BIODEGRADATION OF PRE-TREATED SULFINOL-D WASTE: MONITORING OF NH_3 AND NO_3

NURUL AIN BINTI MOHAMAD SHUKUR

CHEMICAL ENGINEERING
UNIVERSITI TEKNOLOGI PETRONAS

JULY 2009

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by

Nurul Ain binti Mohamad Shurur

Dissertation submitted in partial fulfilment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical Engineering)

JULY 2009

Universiti Teknologi PETRONAS
Bandar Seri Iskandar
31750 Tronoh
Perak Darul Ridzuan

CERTIFICATION OF APPROVAL

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

Approved by,

(AP IR Abd Aziz b Omar)

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

July 2009

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.

Nurul Ain binti Mohamad Shurur

ABSTRACT

Two separate batch reactors were fed with domestic wastewater first and operated in an aerobic and anoxic in a two different reactor. Acclimatization will take place first in order to acclimatize the microorganism for Nitrification takes place in reactor one and denitrification takes place in reactor two. For reactor one, Nitrifying bacteria need to be acclimatized before proceed to treat Fenton pre-treated amine waste and for reactor, Denitrifying bacteria need to be culture and populate in order to run denitrifcation process right after nitrification takes place in reactor one. After doing some observation to make sure the microorganism needed is healthy, Fenton pre-treated amine waste will be put into the reactor for biological treatment. In order to treat nitrogenous compound, nitrification will take place first in reactor one, followed by denitrification process which will happened in reactor two. For expected result, COD and nitrogenous compound will be reduced at the end of the experiment.

ACKNOWLEDGEMENT

First of all, I would like to show my appreciation to my supervisor for Final Year Project (FYP) AP IR Abd Aziz b Omar for his helpful guidance for me in completing this project successfully. And also to my co-supervisor Pn Putri Nazatul Faizura bt Megat Khamaruddin for lot of help. Thank you very much for your support and comments towards me during this one year duration under your supervision.

I would also like to thank my colleagues, Nor Zahidah binti Khalid and Ida Nurhidaya binti Ab Aziz for their help in this project since we are doing same issue (wastewater) for our project.

I would also like to thank Mr. Khalik M Sabil, our FYP coordinator for all his effort in guiding us and always updating the required information needed for our project.

I would also like to record my gratitude to UTP librarian at Information Resource Centre (IRC) for their help in searching the right book to be used as references for this project. Without their help and cooperation, this project might no be completed successfully.

Last but not least, to all who have contributed to this project directly and indirectly of which I might not mentioned here. They include all my colleagues and my family who always give unstoppable support for me.

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CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE PROJECT

Amine based absorbents are frequently used for gas sweetening process. This is resulting to produce wastewater of high chemical oxygen demand (COD) in the range 5000 to 25000 mg/L. Such waste water is difficult to treat by the conventional biological process. As the pre-treatment, this study deals with Fenton's degradation of a wastewater containing Sulfinol-D (amine). Then, biological treatment need to be run in order to reduce NH_3 ,NO_3 and NO_2.

1.2 PROBLEM STATEMENT

- Fenton pre-treated amine waste was found contain some NH_3 and NO_3. It is expected then during biodegradation of pre-treated waste that NH_3 and NO_3 will be formed again.
- It is not recommended to dispose wastewater containing these contaminants as it not environmentally sound practice.

1.3 OBJECTIVES

- To reduce COD , NO_3 and NO_2 level in pre-treated amine waste using biological treatment.
- to determine the effectiveness of COD, NO_3 and NO_2 removal by varying the type and cycle length of treatment .

1.4 FLOW OF STUDY

Throughout this semester, the author needs to follow a certain flow as to execute her project as follow:

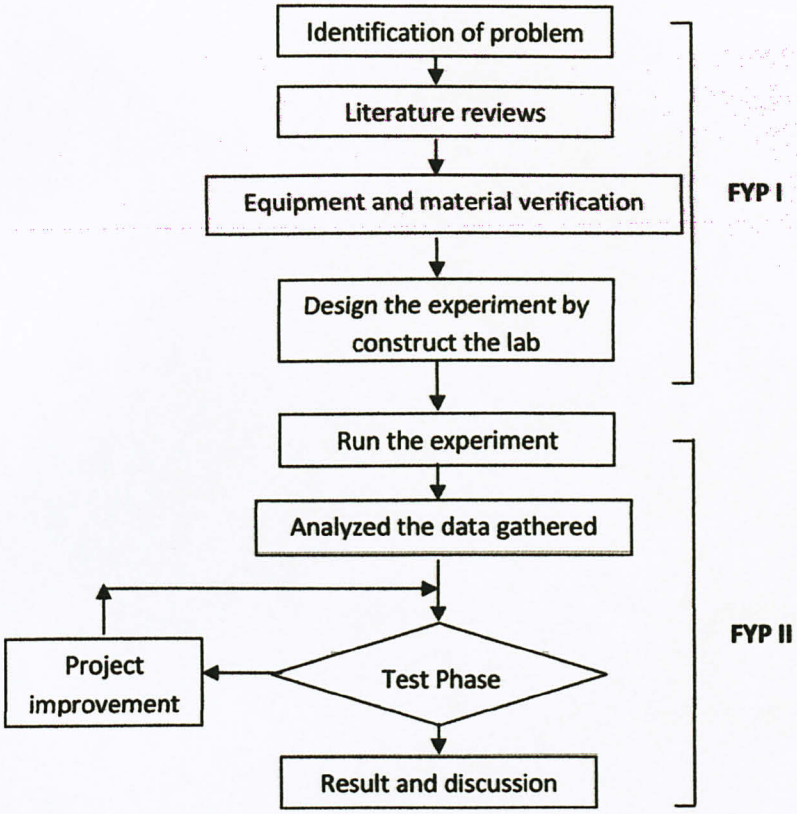


Figure 1: Flow of Study for final year project (FYP) 1 and II

1.5 SCOPE OF STUDY

This projects will required a process for biological nitrogen removal. Sequencing batch reactor (SBR) with two separate reactor which one is for aerobic condition and another fon anoxic condition will be used in order to remove nitrogen biologically. The Sequencing Batch Reactor (SBR) is an activated sludge process designed to operate under non-steady state conditions. An SBR operates in a true batch mode with aeration and sludge settlement both occurring in the two different reactor. The major differences between SBR and conventional continuous-flow, activated sludge system is that the SBR tank carries out the functions of equalization aeration and sedimentation in a time sequence rather than in the conventional space sequence of continuous-flow systems. In addition, the SBR system can be designed with the ability to treat a wide range of influent volumes whereas the continuous system is based upon a fixed influent flow rate. Thus, there is a degree of flexibility associated with working in a time rather than in a space sequence

The majority of the aeration equipment of sequencing batch reactors consist of aerator stone , fine bubble, and coarse bubble aeration systems. The main focus of this report is a nitrogen removal using sequencing batch reactor activated sludge system.

CHAPTER 2
LITERATURE VIEW

2.1 SULFINOL-D (AMINE WASTE WATER)

Sour natural gas can contain undesirable compounds, including H_2S, COS, CS_2 and mercaptans (Goar, 1971). As for that, amine is used in the treatment of sour natural gas and for this study, Sulfinol-D is the tertiary amine that been used in sweetening the gas. It is a highly water soluble compound that has been introduced into soils and ground waters at a number of sour gas processing plant sites.

The Sulfinol-D process uses a physical solvent, tetrahydrothiophene sulfone (sulfolane) and a chemical solvent, diisopropanolamine (DIPA) to remove H_2S, CO_2 and other contaminants from sour natural gas. This process is particularly effective at high H_2S concentrations (Goar, 1971), therefore is useful in MLNG process , where process of the natural gas can contain up to 35% H_2S.

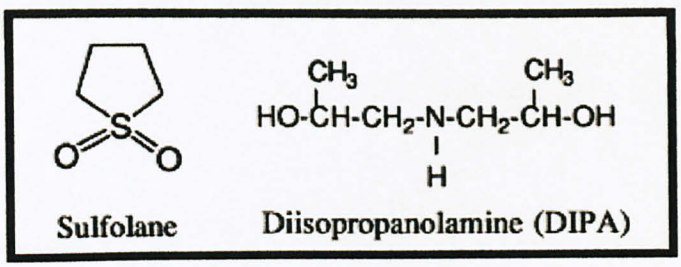


Figure 2: Structures of sulfolane and DIPA, which are the two major components used in the Sulfinol-D

Sulfolane and DIPA (Figure 2) are both highly water soluble compounds, and they have become ground water contaminants with the potential to migrate from the sour gas plant sites.

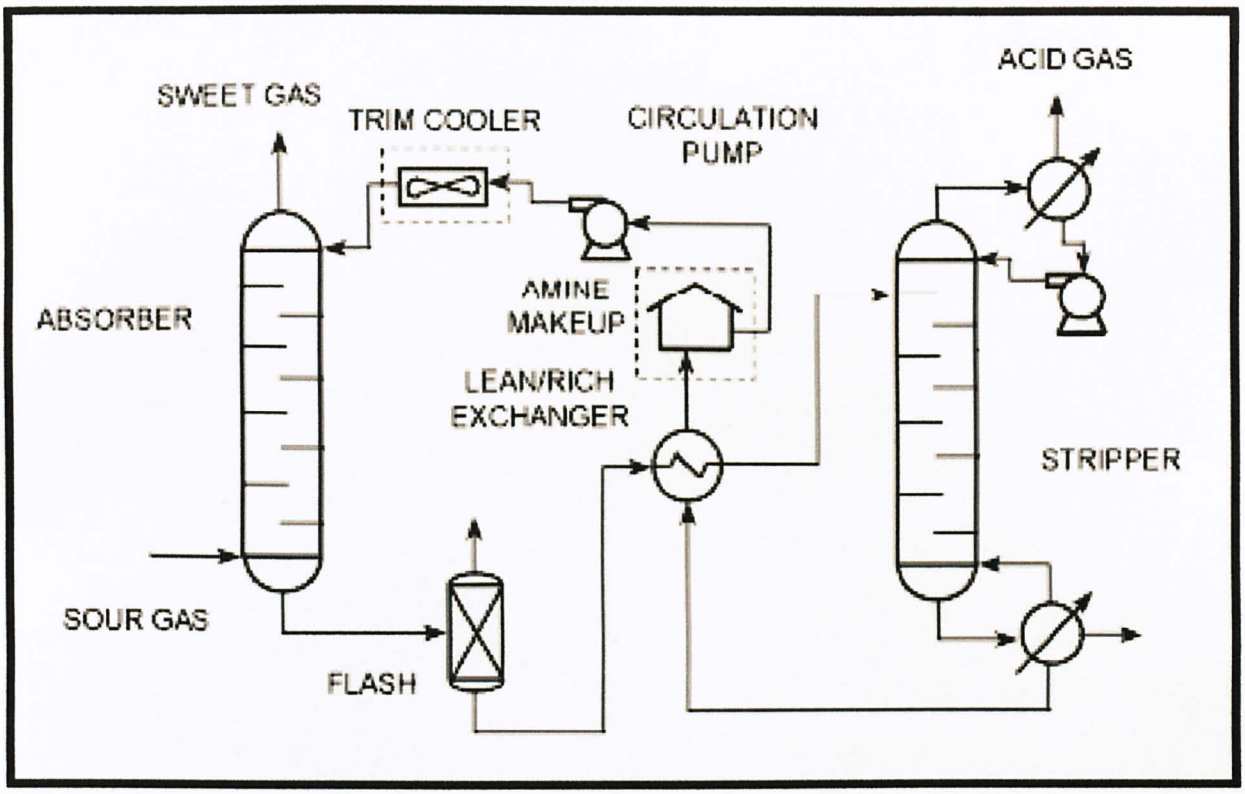


Figure 3: Schematic of simple amine sweetening plant.

