 0.13 | 0.13 | 0.01              | 0.02  | 0.01  | 0.01  |
|              | 0.07                 | 0.11 | 0.08 | 0.09 | 0.97        | 0.98 | 0.95 | 0.97 | 0.16                     | 0.10 | 0.13 | 0.13 | 0.01              | 0.02  | 0.01  | 0.01  |
|              |                      |      |      | 0.09 |             |      |      | 0.97 |                          |      |      | 0.13 |                   |       |       | 0.01  |
| 1.00 a.m.    | 0.39                 | 0.39 | 0.39 | 0.39 | 1.10        | 1.06 | 1.04 | 1.07 | 0.01                     | 0.02 | 0.40 | 0.14 | -0.02             | -0.03 | -0.03 | -0.03 |
|              | 0.39                 | 0.39 | 0.39 | 0.39 | 1.10        | 1.06 | 1.04 | 1.07 | 0.02                     | 0.02 | 0.30 | 0.11 | -0.02             | -0.03 | -0.03 | -0.03 |
|              | 0.39                 | 0.39 | 0.39 | 0.39 | 1.10        | 1.06 | 1.04 | 1.07 | 0.02                     | 0.02 | 0.30 | 0.11 | -0.02             | -0.03 | -0.03 | -0.03 |
|              |                      |      |      | 0.39 |             |      |      | 1.07 |                          |      |      | 0.12 |                   |       |       | -0.03 |
| 8.00 a.m     | 0.53                 | 0.56 | 0.56 | 0.55 | 1.42        | 1.41 | 1.48 | 1.44 | 0.07                     | 0.07 | 0.06 | 0.07 | 0.01              | 0.02  | 0.01  | 0.01  |
|              | 0.54                 | 0.55 | 0.56 | 0.55 | 1.41        | 1.40 | 1.47 | 1.43 | 0.07                     | 0.07 | 0.06 | 0.07 | 0.01              | 0.02  | 0.01  | 0.01  |
|              | 0.53                 | 0.56 | 0.56 | 0.55 | 1.41        | 1.40 | 1.47 | 1.43 | 0.07                     | 0.07 | 0.06 | 0.07 | 0.01              | 0.02  | 0.01  | 0.01  |
|              |                      |      |      | 0.55 |             |      |      | 1.43 |                          |      |      | 0.07 |                   |       |       | 0.01  |
| 10.00 a.m    | 0.60                 | 0.59 | 0.59 | 0.59 | 1.40        | 1.40 | 1.39 | 1.40 | 0.08                     | 0.08 | 0.07 | 0.08 | 0.08              | 0.08  | 0.08  | 0.08  |
|              | 0.60                 | 0.59 | 0.59 | 0.59 | 1.40        | 1.40 | 1.39 | 1.40 | 0.08                     | 0.08 | 0.07 | 0.08 | 0.08              | 0.08  | 0.08  | 0.08  |
|              | 0.60                 | 0.59 | 0.59 | 0.59 | 1.40        | 1.40 | 1.39 | 1.40 | 0.08                     | 0.08 | 0.07 | 0.08 | 0.08              | 0.08  | 0.08  | 0.08  |
|              |                      |      |      | 0.59 |             |      |      | 1.40 |                          |      |      | 0.08 |                   |       |       | 0.08  |



## Appendix 6

Table on the Ammonia content testing for 7 hours (mg/L)

| Testing Time | Sample Type (Result) |      |      |        |             |      |      |        |                          |      |      |        |                   |      |      |        |
|--------------|----------------------|------|------|--------|-------------|------|------|--------|--------------------------|------|------|--------|-------------------|------|------|--------|
|              | 3 Aerobic            |      |      |        | 4 Anaerobic |      |      |        | 5 Aerobic from Anaerobic |      |      |        | 6 Diluted Aerobic |      |      |        |
|              | A                    | B    | C    | Avg    | A           | B    | C    | Avg    | A                        | B    | C    | Avg    | A                 | B    | C    | Avg    |
| 11.00 a.m.   | 0.45                 | 0.44 | 0.44 | 0.75   | 1.01        | 1.01 | 1.01 | 1.01   | 0.57                     | 0.57 | 0.57 | 0.57   | 0.47              | 0.47 | 0.47 | 0.47   |
|              | 0.42                 | 0.42 | 0.42 | 0.75   | 1.02        | 1.02 | 1.02 | 1.02   | 0.62                     | 0.62 | 0.62 | 0.62   | 0.48              | 0.48 | 0.48 | 0.48   |
|              | 0.43                 | 0.43 | 0.44 | 0.75   | 1           | 1    | 1    | 1      | 0.58                     | 0.58 | 0.58 | 0.58   | 0.46              | 0.46 | 0.46 | 0.46   |
|              |                      |      |      | 0.75   |             |      |      | 1.01   |                          |      |      | 0.59   |                   |      |      | 0.47   |
| 12.30 p.m.   | 0.44                 | 0.44 | 0.44 | 0.440  | 1.13        | 1.14 | 1.14 | 1.1367 | 1.01                     | 1.01 | 1.01 | 1.01   | 0.61              | 0.61 | 0.61 | 0.61   |
|              | 0.53                 | 0.53 | 0.53 | 0.530  | 1.44        | 1.44 | 1.44 | 1.44   | 1.05                     | 1.05 | 1.05 | 1.05   | 0.6               | 0.61 | 0.61 | 0.6067 |
|              | 0.72                 | 0.72 | 0.72 | 0.72   | 1.13        | 1.13 | 1.13 | 1.13   | 0.72                     | 0.71 | 0.71 | 0.7133 | 0.61              | 0.61 | 0.61 | 0.61   |
|              |                      |      |      | 0.563  |             |      |      | 1.2356 |                          |      |      | 0.9244 |                   |      |      | 0.6089 |
| 2.00 p.m.    | 0.04                 | 0.04 | 0.04 | 0.04   | 1.07        | 1.07 | 1.07 | 1.07   | 0.4                      | 0.39 | 0.39 | 0.3933 | 0.39              | 0.38 | 0.37 | 0.38   |
|              | 0.05                 | 0.05 | 0.05 | 0.05   | 1.07        | 1.07 | 1.07 | 1.07   | 0.39                     | 0.39 | 0.39 | 0.39   | 0.38              | 0.36 | 0.36 | 0.3667 |
|              | 0.06                 | 0.05 | 0.05 | 0.0533 | 1.07        | 1.07 | 1.07 | 1.07   | 0.4                      | 0.39 | 0.39 | 0.3933 | 0.4               | 0.39 | 0.39 | 0.3933 |
|              |                      |      |      | 0.0478 |             |      |      | 1.07   |                          |      |      | 0.3922 |                   |      |      | 0.38   |
| 3.30 p.m.    | 0                    | 0    | 0    | 0      | 0.96        | 0.96 | 0.96 | 0.96   | 0.23                     | 0.22 | 0.22 | 0.2233 | 0.24              | 0.22 | 0.22 | 0.2267 |
|              | 0                    | 0    | 0    | 0      | 0.95        | 0.95 | 0.95 | 0.95   | 0.23                     | 0.23 | 0.23 | 0.23   | 0.24              | 0.22 | 0.22 | 0.2267 |
|              | 0                    | 0    | 0    | 0      | 0.96        | 0.95 | 0.95 | 0.9533 | 0.23                     | 0.23 | 0.23 | 0.23   | 0.24              | 0.22 | 0.22 | 0.2267 |
|              |                      |      |      | 0      |             |      |      | 0.9544 |                          |      |      | 0.2278 |                   |      |      | 0.2267 |
| 5.00 p.m.    | 0                    | 0    | 0    | 0      | 1.04        | 1.05 | 1.05 | 1.0467 | 0.11                     | 0.12 | 0.12 | 0.1167 | 0.24              | 0.25 | 0.25 | 0.2467 |
|              | 0.01                 | 0.01 | 0.01 | 0.01   | 1.05        | 1.06 | 1.06 | 1.0567 | 0.15                     | 0.15 | 0.15 | 0.15   | 0.24              | 0.24 | 0.24 | 0.24   |
|              | 0.01                 | 0.01 | 0.01 | 0.01   | 1.04        | 1.05 | 1.05 | 1.0467 | 0.12                     | 0.12 | 0.12 | 0.12   | 0.24              | 0.24 | 0.24 | 0.24   |
|              |                      |      |      | 0.0067 |             |      |      | 1.05   |                          |      |      | 0.1289 |                   |      |      | 0.2422 |
| 6.00 p.m.    | 0.05                 | 0.04 | 0.04 | 0.0433 | 1.02        | 1.02 | 1.02 | 1.02   | 0.04                     | 0.04 | 0.04 | 0.04   | 0.23              | 0.22 | 0.22 | 0.2233 |
|              | 0.02                 | 0.02 | 0.02 | 0.02   | 1.03        | 1.03 | 1.03 | 1.03   | 0.04                     | 0.04 | 0.04 | 0.04   | 0.23              | 0.22 | 0.22 | 0.2233 |
|              | 0.02                 | 0.02 | 0.02 | 0.02   | 1.03        | 1.03 | 1.03 | 1.03   | 0.04                     | 0.04 | 0.04 | 0.04   | 0.24              | 0.22 | 0.22 | 0.2267 |
|              |                      |      |      | 0.0278 |             |      |      | 1.0267 |                          |      |      | 0.04   |                   |      |      | 0.2244 |