2.2 FENTON'S REAGENTS

In this work, we explored the chemical oxidation of Sulfinol-D, selected as a model compound for aromatic amines, and the effect by hydroxyl radicals produced from Fenton's reagent. This reagent is a mixture of hydrogen peroxide and ferrous iron that produces OH" radicals according to Walling (1975):

$$H_2O_2 + Fe^{2+} \sim Fe^{3+} + OH^- + OH \ldots\ldots (1)$$

Fenton's reagent possesses **three attractive features** for treating aromatic amines in wastes. **First**, the OH· radicals produced in equation (1) react with organic substances in a rapid manner with second-order rate constants in the range 107-101° $M^{-1}s^{-1}$. Such radicals have proved to effectively react with a variety of compounds such as alcohols, ethers, dyes, chlorinated phenols, pesticides, polycyclic aromatics, etc., in aqueous solutions and waste waters (Haag and Yao, 1992; Kuo, 1992; Pignatello, 1992). **Second**, the reagent components are easy to handle and environmentally friendly since the final decay products (water, oxygen and ferric hydroxide) introduce no further pollution. **Third,** hydrogen peroxide alone is currently used for industrial wastewater treatment to minimize the chemical oxygen demand and the additional cost of ferrous iron is quite low, so the treatment is quite economical. Moreover, ferrous iron can be regenerated electrolytically (Hsiao and Nobe, 1993; Tzedakis *et al.,* 1989).

For the Fenton reaction condition, the higher H_2O_2 concentration will influence the degree of organic mineralization A study done by Matter et al. showed that the influence of higher concentration of Fe^{2+} increases the degree of decomposition of H_2O_2. From past research, the present of H^+ suggests that decomposition of H_2O_2 requires acidic environment for the production of desired ·OH radicals. As for that, the optimum pH is 3, and for pH adjustment, concentrated sulphuric acid, H_2SO_4 and 1 M sodium hydroxide, NaOH are needed to regulate the pH.

Many report have been published on the use of Fenton's reagent to degrade pollutants such as MTBE (Methyl Tert Butyl Ether) [2], aromatic amines [3], pharmaceutical waste [4], petroleum refinery sour water [5], phenol [6] etc. All of these workers has successfully treated the waste water to a certain extend and were able to improved its biodegradability. It also noted that high volume of reagent will be used if complete mineralization of

2.3 ADVANCED OXIDATION PROCESS (AOPs)

Basically, oxidation process means converting to oxide which apply to metals, nonmetals, and organic matter. Oxygen is used as an oxidizer, because it is cheap and easily found which forms about 20% of air. Apparently, contaminants can be oxidized by four common reagents: ozone, hydrogen peroxide, oxygen and air. These procedures may also be combined with ultraviolet (UV) irradiation, ultrasonic vibrator and specific catalysts. A well known example of AOP is the use of Fenton's reagent. Advanced Oxidation Processes, refers to a set of chemical treatment procedures designed to remove organic and inorganic materials in waste water by oxidation. The contaminant materials can be converted into stable inorganic compounds such as water, carbon dioxide and salts.

AOPs, which involve the in situ generation of highly potent chemical oxidants such as hydroxyl radical (OH), have emerged as an important class of technologies for accelerating the oxidation and hence destruction of a wide range of organic contaminants in pollution solids, water and air. (Craig W.Jones, 1999). The hydroxyl radical is a powerful oxidant and a short lived, highly reactive, and non-selective reagent that is easy to produce. It has electrophonic properties and its reactions with appropriate sub-strate molecules are kinetically controlled usually very high second order rate constants, which are often close to the diffusion-controlled limit. (von Sonntag 1996). Kinetic reaction control refers to competing irreversible reactions in which the product composition is determined by the relative rates of product formation.

2.4 BIOLOGICAL TREATMENT

Biological treatment — the use of bacteria and other microorganisms to remove contaminants by assimilating them — has long been a mainstay of wastewater treatment in the chemical process industries (CPI). Because they are effective and widely used, many biological-treatment options are available today. They are, however, not all created

equal, and the decision to install a biological-treatment system requires ample thought. When considering biological waste water treatment for a particular application, it is important to understand the sources of the wastewater generated, typical wastewater composition, discharge requirements, events and practices within a facility that can affect the quantity and quality of the wastewater, and pretreatment ramifications. Consideration of these factors will allow you to maximize the benefits your plant gains from effective biological treatment. Those benefits can include:

• Low capital and operating costs com pared to those of chemical-oxidation processes

• True destruction of organics, versus mere phase separation, such as with air stripping or carbon adsorption

• Oxidation of a wide variety of organic compounds

• Removal of reduced inorganic com pounds, such as sulfides and ammonia, and total nitrogen removal possible through denitrification

• Operational flexibility to handle a wide range of flows and wastewater characteristics • Reduction of aquatic toxicity

SELECTION CRITERIA

Biological-treatment technologies vary greatly in their strengths and weaknesses. The following are application criteria, which are normally relevant in evaluating various biological-treatment options for the CPI:

- ***Bioassay/toxicity control*** — The ability to control and minimize the impact of toxic constituents in wastewater on indicating organisms when the treated water is released
- ***BOD removal efficiency*** — The ability to remove biodegradable, organic compounds
- ***COD removal efficiency*** — The ability to remove chemically oxidizable substances that may or may not be biodegradable
- ***O&M costs*** — The cost to operate and maintain the treatment method
- ***Sludge production*** — The amount of residual biological solids generated by the biological-treatment process
- ***Sludge disposal costs*** — The cost to collect, dewater and dispose of residual sludge from the treatment method, either on-site or off-site
- ***Performance in winter and summer*** — The degree in which high or low ambient-temperatures will affect biological treatment
- ***Performance on high- and low-temperature water*** — The degree in which high and low wastewater temperature will affect biological treatment
- ***Operator attention*** — The relative amount of time required to operate the biological treatment system
- ***Upset recovery*** — The amount of time it takes for a treatment method to recover from upset conditions. Upset conditions are defined as abnormal variations in the flow or characteristics of the wastewater, which can detrimentally affect a biological-treatment system
- ***Expandability*** — The ease of expanding the treatment capacity to accommodate either an overall plant expansion or an increase in loading
- ***Nitrification Efficiency*** — The relative ease of converting ammonia contained in wastewater to nitrates
- ***VOC containment*** — The relative ease with which the biological-treatment equipment can be enclosed to contain and collect VOC emissions
- ***VOC stripping potential*** — The relative ease with which the biological-treatment system will strip volatile organic compounds from the wastewater
- ***Ease of installation*** — The total amount of time and labor required to install the treatment method
- ***Energy efficiency*** — The amount of energy used by a treatment method
- ***Ease of secondary containment*** — The ability and ease with which the treatment system can be provided with secondary containment in case of overflow, spills or leaks
- ***Space requirements*** — The area required by the treatment method ❐

Figure 4 : Selection criteria for biological treatment

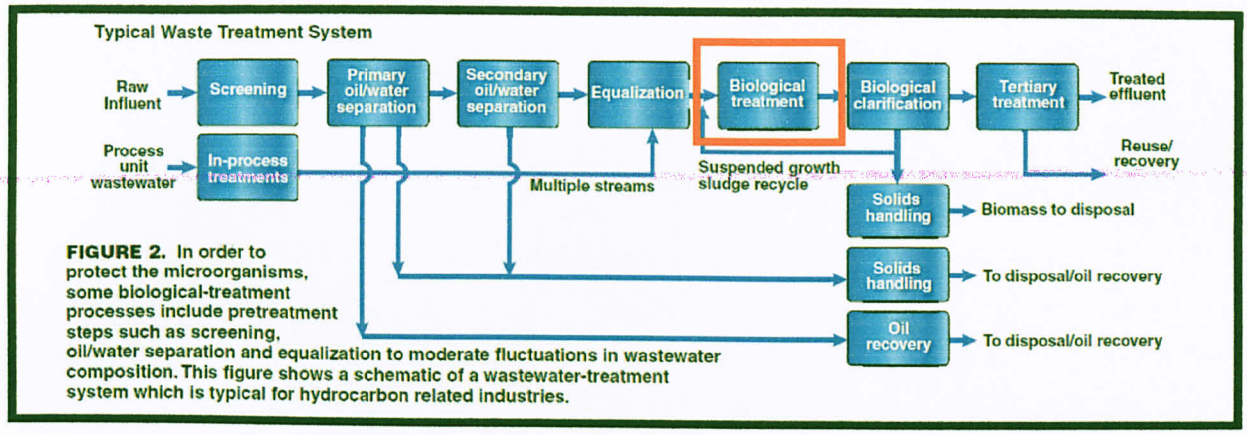


Figure 5 : Typical Waste Treatment System

Objectives

The overall objectives of the biological treatment of wastewater are to :

1. Transform (i.e. oxidize) dissolved and particulate biodegradable constituents into acceptable end products
2. Capture and incorporate suspended and nonsettleable colloidal solids into a biological floc or biofilm
3. Transform or remove nutrients, such as nitrogen (in this project) and phosphorus
4. In some cases, remove specific trace organic and inorganic compound. For industrial wastewater, the objective is to remove or reduce the concentration of organic and inorganic compound. Because some of the constituents and compounds found in industrial wastewater are toxic to microorganisms, pretreatment may be required before the industrial wastewater can be discharged to a municipal collection system.

Application

1. Removal of organic material from wastewater
2. Oxidation of ammonia nitrogen (nitrification)
3. Reduction of oxidised nitrogen (denitrification) to gaseous nitrogen (N_2 and N_2O)
4. Removal of phosphorus
5. Oxidation/ stabilization of organic sludge

How ?

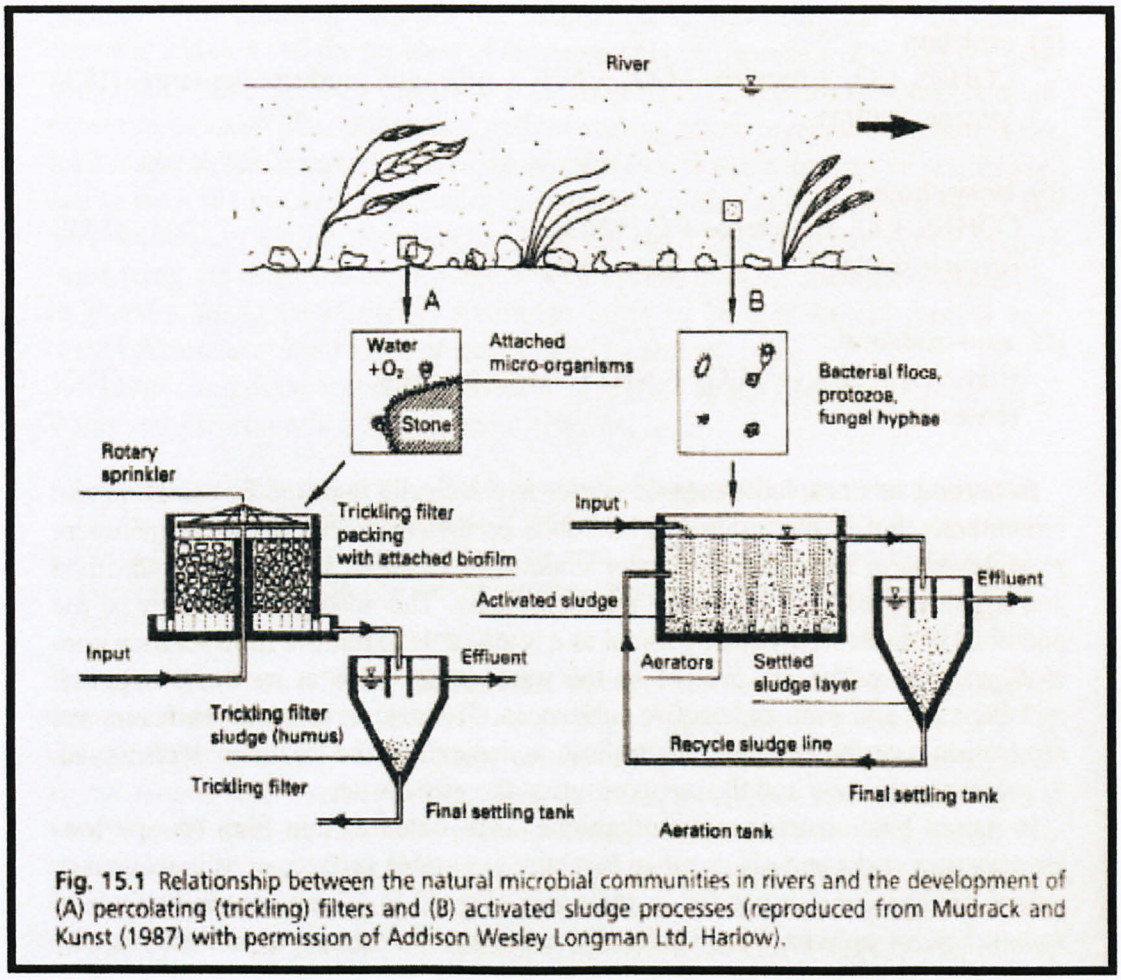


Fig. 15.1 Relationship between the natural microbial communities in rivers and the development of (A) percolating (trickling) filters and (B) activated sludge processes (reproduced from Mudrack and Kunst (1987) with permission of Addison Wesley Longman Ltd, Harlow).

Figure 6 : Relationship between the natural microbial communities in rivers and the development of (A) percolating (trickling) and (B) activated sludge process

(reproduced from Mudrack and Kunst (1987) with permission of Addison Wesley Longman Ltd, Harlow)

Role of microorganism in Wastewater Treatment

The removal of dissolved and particulate carbonaceous BOD and the stabilization of organic matter found in wastewater is accomplished biologically using a variety of microorganisms, principally bacteria. Microorganism are used to oxidize (i.e. convert) the dissolved and particulate carbonaceous organic matter into simple end products and additional biomass, as represented by the following equation for the aerobic biological oxidation of organic matter

v_1 (organic material) + v_2O2 + v_3NH_3 + v_4PO4_3- $\rightarrow$ v_5 (new cells) + v_6CO_2 + v_7H2_0

where v_i the stoichiometric coefficient

In the equation, oxygen, ammonia, and phosphate are used to represent the nutrient needed for the conversion of the organic matter to simple and products [i.e. carbon dioxide and water]. The term shown over the directional arrow used to denote the fact that microorganisms are needed to carry out the oxidation process. The term new cells is used to represent the biomass produced as a result of the organic matter. Microorganisms are also used to remove nitrogen and phosphorus in wastewater treatment processes. Specific bacteria are capable of oxidizing ammonia (nitrification) to nitrite and nitrate, while other bacteria can reduced the oxidized nitrogen to gaseous nitrogen. For phosphorus removal, biological processes are configured to encourage the growth of bacteria with the ability to take up and store large amounts of inorganic phosphorus.

2.5 THE ACTIVATED SLUDGE PROCESS

The activated sludge process is the most commonly used system for the treatment of municipal wastewater, and it is probably the most versatile and effective of all wastewater treatment processes. The treatment wastes is biological in that it uses microscopic organisms to degrade or remove wastes. The process consists of at least one aeration tank and one clarifier .

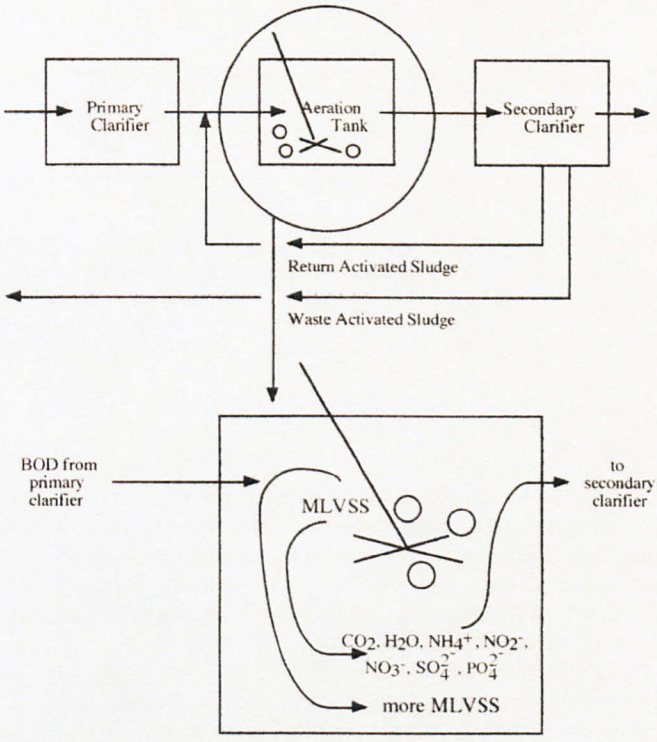


Figure 5.1 *The activated sludge process. In the activated sludge process, a tank (aeration tank) or series of tanks is used to degrade or oxidize wastes. The oxidation of the wastes is achieved by mixing the wastes, with air and a high concentration of bacteria, for sufficient time. When the wastes are oxidized more bacteria are produced. The bacteria in the form of floc particles leave the aeration tank and enter a clarifier. Here the floc particles settle to the bottom of the clarifier and are returned to the aeration tank to degrade more incoming wastes or are removed from the process. If a clarifier is placed upstream of the aeration tank, the clarifier is called a "primary clarifier." The purpose of this clarifier is to remove heavy solids and fats, oils, and grease. The clarifier following the aeration tank usually is called a "secondary clarifier." Wastes oxidized in the aeration tank are converted to carbon dioxide (CO_2), water (H_2O), ammonium ions (NH_4^+), nitrite ions (NO_2^-), nitrate ions (NO_3^-), sulfate ions (SO_4^{2-}), phosphate ions (PO_4^{2-}), and more bacterial cells (MLVSS).*

Figure 7 : The activated sludge process

Often a sedimentation tank or clarifier is placed upstream of the activated sludge processes. The purpose of this first or primary clarifier is to remove floating materials such as oils and greases and heavy solids that settle to the bottom of the clarifier. If a primary clarifier is placed upstream of the activated sludge process, the clarifier following the aeration tank is secondary clarifier.

The aeration tank is a biological reactor or amplier where relatively large numbers of bacteria are provided with dissolved oxygen and carbonaceous and nitrogenous wastes. In the presence of dissolved oxygen, the bacteria degrade the carbonaceous and nitrogenous wastes. The degradation of the wastes by the bacteria (biological reactor) result in the growth of the bacterial population (biological amplifier).

The wastes that are degraded by the bacteria are the substrates used to obtain carbon and energy. The term given for the substrates is biochemical oxygen demand (BOD). The BOD is the amount of dissolved oxygen measured in milligrams per liter (mg/l) required by the organisms, primarily bacteria, to oxidize (degrade) the wastes to simple inorganic compounds and more bacterial cells.

Table 1 : Inorganic products released in the aeration tank from the oxidation of proteins.

Element contained in proteins	**Inorganics product Released**
Carbon	Carbon dioxide (CO_2)
Hydrogen	Water (H_2O)
Nitrogen	Ammonium ion (NH_4^+)
Oxygen	Water (H_2O)
Phosphorus	Phosphate ion (PO_4^{2-})
Sulfur	Sulfate ion (SO_4^{2-})

Ultimately, under appropriate operational conditions and adequate aeration time, the bacteria convert the substrate to simplistic products through biochemical reactions. Some organic-nitrogen compounds such as proteins contain carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulfur. The sulfur is found in thiol groups (-SH) bonded to the proteins. When proteins are degraded in the aeration tank by bacteria, the bacteria obtain carbon for growth, energy for the cellular activity, and release inorganic products in the aeration tank (refer table).