## Appendix 7

**Table on the Nitrate content testing for 7 hours (mg/L)**

| Testing Time | Sample Type (Result) |      |      |      |             |      |      |      |                          |       |       |       |                   |      |      |      |
|--------------|----------------------|------|------|------|-------------|------|------|------|--------------------------|-------|-------|-------|-------------------|------|------|------|
|              | 3 Aerobic            |      |      |      | 4 Anaerobic |      |      |      | 5 Aerobic from Anaerobic |       |       |       | 6 Diluted Aerobic |      |      |      |
|              | A                    | B    | C    | Avg  | A           | B    | C    | Avg  | A                        | B     | C     | Avg   | A                 | B    | C    | Avg  |
| 11.00 a.m.   | 1.20                 | 1.20 | 1.20 | 1.20 | 1.00        | 0.90 | 1.00 | 0.97 | 2.90                     | 3.00  | 3.00  | 2.97  | 4.00              | 4.00 | 3.90 | 3.97 |
|              | 0.90                 | 0.90 | 0.90 | 0.90 | 0.90        | 0.80 | 0.80 | 0.83 | 2.70                     | 2.70  | 2.70  | 2.70  | 4.30              | 4.40 | 4.30 | 4.33 |
|              | 1.10                 | 1.10 | 1.10 | 1.10 | 1.30        | 1.20 | 1.30 | 1.27 | 3.90                     | 4.40  | 4.20  | 4.17  | 7.30              | 7.50 | 7.50 | 7.43 |
|              |                      |      |      | 1.07 |             |      |      | 1.02 |                          |       |       | 3.28  |                   |      |      | 5.24 |
| 12.30 p.m.   | 0.90                 | 0.90 | 0.90 | 0.90 | 1.70        | 1.70 | 1.60 | 1.67 | 4.40                     | 4.50  | 4.40  | 4.43  | 3.20              | 3.20 | 3.20 | 3.20 |
|              | 1.00                 | 0.90 | 0.90 | 0.93 | 1.80        | 1.70 | 1.80 | 1.77 | 4.90                     | 4.90  | 4.90  | 4.90  | 1.50              | 1.40 | 1.50 | 1.47 |
|              | 1.50                 | 1.50 | 1.50 | 1.50 | 2.00        | 1.70 | 1.60 | 1.77 | 3.00                     | 3.10  | 3.10  | 3.07  | 1.60              | 1.50 | 1.60 | 1.57 |
|              |                      |      |      | 1.11 |             |      |      | 1.73 |                          |       |       | 4.13  |                   |      |      | 2.08 |
| 2.00 p.m.    | 1.30                 | 1.30 | 1.30 | 1.30 | 1.00        | 1.00 | 1.00 | 1.00 | 4.80                     | 5.00  | 5.00  | 4.93  | 1.20              | 1.00 | 1.10 | 1.10 |
|              | 0.50                 | 0.40 | 0.40 | 0.43 | 0.30        | 0.20 | 0.20 | 0.23 | 5.90                     | 6.20  | 6.20  | 6.10  | 2.10              | 2.00 | 2.00 | 2.03 |
|              | 3.10                 | 3.10 | 3.10 | 3.10 | 1.00        | 1.00 | 1.00 | 1.00 | 7.00                     | 7.30  | 7.50  | 7.27  | 2.40              | 2.40 | 2.40 | 2.40 |
|              |                      |      |      | 1.61 |             |      |      | 0.74 |                          |       |       | 6.10  |                   |      |      | 1.84 |
| 3.30 p.m.    | 0.20                 | 0.20 | 0.10 | 0.17 | 0.00        | 0.00 | 0.00 | 0.00 | 8.30                     | 8.50  | 8.70  | 8.50  | 2.10              | 2.00 | 2.00 | 2.03 |
|              | 0.00                 | 0.00 | 0.00 | 0.00 | 0.00        | 0.00 | 0.00 | 0.00 | 8.50                     | 8.70  | 8.70  | 8.63  | 1.30              | 1.50 | 1.30 | 1.37 |
|              | 0.70                 | 0.70 | 0.70 | 0.70 | 0.00        | 0.00 | 0.00 | 0.00 | 9.60                     | 9.70  | 9.60  | 9.63  | 2.20              | 2.30 | 2.10 | 2.20 |
|              |                      |      |      | 0.29 |             |      |      | 0.00 |                          |       |       | 8.92  |                   |      |      | 1.87 |
| 5.00 p.m.    | 1.70                 | 1.40 | 1.30 | 1.47 | 0.80        | 0.60 | 0.70 | 0.70 | 12.80                    | 12.70 | 12.90 | 12.80 | 2.30              | 2.40 | 2.40 | 2.37 |
|              | 1.60                 | 1.50 | 1.30 | 1.47 | 1.00        | 1.00 | 1.00 | 1.00 | 10.60                    | 10.50 | 10.80 | 10.63 | 3.50              | 3.40 | 3.30 | 3.40 |
|              | 2.20                 | 1.70 | 1.80 | 1.90 | 1.00        | 0.70 | 0.80 | 0.83 | 11.00                    | 10.50 | 10.70 | 10.73 | 3.90              | 3.90 | 3.90 | 3.90 |
|              |                      |      |      | 1.61 |             |      |      | 0.84 |                          |       |       | 11.39 |                   |      |      | 3.22 |
| 6.00 p.m.    | 0.00                 | 0.00 | 0.00 | 0.00 | 6.00        | 0.80 | 0.90 | 2.57 | 9.00                     | 9.20  | 9.40  | 9.20  | 2.80              | 2.70 | 2.80 | 2.77 |
|              | 0.10                 | 0.20 | 0.20 | 0.17 | 0.60        | 0.80 | 0.90 | 0.77 | 6.90                     | 7.30  | 7.40  | 7.20  | 2.30              | 2.20 | 2.30 | 2.27 |
|              | 0.00                 | 0.00 | 0.00 | 0.00 | 3.00        | 3.40 | 3.30 | 3.23 | 12.30                    | 12.60 | 12.60 | 12.50 | 3.70              | 3.70 | 3.80 | 3.73 |
|              |                      |      |      | 0.06 |             |      |      | 2.19 |                          |       |       | 9.63  |                   |      |      | 2.92 |

## Appendix 8

**Table on the mg of Ammonia over mg of MLVSS testing for 7 hours (mg/L)**

| Hours | Sample Type |        |               |               |         |        |               |               |          |        |               |               |         |        |               |               |
|-------|-------------|--------|---------------|---------------|---------|--------|---------------|---------------|----------|--------|---------------|---------------|---------|--------|---------------|---------------|
|       | A           |        |               |               | B       |        |               |               | C        |        |               |               | D       |        |               |               |
|       | Ammonia     | MLVSS  | Volume Sample | Ammonia/MLVSS | Ammonia | MLVSS  | Volume Sample | Ammonia/MLVSS | Ammonia  | MLVSS  | Volume Sample | Ammonia/MLVSS | Ammonia | MLVSS  | Volume Sample | Ammonia/MLVSS |
| 0.5   | 0.75        | 558.33 | 10 ml         | 0.001343      | 1.01    | 90.00  | 10 ml         | 0.011222      | 0.59     | 370.00 | 10 ml         | 0.001595      | 0.47    | 528.33 | 10 ml         | 0.00089       |
| 2     | 0.56333     | 641.67 | 10 ml         | 0.000878      | 1.23556 | 151.67 | 10 ml         | 0.008147      | 0.924444 | 253.33 | 10 ml         | 0.003649      | 0.60889 | 666.67 | 10 ml         | 0.000913      |
| 3.5   | 0.04778     | 765.00 | 10 ml         | 6.25E-05      | 1.07    | 283.33 | 10 ml         | 0.003776      | 0.392222 | 238.33 | 10 ml         | 0.001646      | 0.38    | 503.33 | 10 ml         | 0.000755      |
| 5     | 0           | 648.33 | 10 ml         | 0             | 0.95444 | 263.33 | 10 ml         | 0.003624      | 0.227778 | 305.00 | 10 ml         | 0.000747      | 0.22667 | 435.00 | 10 ml         | 0.000521      |
| 6.5   | 0.00667     | 208.33 | 10 ml         | 3.2E-05       | 1.05    | 36.11  | 10 ml         | 0.029077      | 0.128889 | 158.33 | 10 ml         | 0.000814      | 0.24222 | 56.67  | 10 ml         | 0.004275      |
| 8.00  | 0.02778     | 262.78 | 10 ml         | 0.000106      | 1.02667 | 67.22  | 10 ml         | 0.015273      | 0.04     | 110.00 | 10 ml         | 0.000364      | 0.22444 | 56.67  | 10 ml         | 0.003961      |



## Appendix 9

**Table on Nitrate content based on Sampling Day and Type of Sample from Acclimatization to Refinery Wastewater Addition**