Ammonium ions that produced in the sewer system and the aeration tank through hydrolysis and deamination are the substrate for the bacteria that oxidize nitrogen in the form of ammonium ions by bacteria is nitrification. When ammonium ions are oxidized, the bacteria obtain energy and release nitrite ions in the aeration tank.

The nitrite ions that are produced in the aeration tank are the substrate for the bacteria that oxidize nitrogen in the form of nitrite ions. The oxidation of nitrite ions by bacteria is nitrification. When nitrite ions is oxidized, the bacteria obtain energy and release nitrate ions in the aeration tank.

When bacterial cells oxidize substrate in the aeration tank, reproduction occurs or an increase in the bacterial population results. Bacteria represent a portion of solids in the aeration tank. Therefore, as the bacterial population increases through reproduction, the solids inventory in the aeration tank also increases..

Solids in the aeration tank are referred to as sludge. Because the sludge is aerated, and the bacteria become very active during aeration, the term "activated sludge" is used to describe the process where bacterial solids are active in the purification of the wastes within the aeration tank.

As the bacteria in the aeration tank age, many bacteria stick together to form floc particles or large solids. These particles contain a large number and diversity of bacteria that degrade the wastes in the aeration tank. As the solids flow into secondary clarifier and a clear supernatant develops above the settled solids. After additional treatment, the supernatant is discharged to to the receiving water.

The settled solids may be returned to the aeration tank or may be removed from the activated sludge process. The removal of solids from the activated sludge system is referred to as wasting. Solids are "wasted" to another treatment unit for additional treatment and disposal.

There are several operational factors that can be used to monitor and regulate the activated sludge process. The factor include F/M and MCRT. These factors are critical for monitoring and regulating nitrification and denitrification in the activated sludge process.

F/M

F/M is the food-to-microorganism ratio. This factor measures the quantity of BOD or food (the"F" in F/M) available per day per quantity of bacteria or microorganisms (the "M" in F/M). the F/M decreases when less food enters an activated sludge process or fewer bacteria are wasted from an activated sludge processes.

$$\frac{F}{M} = \frac{QS_0}{VX} = \frac{S_0}{\tau X}$$

MCRT

The MCRT is the mean cell residence time as measured in days. The MCRT is the average time the solids or bacteria are retained in a activated sludge process. The higher the MCRT is the older bacteria are.

The MCRT is increased in an activated sludge process by decreasing the quantity of solid wasted. The MCRT is decreased in an activated sludge process by increasing the quantity of solids wasted.

2.6 NITRIFICATION

Biological nitrification is the conversion or oxidation of ammonium ions to nitrite ions and then to nitrate ions. During the oxidation of ammonium ions and nitrite ions, oxygen is added to the ions by a unique group or organism, the nitrifying bacteria

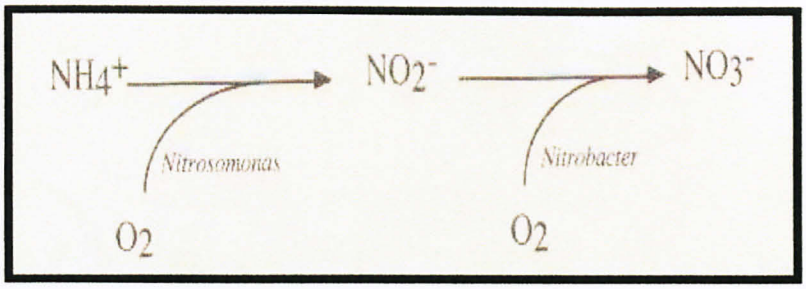


Figure 8 : Biological nitrification

Table 2 : Organism in the aeration tank that are capable of nitrification.

Organism	Genus
Actinomycetes	*Myocbacterim* *Nocardia* *Streptomyces*
Algae	*Chlorella*
Bacteria	*Arthrobacter* *Bacillus* *Nitrobacter* *Nitrosomonas* *Proteus* *Pseudomonas* *Vibrio*
Fungi	*Aspergillus*
Protozoa	*Epistylis* *Vorticella*

Nitrifications occurs in nature and in activated sludge processes. Nitrification in soil is especially important in nature , because nitrogen in absorbed by plants as a nutrients in the form of nitrate ions. Nitrification in water may be required for regulatory purposes or may be contribute to operational problems.

Although ammonium ions and ammonia are reduced forms of nitrogen, that is, are not bonded to oxygen, it is the ammonium ion, not ammonia, that is oxidized during nitrification. The quantities of ammonium ions and ammonia in an aeration tank are dependant on the pH and temperature of the activated sludge .In the temperature range 10°C to 20°C and pH range 7 to 8.5, which are typical of most activated sludge processes, about 95% of the reduced form of nitrogen is present as ammonium ions.

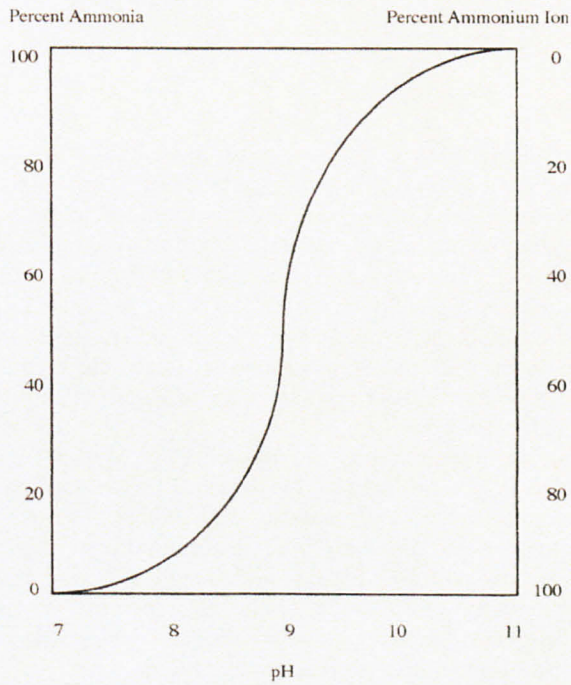


Figure 6.2 *pH and the conversion of ammonia and ammonium ions. The relative quantities of ammonia and ammonium ions in wastewater or the activated sludge process are determined by the pH of the wastewater or aeration tank. As the pH of the wastewater decreases, ammonium ions are favored. As the pH of the wastewater increases, ammonia is favored. At a pH value of 9.4 or higher, ammonia is strongly favored.*

Figure 9 : Affect oh pH and temperature in activated sludge process

The oxidation of ammonium ions and nitrite ions is achieved through the addition of dissolved oxygen within bacterial cells. Because nitrification or the biochemical reactions of oxygen addition occur inside biological cells, nitrification occurs through biochemical reactions.

The ammonium ions produced in the wastewater from hydrolysis of urea and degradation of organic-nitrogen compounds. Hydrolysis and degradation of organic-nitrogen compound results in the release of amino groups and the production of ammonium ions.

Although there are many organisms that are capable of oxidizing ammonium ions and nitrite ions (Table 2) , the principle organisms responsible for most, if not all, nitrification in the activated sludge process are two genera of Nitrifying Bacteria, *Nitrosomonas* and *Nitrobacter* (see figure 10). These genera of bacteria posses special enzymes and cellular structures that permit them to achieve significant nitrification.

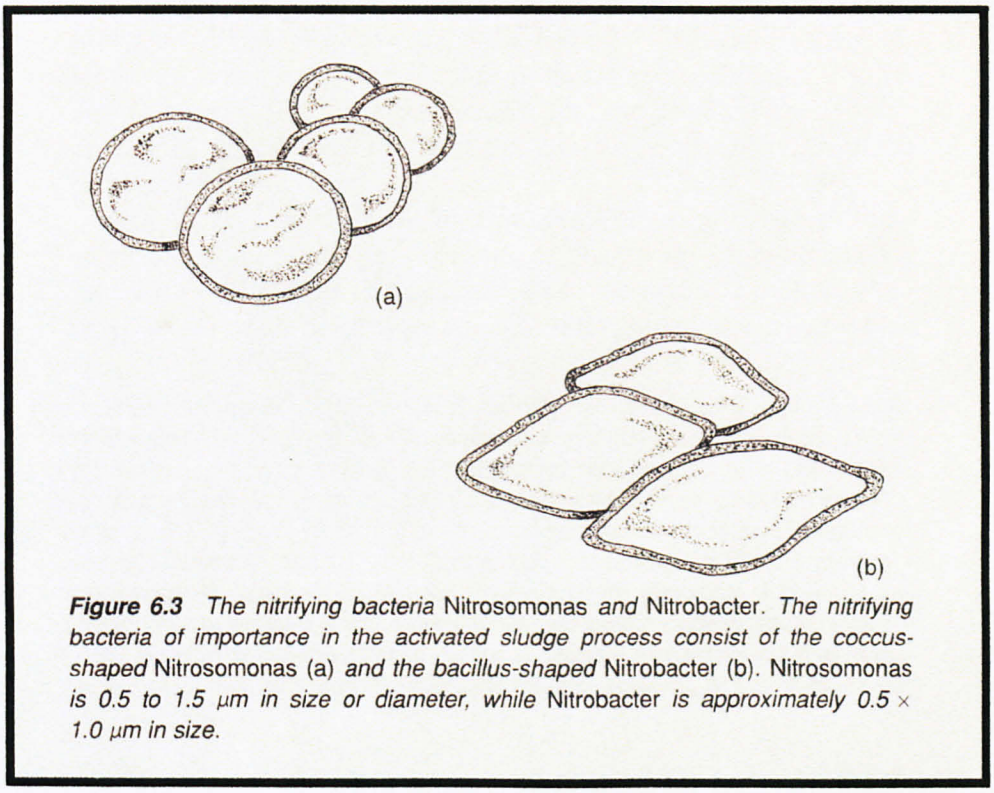


Figure 6.3 *The nitrifying bacteria* Nitrosomonas *and* Nitrobacter. *The nitrifying bacteria of importance in the activated sludge process consist of the coccus-shaped* Nitrosomonas (a) *and the bacillus-shaped* Nitrobacter (b). Nitrosomonas *is 0.5 to 1.5 μm in size or diameter, while* Nitrobacter *is approximately 0.5 × 1.0 μm in size.*

Figure 10 : Nitrifying bacteria

The genera of nitrifying bacteria that oxidize ammonium ions to nitrite ions are prefixed Nitroso- (such as *Nitrosomonas*) and the genera of nitrifying bacteria that oxidize nitrite ions to nitrate ions are prefixed Nitro (such as *Nitrobacter*). Nitrification by organisms other than nitrifying bacteria occurs at relatively low rates, and it is not associated with cellular growth or reproduction.

The rate nitrification achieved by nitrifying bacteria is often 1,000 to 10,000 times greater than the rate nitrification achieved by other organisms. Besides the nitrifying bacteria, there are two protozoa that are present in relatively large numbers during rapid nitrification. These protozoa are *Epistylist* and *Vorticella* (see figure 11). However, it is not known if these protozoa are capable of a rapid rate of nitrification, or if they simple grow in large numbers under operational conditions that are optimal for nitrification to occur through nitrifying bacteria.

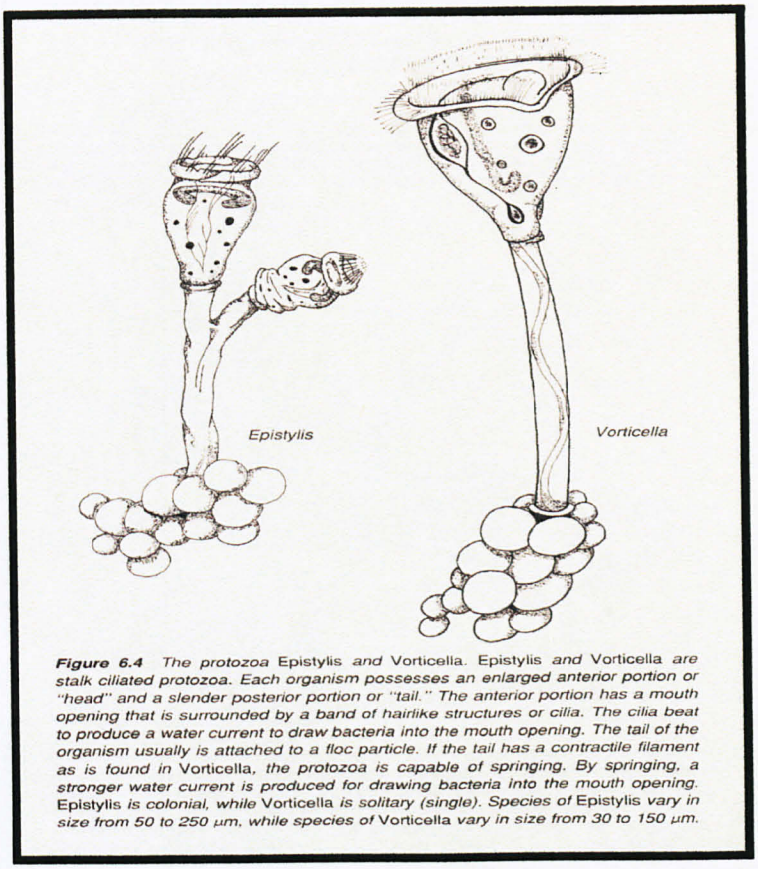


Figure 6.4 *The protozoa* Epistylis *and* Vorticella. Epistylis *and* Vorticella *are stalk ciliated protozoa. Each organism possesses an enlarged anterior portion or "head" and a slender posterior portion or "tail." The anterior portion has a mouth opening that is surrounded by a band of hairlike structures or cilia. The cilia beat to produce a water current to draw bacteria into the mouth opening. The tail of the organism usually is attached to a floc particle. If the tail has a contractile filament as is found in* Vorticella, *the protozoa is capable of springing. By springing, a stronger water current is produced for drawing bacteria into the mouth opening.* Epistylis *is colonial, while* Vorticella *is solitary (single). Species of* Epistylis *vary in size from 50 to 250 µm, while species of* Vorticella *vary in size from 30 to 150 µm.*

Figure 11 : The protozoa Epistylist and Vorticella

Although activated sludge processes are used for nitrification, these processes are not ideal for nitrification. Due to the large population size and growth of organotrophs in the aeration tank as compared to the small population size and slow growth of nitrifying bacteria, the population size of nitrifying bacteria is gradually diluted, making it difficult to achieve and maintain desired nitrification. Approximately 90% to 97% of the bacteria in the activated sludge process consist of organotrophs, while the remaining 3% to 10% of the bacteria are nitrifiers.

2.7 DENITRIFICATION

The term "denitrification" was first used in France in1886 to describe the use of nitrate ions by some bacteria to degrade substrate. The bacterial use of nitrate ions (and nitrite ions) to degrade substrate actually evolved before the use of free molecular oxygen.

Wastewater denitrification describes the use of nitrite ions or nitrate ions by facultative anaerobes (denitrifying bacteria) to degrade cBOD. Although denitrification often is combined with aerobic nitrification to remove various forms of nitrogenous compounds from wastewater, denitrification occurs whenever an **anoxic** condition exists. Therefore denitrification can promote favorable operational conditions or can contribute to operational problems.

Anoxic : means "without oxygen". Microorganism use the fixed oxygen in nitrate compounds as source of energy. The process produces more organism and remove nitrogen from wastewater by converting it to nitrogen gas that is released into the air.

Facultative anaerobes make up approximately 80% of the bacteria within an activated sludge process. These organisms have the enzymatic ability to use free molecular oxygen, nitrite ions, or nitrate ions to degrade cBOD. Facultative anaerobes prefer and use free molecular oxygen when it is available. The use of free molecular oxygen provides the bacteria with more energy for cellular activity, growth , and reproduction than does the use nitrite ions or nitrate ions.

Bacterial degradation of cBOD is "respiration". Respiration may be aerobic (oxic) or anaerobic. Aerobic respiration occurs when free molecular oxygen is available and is used to degrade cBOD, such as glucose ($C_6H_{12}O_6$)

$$C_6H_{12}O_6 + O_2 \rightarrow 6CO_2 + 6H_2O$$

Anaerobic respiration occurs when free molecular oxygen is not available and another molecule is used to degrade cBOD. Molecules other than free molecular oxygen that can be used to degrade cBOD include nitrite ions and nitrate ions. The molecule used for the degradation of cBOD is dependent on its availability, the presence of other molecules, and the enzymatic ability of the bacterial population. If nitrite ions or nitrate ions are used to degrade cBOD, such as five carbon sugar, this form of respiration is temed "anoxic"

$$C_5H_{10}O_5 + 4NO_3^- \rightarrow 5CO_2 + 7H_2O + 2N_2$$

During anoxic respiration, nitrite ions and nitrate ions are reduced (oxygen removed from the ions) through several biochemical steps or reactions. The principle gaseous end product of the biochemical reactions is molecular nitrogen.

Anoxic respiration or denitrification is termed "dissimilatory" nitrite or nitrate reduction, because nitrite ions and nitrate ions, respectively, are reduced to from molecular nitrogen. The nitrogen in the nitrate ions is loss to the atmosphere as a gas.

Nitrification does not remove nitrogen from wastewater, it simply transforms it from ammonium ions to nitrate ions. Denitrification removes nitrogen from wastewater by converting it to insoluble gases that escape to the atmosphere. Besides molecular nitrogen, nitrous oxide (N_2O) is produced during denitrification from nitrite ions and nitrate ions. This nitrogen-containing gas is insoluble in wastewater and escapes to the atmosphere.

When nitrite ions and nitrate ions are reduced to ammonium ions inside the bacteria cell, the nitrogen in the ammonium ions is incorporated into cellular material. This reduction of nitrogen is termed "assimilatory" nitrite or nitrate reduction .

Assimilatory nitrite reduction and assimilatory nitrate reduction do not remove nitrogen from wastewater.

2.8 COD

In environmental chemistry, the chemical oxygen demand (COD) test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water (e.g. lakes and rivers), making COD a useful measure of water quality. It is expressed in milligrams per liter (mg/L), which indicates the mass of oxygen consumed per liter of solution. Older references may express the units as parts per million (ppm).

Using potassium dichromate

Potassium dichromate is a strong oxidizing agent under acidic conditions. (Acidity is usually achieved by the addition of sulfuric acid.) The reaction of potassium dichromate with organic compounds is given by:

$$C_nH_aO_bN_c + dCr_2O_7^{2-} + (8d + c)H^+ \rightarrow nCO_2 + \frac{a + 8d - 3c}{2}H_2O + cNH_4^+ + 2dCr^{3+}$$

where $d = 2n/3 + a/6 - b/3 - c/2$. Most commonly, a 0.25 N solution of potassium dichromate is used for COD determination, although for samples with COD below 50 mg/L, a lower concentration of potassium dichromate is preferred.

In the process of oxidizing the organic substances found in the water sample, potassium dichromate is reduced (since in all redox reactions, one reagent is oxidized and the other is reduced), forming Cr^{3+}. The amount of Cr^{3+} is determined after oxidization is complete, and is used as an indirect measure of the organic contents of the water sample.