| Sampling Day | Sample Type (Result) Nitrate |       |       |       |            |       |       |       |            |       |       |       |            |       |       |       |
|--------------|------------------------------|-------|-------|-------|------------|-------|-------|-------|------------|-------|-------|-------|------------|-------|-------|-------|
|              | Influent A                   |       |       |       | Influent B |       |       |       | Effluent A |       |       |       | Effluent B |       |       |       |
|              | A                            | B     | C     | Avg   | A          | B     | C     | Avg   | A          | B     | C     | Avg   | A          | B     | C     | Avg   |
| 1            | 3.80                         | 4.20  | 3.20  | 3.73  | 3.20       | 3.30  | 3.60  | 3.37  | 15.40      | 14.30 | 14.90 | 14.87 | 13.20      | 12.90 | 12.50 | 12.87 |
|              |                              |       |       | 3.73  |            |       |       | 3.37  |            |       |       | 14.87 |            |       |       | 12.87 |
| 2            | 4.20                         | 2.70  | 2.80  | 3.23  | 1.40       | 3.40  | 4.60  | 3.13  | 14.90      | 15.10 | 13.10 | 14.37 | 10.30      | 11.10 | 10.20 | 10.53 |
|              |                              |       |       | 3.23  |            |       |       | 3.13  |            |       |       | 14.37 |            |       |       | 10.53 |
| 3            | 3.40                         | 2.80  | 2.10  | 2.77  | 2.60       | 2.10  | 2.80  | 2.50  | 13.90      | 13.40 | 10.10 | 12.47 | 8.30       | 8.10  | 7.80  | 8.07  |
|              |                              |       |       | 2.77  |            |       |       | 2.50  |            |       |       | 12.47 |            |       |       | 8.07  |
| 4            | 1.10                         | 0.70  | 0.70  | 0.83  | 1.10       | 1.10  | 1.20  | 1.13  | 46.10      | 31.40 | 30.00 | 35.83 | 22.90      | 23.10 | 20.90 | 22.30 |
|              |                              |       |       | 0.83  |            |       |       | 1.13  |            |       |       | 35.83 |            |       |       | 22.30 |
| 5            | 1.30                         | 1.10  | 0.90  | 1.10  | 1.40       | 1.20  | 0.90  | 1.17  | 19.90      | 17.30 | 20.30 | 19.17 | 20.90      | 16.50 | 19.00 | 18.80 |
|              |                              |       |       | 1.10  |            |       |       | 1.17  |            |       |       | 19.17 |            |       |       | 18.80 |
| 6            | 5.60                         | 6.40  | 6.20  | 6.07  | 5.40       | 6.50  | 5.80  | 5.90  | 13.20      | 11.60 | 12.20 | 12.33 | 10.10      | 10.50 | 10.30 | 10.30 |
|              |                              |       |       | 6.07  |            |       |       | 5.90  |            |       |       | 12.33 |            |       |       | 10.30 |
| 7            | 7.90                         | 7.70  | 6.20  | 7.27  | 4.70       | 5.10  | 5.60  | 5.13  | 12.60      | 13.70 | 13.50 | 13.27 | 8.10       | 10.40 | 11.70 | 10.07 |
|              |                              |       |       | 7.27  |            |       |       | 5.13  |            |       |       | 13.27 |            |       |       | 10.07 |
| 8            | 10.30                        | 13.20 | 13.40 | 12.30 | 11.20      | 11.40 | 11.10 | 11.23 | 12.10      | 11.20 | 11.30 | 11.53 | 9.80       | 8.70  | 9.20  | 9.23  |
|              |                              |       |       | 12.30 |            |       |       | 11.23 |            |       |       | 11.53 |            |       |       | 9.23  |



|     |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |       |
|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 9   | 33.90 | 34.00 | 33.90 | 33.93 | 48.90 | 49.00 | 49.10 | 49.00 | 13.70 | 13.40 | 13.70 | 13.60 | 13.20 | 13.40 | 13.30 | 13.30 |
|     |       |       |       | 33.93 |       |       |       | 49.00 |       |       |       | 13.60 |       |       |       | 13.30 |
| 10  | 34.10 | 33.80 | 33.80 | 33.90 | 45.10 | 44.90 | 44.80 | 44.93 | 15.60 | 14.30 | 15.10 | 15.00 | 14.10 | 15.10 | 15.30 | 14.83 |
|     |       |       |       | 33.90 |       |       |       | 44.93 |       |       |       | 15.00 |       |       |       | 14.83 |
| 11  | 33.50 | 32.10 | 33.80 | 33.13 | 44.10 | 44.50 | 45.10 | 44.57 | 15.30 | 15.10 | 15.10 | 15.17 | 14.10 | 14.50 | 14,6  | 14.30 |
|     |       |       |       | 33.13 |       |       |       | 44.57 |       |       |       | 15.17 |       |       |       | 14.30 |
| RA1 | 2.70  | 3.30  | 3.10  | 3.03  | 4.10  | 3.20  | 3.20  | 3.50  | 6.20  | 11.80 | 14.50 | 10.83 | 5.90  | 3.80  | 6.90  | 5.53  |
|     |       |       |       | 3.03  |       |       |       | 3.50  |       |       |       | 10.83 |       |       |       | 5.53  |
| RA2 | 3.10  | 3.20  | 2.90  | 3.07  | 3.30  | 3.40  | 3.50  | 3.40  | 10.10 | 9.80  | 10.20 | 10.03 | 6.50  | 6.60  | 6.10  | 6.40  |
|     |       |       |       | 3.07  |       |       |       | 3.40  |       |       |       | 10.03 |       |       |       | 6.40  |
| RA3 | 3.40  | 2.80  | 2.10  | 2.77  | 2.60  | 2.10  | 2.80  | 2.50  | 9.80  | 9.10  | 9.50  | 9.47  | 5.40  | 5.90  | 5.80  | 5.70  |
|     |       |       |       | 2.77  |       |       |       | 2.50  |       |       |       | 9.47  |       |       |       | 5.70  |
| RA4 | 3.10  | 3.50  | 2.90  | 3.17  | 4.10  | 4.50  | 4.30  | 4.30  | 9.30  | 9.40  | 9.10  | 9.27  | 5.10  | 5.30  | 5.10  | 5.17  |
|     |       |       |       | 3.17  |       |       |       | 4.30  |       |       |       | 9.27  |       |       |       | 5.17  |
| RA5 | 1.30  | 1.10  | 0.90  | 1.10  | 1.40  | 1.20  | 0.90  | 1.17  | 8.10  | 8.15  | 7.90  | 8.05  | 4.30  | 4.50  | 4.30  | 4.37  |
|     |       |       |       | 1.10  |       |       |       | 1.17  |       |       |       | 8.05  |       |       |       | 4.37  |
| RA6 | 1.40  | 1.50  | 1.10  | 1.33  | 1.30  | 0.80  | 1.40  | 1.17  | 7.90  | 8.10  | 7.80  | 7.93  | 3.00  | 3.90  | 3.00  | 3.30  |
|     |       |       |       | 1.33  |       |       |       | 1.17  |       |       |       | 7.93  |       |       |       | 3.30  |