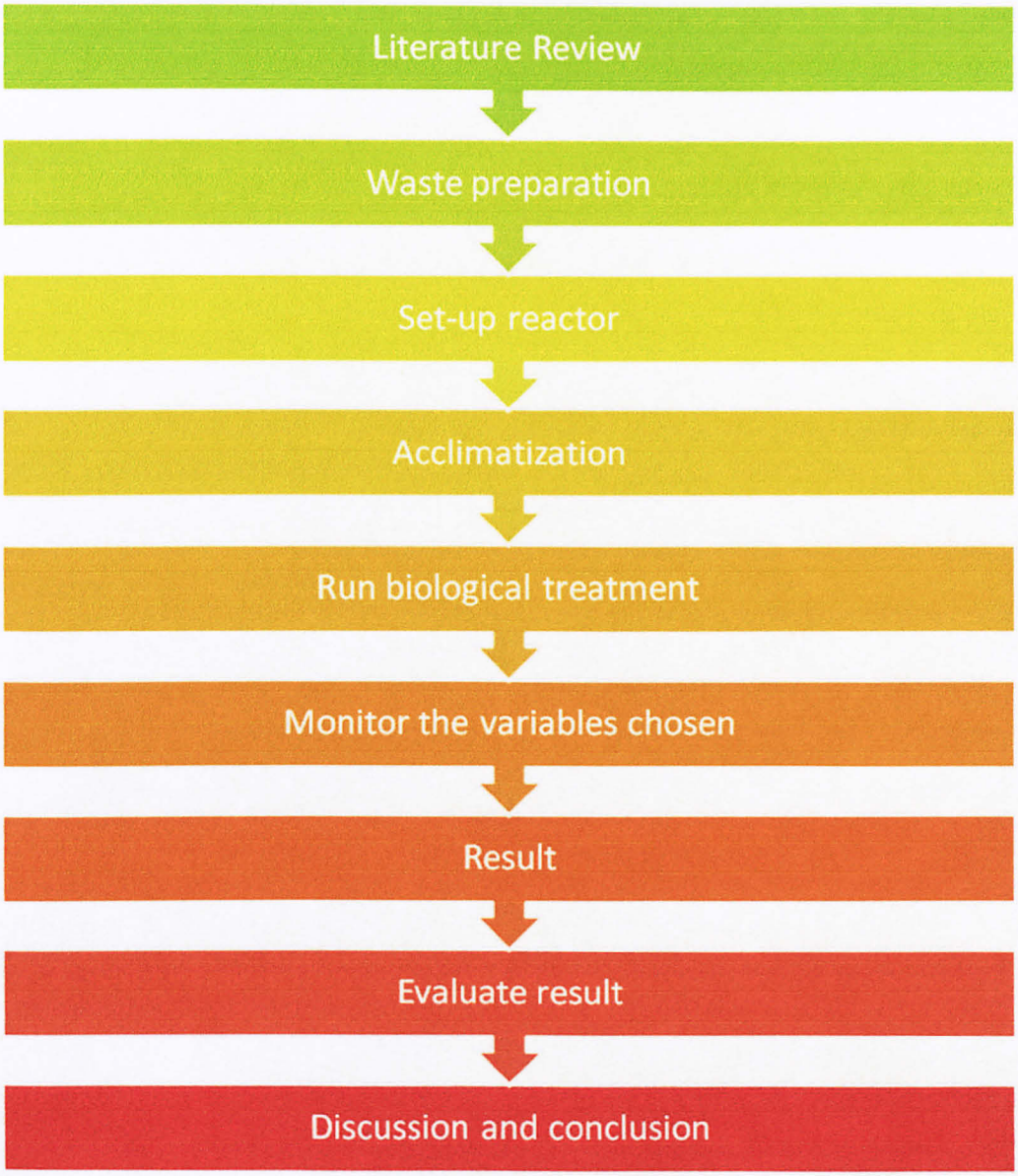
Blanks

Because COD measures the oxygen demand of organic compounds in a sample of water, it is important that no outside organic material be accidentally added to the sample to be measured. To control for this, a so-called blank sample is required in the determination of COD (and BOD, for that matter). A blank sample is created by adding all reagents (e.g.

acid and oxidizing agent) to a volume of distilled water. COD is measured for both the water and blank samples, and the two are compared. The oxygen demand in the blank sample is subtracted from the COD for the original sample to ensure a true measurement of organic matter.

CHAPTER 3

METHODOLOGY



3.1 Literature Review

This is the steps to gain the information on the related theory involved for this project. Basically, the research is narrow down on biological treatment where nitrification and denitrification takes place in order to reduce COD at the end of project

3.2 Waste preparation

Fenton's reagent reaction

1. Experiment were conducted in the batch mode in a 1 L jacketed glass reactor with provisions for sampling, temperature and pH probes.
2. The reactor was placed on a magnetic stirrer
3. Water was passed through the jacket during the reaction in order to maintain the solution temperature at 30°C
4. 500 ml of Sulfinol-D solution prepared to the required concentration and charged into the reactor
5. The pH solution was corrected before mixing in a weighted amount of $FeSO_4.7H_20$ crystals.
6. Then calculated amount of 30% H_2O_2 was added slowly in order to avoid excessive foaming
7. Sample were taken periodically throughout the experiment to determined COD. COD analysis were done using HACH 8000 using DR5000 spectrophotometer.

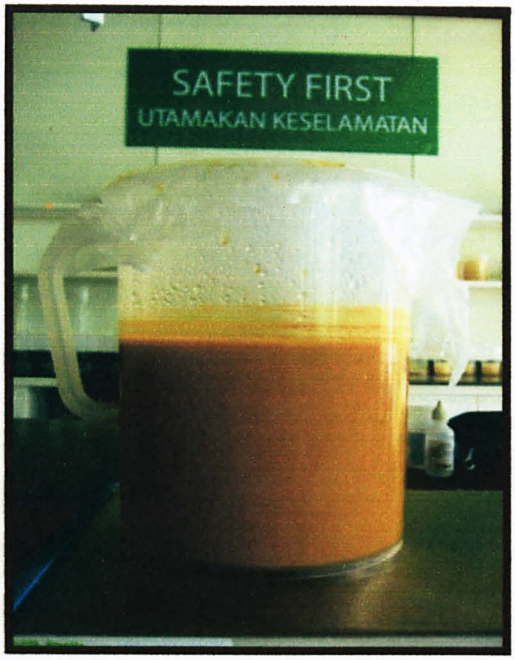


Figure 12 : Fenton pre-treated amine waste

3.3 Set-up Reactor

For this experiment, two set reactor had been set-up in order to run the biological treatment for Fenton pre-treated amine waste. One reactor is set up for aerobic condition where excess air will be provided into the reactor for microorganism to live and to favor nitrification process. Another reactor is set-up for anoxic condition where air is provided less just to make the microorganism use the fixed oxygen in nitrate compounds as source of energy. For anoxic condition excess air is not needed since denitrification are not favorable with the excess air.

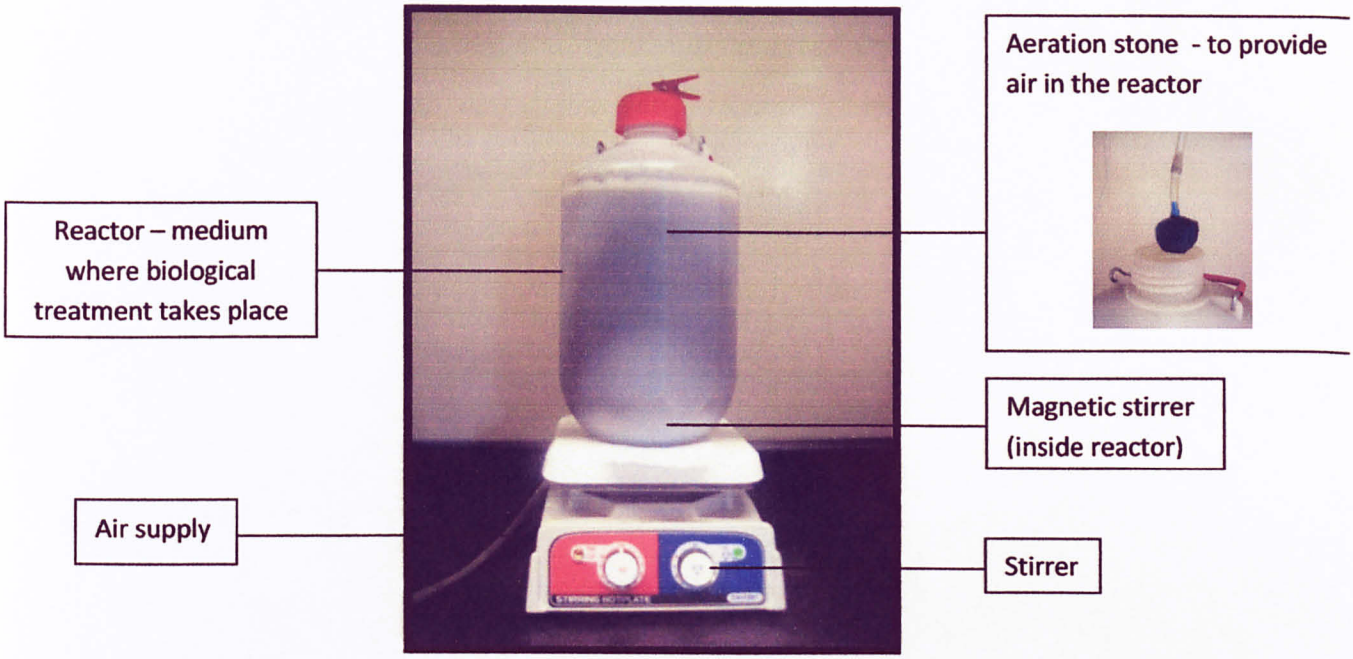


Figure 13 : Reactor for Acclimatization and biological treatment process

3.4 Acclimatization

- The constituents in ecosystem are in a dynamic steady state. This means when there is a change in the feed, the bacteria that have the capability to secrete the appropriate enzyme for ingestion will be more dominant. They will grow and multiply. This is known as adaptation or acclimation.
- Acclimatization or acclimation is the process of an organism adjusting to change in its environment, allowing it to survive changes in temperature, water and food availability, other stresses and often relates to seasonal weather changes. Acclimatization occurs in a short time, (days to weeks) and within one organism's lifetime (compare adaptation). This may be a discrete occurrence or may instead represent part of a periodic cycle. In this case, activated sludge from UTP domestic wastewater were taken and had been acclimatized first before proceed to

treat Fenton pre-treated amine waste. To acclimatized the microorganism in the activated sludge, ammonia solution had been added to the reactor for microorganism to adjusting themselves with new waste (Fenton pre-treated amine waste) which is contains high concentration of nitrogenous matter.

3.5 Biological treatment

After acclimatization had been done for almost two weeks, depends of the healthiness and population of microorganism, the microorganism will be ready to do the biological process Fenton pre-treated amine waste.

1. The domestic wastewater will be pumped out from reactor in order to replace the wastewater to Fenton pre-treated amine waste for biological treatment takes place. Before that, the reactor need to be settled first, to settled/separate activated sludge at the bottom of the reactor and the treated water in the first layer. The treated water need to be suck out from the reactor and replace with the prepared waste.

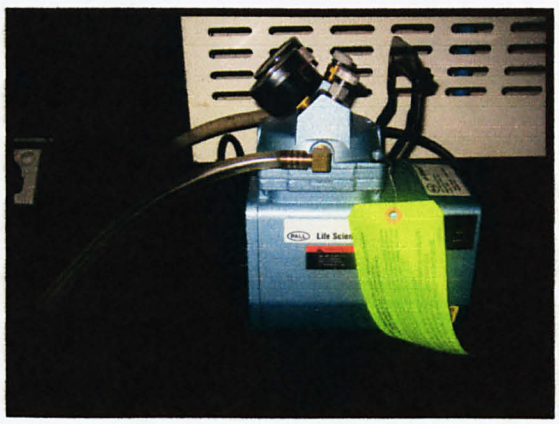


Figure 14 : Pump to suck out domestic wastewater to replace with Fenton pre-treated amine waste

2. After Fenton pre-treated amine waste had been put inside the reactor, the biological treatment need to be run according to the parameter that had been set up.

3.6 Other equipment used in the experiment

DR5000 Spectrophotometer

This project has been using DR5000 spectrophotometer which involves the spectroscopy of photons in the UV-visible region. The spectrophotometer is a complex instrument used in measuring the absorbance of bio-molecules within the ultraviolet and visible light spectrum, similar to the one found in the laboratory. It is a conglomerate of light sources, wavelength selectors, optical systems, sample chambers, photo detectors, and meters functioning together to perform a specific task – to measure the COD contains of a sample.

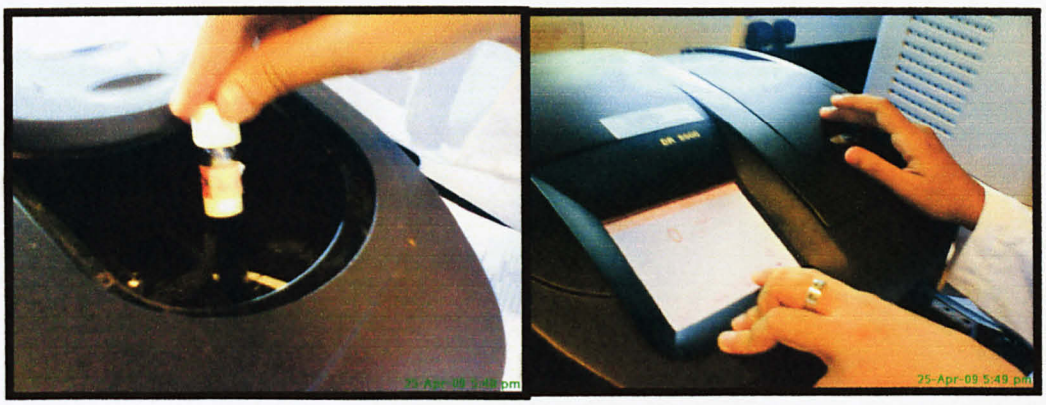


Figure 15: DR5000 spectrophotometer

HACH 8000 COD vials

The HACH 8000 COD vials are used as the medium to read COD contain in waste water sample. The mg/L COD results are defined as the mg of O_2 consumed per liter of sample under conditions of this procedure. In this procedure, the sample is heated for two hours with a strong oxidizing agent, potassium dichromate. Oxidizable organic compounds react, reducing the dichromate ion (Cr_2O_{72}–) to green chromic ion (Cr^{3+}). When the 3–150 mg/L colorimetric method is used, the amount of Cr6+ remaining is determined. When the 20–1500 mg/L colorimetric method is used, the amount of Cr^{3+} produced is determined. The COD reagent also contains silver and mercury ions. Silver is a catalyst, and mercury is used to complex chloride interferences. Test results for the 3 to 150 mg/L range are measured at 420 nm. Test results for the 20 to 1,500 mg/L COD range are measured at 620 nm.

Figure 16: COD sample in HACH 8000

pH Meter

pH meter is an electronic instrument used to measure the pH which means the level of acidity and alkalinity of a liquid. For this project, pH became one of the elements that need to be considered as the result. A typical pH meter consists of a special measuring probe like a glass electrode that is connected to an electronic meter. The

electronic meter will measure and display the pH reading for that solution. While taking the measurement, pH meter should be calibrated before and after each of the reading since the glass electrode does not give a reproducible e.m.f. over longer periods of time.

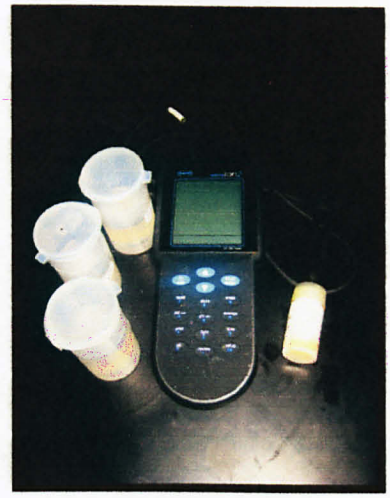


Figure 17 : pH Meter

Ion Chromatography

A form of liquid chromatography where retention is predominantly controlled by ionic interactions between the ions of the solute and *counter ions* that are situated in, or on, the stationary phase. For example, to separate organic acids, it is the negatively charges acid ions that need to be selectively retained. It follows that the stationary phase must contain immobilized positively charged cations as counter ions to interact with the acid ions to retain them. Conversely, to separate cations, the stationary phase must contain immobilized anions as counter ions with which the cations can interact. Ion exchange stationary phases are available in mainly two forms. One form (probably the most popular) consists of cross-linked polystyrene polymer beads of an appropriate size which has been suitably treated to link ionic groups to the surface. The other form is obtained by chemically bonding ionic groups to silica gel by a process similar to that used to produce bonded phases. These materials are called ion exchange media, a term which has given rise to the term ion *exchange chromatography* as an alternative to ion chromatography.

Ionic substances can also be adsorbed on the surface of a reverse phase media and act as an *adsorbed* ion exchanger. The mobile phase is made to contain a small percentage of a soluble organic ionic material (e.g. tetrabutyl ammonium dihydrogen phosphate or n-octyl sulphonate). These substances are adsorbed onto the surface by dispersive interactions between the alkyl groups of the agent and those of the bonded phase and act as counterions. In general ion chromatography is one of the more difficult types of liquid chromatography to exlpoit and is most often used for analysis of anions for which there are no other rapid analytical methods. [2]

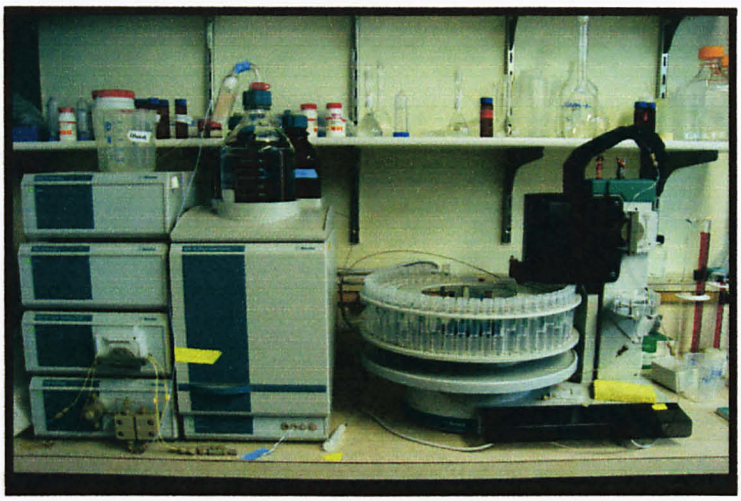


Figure 18 : Ion chromatography equipment set

Technique :

A sample is introduced, either manually or with an autosampler, into a sample loop of known volume. A buffered aqueous solution known as the mobile phase carries the sample from the loop onto a column that contains some form of stationary phase material. This is typically a resin or gel matrix consisting of agarose or cellulose beads with covalently bonded charged functional groups. The target analytes (anions or cations) are retained on the stationary phase but can be eluted by increasing the concentration of a similarly charged species that will displace the analyte ions from the stationary phase. For example, in cation exchange chromatography, the positively charged analyte could be displaced by the addition of positively charged sodium ions. The analytes of interest must

then be detected by some means, typically by conductivity or UV/Visible light absorbance.

In order to control an IC system, a chromatography data system (CDS) is usually needed. In addition to IC systems, some of these CDSs can also control gas chromatography (GC) and HPL.

CHAPTER 4

RESULT AND DISCUSSION

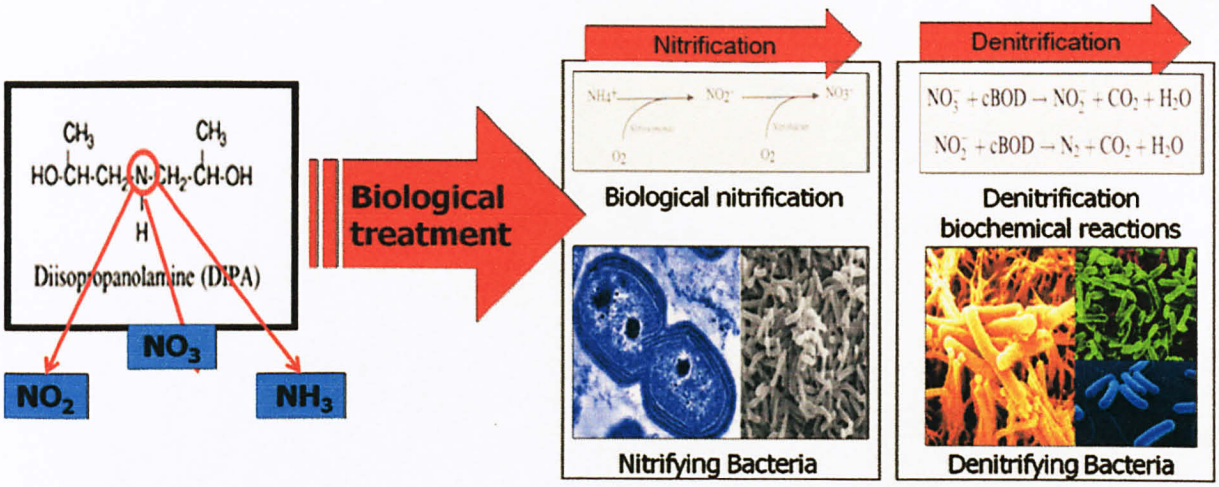
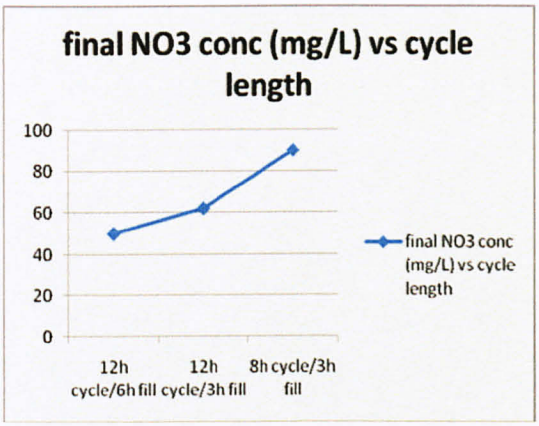