## Appendix 10

**Table on Ammonia content based on Sampling Day and Type of Sample from Acclimatization to Refinery Wastewater Addition**

| Testing Day            | Sample Type (Result) Ammonia |      |      |      |            |      |      |      |            |      |      |      |            |      |      |      |
|------------------------|------------------------------|------|------|------|------------|------|------|------|------------|------|------|------|------------|------|------|------|
|                        | Influent A                   |      |      |      | Influent B |      |      |      | Effluent A |      |      |      | Effluent B |      |      |      |
|                        | A                            | B    | C    | Avg  | A          | B    | C    | Avg  | A          | B    | C    | Avg  | A          | B    | C    | Avg  |
| Monday                 | 2.21                         | 2.11 | 2.22 | 2.18 | 2.56       | 2.47 | 2.55 | 2.53 | 0.21       | 0.22 | 0.21 | 0.21 | 0.27       | 0.28 | 0.27 | 0.27 |
|                        |                              |      |      | 2.18 |            |      |      | 2.53 |            |      |      | 0.21 |            |      |      | 0.27 |
| Tuesday                | 2.35                         | 2.31 | 2.32 | 2.33 | 2.68       | 2.30 | 2.64 | 2.54 | 0.23       | 0.21 | 0.23 | 0.22 | 0.31       | 0.33 | 0.32 | 0.32 |
|                        |                              |      |      | 2.33 |            |      |      | 2.54 |            |      |      | 0.22 |            |      |      | 0.32 |
| Wednesday              | 2.57                         | 2.44 | 2.55 | 2.52 | 2.78       | 2.79 | 2.69 | 2.75 | 0.39       | 0.38 | 0.39 | 0.39 | 0.68       | 0.66 | 0.71 | 0.68 |
|                        |                              |      |      | 2.52 |            |      |      | 2.75 |            |      |      | 0.39 |            |      |      | 0.68 |
| Thursday               | 3.84                         | 3.86 | 3.90 | 3.87 | 3.31       | 3.37 | 3.33 | 3.34 | 0.41       | 0.51 | 0.50 | 0.47 | 0.77       | 0.72 | 0.53 | 0.67 |
|                        |                              |      |      | 3.87 |            |      |      | 3.34 |            |      |      | 0.47 |            |      |      | 0.67 |
| Friday                 | 3.57                         | 3.57 | 3.64 | 3.59 | 3.58       | 3.55 | 3.66 | 3.60 | 0.51       | 0.53 | 0.48 | 0.51 | 0.76       | 0.71 | 0.81 | 0.76 |
|                        |                              |      |      | 3.59 |            |      |      | 3.60 |            |      |      | 0.51 |            |      |      | 0.76 |
| Monday<br>15.3.2010    | 1.10                         | 1.20 | 1.09 | 1.13 | 3.41       | 3.21 | 3.41 | 3.34 | 0.02       | 0.02 | 0.01 | 0.02 | 0.00       | 0.01 | 0.01 | 0.01 |
|                        |                              |      |      | 1.13 |            |      |      | 3.34 |            |      |      | 0.02 |            |      |      | 0.01 |
| Wednesday<br>17.3.2010 | 1.06                         | 1.05 | 1.09 | 1.07 | 3.63       | 3.52 | 3.63 | 3.59 | 0.01       | 0.01 | 0.02 | 0.01 | 0.01       | 0.00 | 0.01 | 0.01 |
|                        |                              |      |      | 1.07 |            |      |      | 3.59 |            |      |      | 0.01 |            |      |      | 0.01 |
| Friday<br>19.3.2010    | 1.03                         | 1.04 | 1.04 | 1.04 | 3.51       | 3.49 | 3.52 | 3.51 | 0.01       | 0.01 | 0.00 | 0.01 | 0.00       | 0.00 | 0.01 | 0.00 |
|                        |                              |      |      | 1.04 |            |      |      | 3.51 |            |      |      | 0.01 |            |      |      | 0.00 |
| Monday<br>22.3.2010    | 0.98                         | 0.96 | 0.97 | 0.97 | 1.00       | 1.00 | 1.00 | 1.00 | 0.00       | 0.00 | 0.00 | 0.00 | 0.00       | 0.00 | 0.00 | 0.00 |
|                        |                              |      |      | 0.97 |            |      |      | 1.00 |            |      |      | 0.00 |            |      |      | 0.00 |
| Wednesday<br>24.3.2010 | 0.96                         | 0.91 | 0.91 | 0.93 | 1.02       | 1.01 | 1.01 | 1.01 | 0.00       | 0.00 | 0.00 | 0.00 | 0.00       | 0.01 | 0.00 | 0.00 |
|                        |                              |      |      | 0.93 |            |      |      | 1.01 |            |      |      | 0.00 |            |      |      | 0.00 |

|           |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|-----------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Friday    | 0.78 | 0.81 | 0.81 | 0.80 | 0.98 | 0.97 | 0.99 | 0.98 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.01 | 0.00 | 0.01 |
| 26.3.2010 |      |      |      | 0.80 |      |      |      | 0.98 |      |      |      | 0.00 |      |      |      | 0.01 |
| 22.4.2010 | 2.34 | 2.33 | 2.26 | 2.31 | 2.21 | 2.34 | 2.26 | 2.27 | 0.38 | 0.33 | 0.36 | 0.36 | 0.36 | 0.34 | 0.36 | 0.35 |
|           |      |      |      | 2.31 |      |      |      | 2.27 |      |      |      | 0.36 |      |      |      | 0.35 |
| 24.4.2010 | 2.27 | 2.36 | 2.37 | 2.33 | 2.17 | 2.36 | 2.34 | 2.29 | 0.30 | 0.29 | 0.35 | 0.31 | 0.27 | 0.36 | 0.34 | 0.32 |
|           |      |      |      | 2.33 |      |      |      | 2.29 |      |      |      | 0.31 |      |      |      | 0.32 |
| 26.4.2010 | 2.41 | 2.44 | 2.38 | 2.41 | 4.44 | 4.37 | 4.51 | 4.44 | 0.31 | 0.33 | 0.31 | 0.32 | 0.31 | 0.35 | 0.36 | 0.34 |
|           |      |      |      | 2.41 |      |      |      | 4.44 |      |      |      | 0.32 |      |      |      | 0.34 |
| 28.4.2010 | 2.41 | 2.31 | 2.37 | 2.36 | 7.71 | 7.64 | 7.69 | 7.68 | 0.30 | 0.39 | 0.37 | 0.35 | 1.69 | 1.66 | 1.60 | 1.65 |
|           |      |      |      | 2.36 |      |      |      | 7.68 |      |      |      | 0.35 |      |      |      | 1.65 |
| 30.4.2010 | 2.26 | 2.28 | 2.31 | 2.28 | 7.81 | 7.76 | 7.58 | 7.72 | 0.25 | 0.29 | 0.28 | 0.27 | 1.57 | 1.58 | 1.54 | 1.56 |
|           |      |      |      | 2.28 |      |      |      | 7.72 |      |      |      | 0.27 |      |      |      | 1.56 |
| 3.5.2010  | 2.43 | 2.34 | 2.31 | 2.36 | 7.65 | 7.75 | 7.70 | 7.70 | 0.33 | 0.31 | 0.34 | 0.33 | 1.49 | 1.46 | 1.47 | 1.47 |
|           |      |      |      | 2.36 |      |      |      | 7.70 |      |      |      | 0.33 |      |      |      | 1.47 |



## Appendix 11

**Table on Phosphorus content based on Sampling Day and Type of Sample from Acclimatization to Refinery Wastewater Addition**

| Sampling day | IA    | IB    | EA    | EB    |
|--------------|-------|-------|-------|-------|
| 1            | 9.80  | 10.23 | 8.65  | 9.27  |
| 2            | 10.62 | 11.32 | 9.98  | 10.50 |
| 3            | 21.78 | 16.73 | 15.45 | 15.12 |
| 4            | 23.73 | 15.28 | 19.75 | 11.42 |
| 5            | 5.82  | 7.80  | 1.78  | 1.33  |
| 6            | 6.08  | 6.37  | 1.77  | 1.58  |
| 7            | 6.43  | 4.50  | 2.75  | 6.03  |
| 8            | 6.63  | 5.73  | 2.30  | 5.42  |
| 9            | 6.75  | 7.63  | 4.75  | 5.97  |
| 10           | 6.85  | 7.08  | 4.80  | 5.97  |
| 11           | 5.65  | 6.07  | 4.05  | 5.48  |
| R1           | 5.55  | 2.65  | 6.9   | 6     |
| R2           | 1.35  | 2.75  | 5.8   | 5.75  |
| R3           | 2.05  | 3.5   | 6.55  | 9.15  |
| R4           | 5.3   | 4.95  | 7.2   | 13.1  |
| R5           | 5.65  | 5.15  | 6.8   | 11.6  |
| R6           | 6.1   | 5.25  | 7.35  | 12.75 |

## Appendix 12

### Methodology for Ammonia Content Testing using Nessler Method

Before any testing can be conducted, the lab has to be prepared first. The tools, equipment, consumables must be prepared firsthand before conducting any experiment. Details about the equipment and material used will be discussed in latter section.