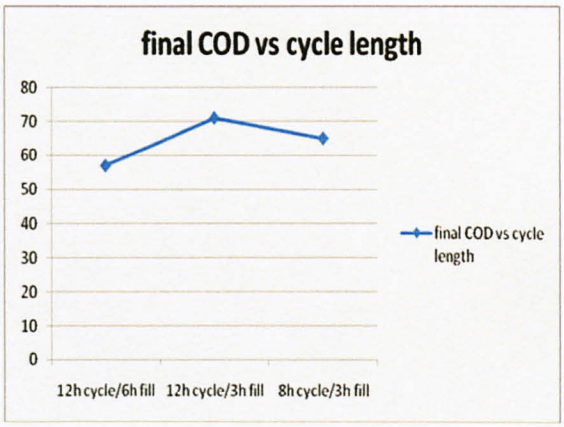
Table 3 : operating parameters and experimental conditions for batch reactor

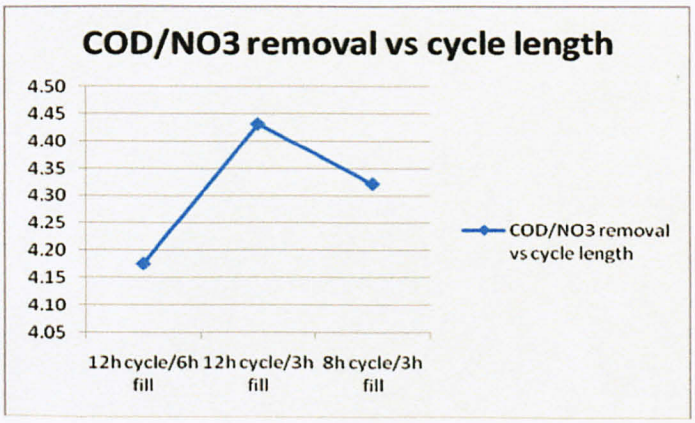
cycle length (h)	12	8	6
total reactor volume (L)	5	5	5
liquid volume/cycle (L)	2	2	2
operating sequence fill	6	3	2
react/recirculation	4	3	2
settling time	2	2	2

Table 4 : results during experiment

Cycle length		Initial	Final
12h cycle/6h fill	COD	960	41
	NO_3	265	45
	NO_2		ND
12h cycle/3h fill	COD	960	56
	NO_3	265	61
	NO_2		1
8h cycle/3h fill	COD	960	117
	NO_3	265	70
	NO_2		6







Based on the result obtained, COD reduction had been achieved for every cycle length where for 12h cycle/6h fill can reduced up to 919 mg/L, 12h cycle/3h fill can reduced up to 904 mg/L and 8h cycle/3h fill can reduced up to 843 mg/L. Same goes to reduction of NO_3 compound where for 12h cycle/6h fill can reduced up to 220 mg/L, 12h cycle/3h fill can reduced up to 204 mg/L and 8h cycle/3h fill can reduced up to 195 mg/L. According to graph obtained for observed COD/NO_3 removal for 12h cycle/3h is the highest removal among three cycle length.

After had been put under each of cycle length, for reactor one, nitrification is success to occur because of :

Nitrifying bacteria

Nitrifying bacteria live in large variety of habitats including fresh water, potable water, wastewater, marine water, brackish water, and soil. Nitrifying bacteria are known by many names that are derived from the carbon and energy substrates.
Although some genera of nitrifying bacteria are capable of using some organic compounds to obtain carbon, the principal genera of nitrifying bacteria in the activated sludge process, *Nitrosomonas* and *Nitrobacter,* use carbon dioxide or inorganic carbon as their carbon source for the synthesis of cellular material. For each molecule of carbon dioxide assimilated into cellular material by nitrifying bacteria, approximately 30 molecules of ammonium ions or 100 molecules of nitrite ions must be oxidized.

Due to the relatively large quantity of ammonium ions and nitrite ions needed to assimilate carbon dioxide, nitrifying bacteria have a very low reproductive rate. Even under the best conditions the reproductive rate of nitrifying bacteria are minimal. In the activated sludge process, nitrifying bacteria are able to increase in number only if their reproductive rate is grater than their removal rate through sludge wasting and discharge in the final fluent. Therefore a high MCRT is required to increase the number of nitrifying bacteria in the activated sludge process.

For the growth of one pound dry cells, *Nitrosomonas* must oxidize 30 pounds of ammonium ions, while *Nitrobacter* must oxidize 100 pounds of nitrite ions. In contrast, for the growth of one pound of dry cells, the organotrophic bacterium *Escherichia Coli* must oxidize only two pounds glucose.

Nitrifying bacteria obtain their energy by oxidizing inorganic substrates, namely ammonium ions and nitrite ions. Nitrite ions that are the product of the oxidation of ammonium ions by *Nitrosomonas* serve as the substrate for *Nitrobacter* to have an energy substrate.

Nitrifying bacteria belong in the family Nitrobacteracae. With some exceptions, bacteria in this family obtain carbon by assimilating carbon dioxide to a 5-carbon sugar, ribulose diphosphate, to produce a 6-carbon sugar, glucose. The assimilation of carbon dioxide results in the production of cellular material. When carbon dioxide is reduced by the addition of hydrogen.

There are two energy-yielding, biochemical reactions that occur during nitrification. More energy is derived from the first biochemical reaction, that is, the oxidationof ammonium ions, than the second biochemical reaction, that is, the oxidation of nitrite ions.

$$NH_4^+ + 1.5O_2 \text{ - } \textit{Nitrosomonas} \rightarrow NO_2^- + 2H^+ + H_2O + \text{energy}$$

$$NO_2^- + 0.5\ O_2 \text{ - } \textit{Nitrobacter} \rightarrow NO_3^- + \text{energy}$$

The energy yielding reactions occur inside the bacterial cells, and both reactions involve the use of free molecular oxygen. Since an accumulation of nitrite ions usually does not occur, the overall reaction for nitrification is a combination of the two energy-yielding reactions

$$NH_4^+ + 2O_2 \text{ - Nitrifying bacteria} \rightarrow NO_3^- + 2H^+ + H_2O$$

There are several intermediate compounds such as hydroxylamine (NH_2OH) that are produced during nitrification. However, these compounds are short-lived and therefore are not presented in equations that describe the energy-yielding reaction of nitrification.

Although ammonium ions are used as an energy source by nitrifying bacteria, not all of the ammonium ions taken inside the bacterial cells are nitrified. Some of the ammonium ions are used as a nutrient source for nitrogen and are assimilated into new cell material ($C_5H_7O_2N$). the growth of new cells in the activated sludge process is referred to as an increase in the mixed liquor volatile suspended solids (MLVSS)

$$4CO_2 + HCO_3^- + NH_4^+ + 4\,H_2O \rightarrow C_5H_7O_2N + 5O_2 + 3H_2O$$

Carbon dioxide serves as the carbon source for the synthesis of cellular material and is made available to nitrifying bacteria as bicarbonate alkalinity. This alkalinity is produced when carbon dioxide dissolves in wastewater.

In the second reactor, denitrification also success to occur, which were helped by these microorganism :

Denitrifying bacteria

Although several groups of organisms are capable of denitrification, including fungi and the protozoa *Loxodes*, most denitrifying organisms consist of facultative anaerobic bacteria. The bacteria degrade cBOD using nitrite ions and nitrate ions in the absence of free molecular oxygen. The bacteria degrade cBOD in order to obtain energy for cellular activity and carbon for cellular synthesis (growth and reproduction)

A relatively large number of genera of facultative anaerobes are capable of denitrification. Most denitrifiers reduce nitrate ions to molecular nitrogen without accumulation of intermediates. However, some denitrifiers lack key enzyme systems does permit the production and accumulation of intermediates

Although there are genera numerous genera of denitrifying bacteria, all denitrifying genera do not contain large numbers of species, and all denitrifying bacteria do not respire similarly. The genera *Alcaligenes, Bacillus,* and *Pseudmonas* contain the largest number of denitrifying bacteria.

Many genera of denitrifying bacteria can use nitrite ions to degrade cBOD, some genera such as *Enterobacter* and *Escherichia* can use only nitrate ions. Other genera such as *Alcaligenes* can use only nitrite ions. The use of nitrate ions in this manner is known as nitrate respiration, while the use of nitrite ions is known as nitrite respiration. The reduction of nitrate ions to only nitrite ions during denitrification may result in an accumulation of nitrite ions. This form of respiration in a secondary clarifier may result in the production of the "chlorine sponge".

The enzymatic machinery needed for denitrification is formed only under anoxic condition or the presence of a low oxygen concentration. However, the production of the enzymatic machinery for denitrification is accomplished quickly.

For biochemical pathway and respiration, it refers to the sequential steps of chemical reactions occurring inside the bacterial cells as nitrite ions are reduced to molecular nitrogen during cBOD degradation. The overall degradation of cBOD using nitrate ions can be expressed in two, simplistic biochemical reactions

$$NO_3^- + cBOD \rightarrow NO_2^- + CO_2 + H_2O$$

$$NO_2^- + cBOD \rightarrow N_2 + CO_2 + H_2O$$

The biochemical pathway for denitrification involves a stepwise conversion of nitrate ions to molecular nitrogen. These steps are the conversion of nitrate ions to nitrite ions, the conversion of nitrite ions to nitric oxide (NO), the coversion of nitric oxide to nitrous oxide (N_2O), and the conversion of nitrous oxide to molecular nitrogen.

COD and nitrate stoichiometry

Theoretically, reduction of 1g NO_3^-N to nitrogen requires 2.86g COD. The observed ratios during denitrification are, however, higher as organic carbon is also utilized for the cell growth. Widely varying ratios, ranging from 3.45 to 5.34 have been reported in literature . Organic carbon source used in the studies was methanol. Fang and Zhou have reported a ratio of 3.34 for complete denitrification using sucrose, phenol and mcresol as substrates in up flow granular sludge blanket reactor.

CHAPTER 5
CONCLUSIONS AND RECCOMENDATION

Long duration time is needed in order to run this experiment since I am having difficulties to acclimatize the microorganism. I need to run a lot of batch since the microorganism is easily dead in the middle of the acclimatization.
Acclimatization need to be run properly in order to get health microorganism and to culture wanted microorganism such as nitrifying bacteria and denitrifying bacteria.

- COD, NO_3 and NO_2 level in Fenton pre-treated amine waste can be treated and reduced using biological treatment (Nitrification and denitrification process) in order to remove various forms of nitrogenous compounds from wastewater.
- Effectiveness of COD, NO_3 and NO_2 can be determined by varying cycle length of treatment.

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