Before conducting the experiment, the wastewater sample needed to be diluted as we need to save as much as wastewater as possible. The sample is diluted with ratio 1:5 with 5 part of distilled water. Prepare enough samples for 3 times of testing as we will need accurate results.

After the sample is ready, STORED PROGRAMS on the Photospectrometer is pressed and the code for the test is selected, N Ammonia, Ness.

A 25 ml mixing graduated cylinder is filled with the wastewater up to the 25 ml mark.

A 25 ml mixing graduated cylinder is filled with deionized water up to the 25 ml mark. This will be used for the blank sample.

3 drops of mineral stabilizer are added to each cylinder. The cylinders are inverted several times to ensure proper mixing.

3 drops of Polyvinyl Alcohol Dispersing Agent is added to each cylinder. The cylinders are inverted several times to ensure proper mixing.

1.0 ml of Nessler Reagent is pipetted into each cylinder. The cylinders are inverted several times to ensure proper mixing.

The Timer is set for one minute and pressed.

The samples are poured into 10 ml square sample cell.

When the timer expires, the blank is inserted into the cell holder with the fill line facing right.

The ZERO button is pressed.

The prepared sample is wiped and inserted into the cell holder with the fill line facing right.

The READ button is pressed and the result is recorded.

Repeat the procedures for another 2 times to ensure accurate results are obtained.

## **Appendix 13**

### **Methodology for Nitrate content Testing using the Powder Pillow method**

The required sample is taken from the beaker containing the wastewater samples taken for the interval.

After the sample is ready, STORED PROGRAMS on the Photospectrometer is pressed and the code for the test is selected, 355 N, Nitrate HR PP

The sample is poured into 10 ml square sample cell.

The contents of one NitraVer 5 Nitrate Reagent Powder Pillow are added into the square sample cell.

The Timer is set for one minute and pressed

The cell is shaken vigorously until the timer expires

When the timer expires, the timer is set to 5 minutes and pressed again. A five minutes reaction period will begin

When the timer expires, a second square sample cell is filled with 10 ml of sample. This will be used for the Blank sample

The blank is wiped and the blank is inserted into the cell holder with the fill line facing right.

The ZERO button is pressed

The prepared sample is wiped and inserted into the cell holder with the fill line facing right.

The READ button is pressed and the result is recorded.

Repeat the procedures for another 2 times to ensure accurate results are obtained.

## **Appendix 14**

### **Methodology for MLSS and MLVSS Testing**

Before conducting the experiment, the wastewater sample needed to be diluted as we need to save as much as wastewater as possible. The sample is diluted with ratio 1:5 with 5 part of distilled water. Prepare enough samples for 3 times of testing as we will need accurate results.

Preparation of glass-fiber filter disk: If pre-prepared glass fiber filter disks are used, eliminate this step. Insert disk with wrinkled side up in filtration apparatus.

Apply vacuum and wash disk with three successive 20-mL portions of reagent-grade water.

Continue suction to remove all traces of water, turn vacuum off, and discard washings.

Remove filter from filtration apparatus and transfer to an inert aluminum weighing dish.



Dry in an oven at 103 to 105°C for 1 h.

If volatile solids are to be measured, ignite at 550°C for 15 min in a muffle furnace.

Cool in desiccator to balance temperature and weigh.

Repeat cycle of drying or igniting, cooling, desiccating, and weighing until a constant weight is obtained or until weight change is less than 4% of the previous weighing or 0.5 mg, whichever is less. Store in desiccator until needed.

## **Appendix 15**

### **TOOLS AND EQUIPMENTS**

#### **General Equipments**

- 1) Biosys Sequencing Batch Reactor to degrade the Petroleum Wastewater
- 2) High Strength Wastewater (Petroleum Wastewater)
- 3) Reactor with Baffle
- 4) Aerator
- 5) Mixer

#### **Ammonia Testing using Nessler Method**

- 1) 25 ml graduated cylinder
- 2) Nessler Reagent
- 3) Deionized Water
- 4) Mineral Stabilizer
- 5) Polyvinyl Alcohol Dispersing Agent
- 6) Photospectrometer
- 7) Square Sample Cell

#### **Nitrate Testing using Powder Pillow method**

- 1) NitraVer 5 Nitrate Reagent Powder Pillow
- 2) Square Sample Cell
- 3) Beaker
- 4) Photospectrometer
- 5) Stopper

#### **MLSS and MLVSS Testing**

- 1) 47 mm filter paper

- 2) Filter Holder
- 3) Filtering Flask
- 4) Drying Oven
- 5) Desiccators
- 6) Tweezers
- 7) Measurement cylinder
- 8) Furnace set to 550 C