Removal of ammonia in the sulfide-rich wastewater using Conventional Activated Sludge process.

by

Suhaila binti Abdul Halim

ID: 8469

Dissertation submitted in partial fulfillment of the requirements for the

Bachelor of Engineering (Hons)

(Chemical Engineering)

JANUARY 2009

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CERTIFICATION OF APPROVAL

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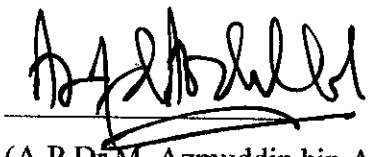

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ABSTRACT

The objective of this research is to study the performance of conventional activated sludge in removing ammonia for the treatment of high sulfide wastewater. The variables in this project are Sludge Retention Time (SRT) and various concentration of sulfide in the wastewater. The scope of this project is to study the effect of SRT on activated sludge process in removing ammonia under different sulfide concentration. The approach involves using waste water from Sewage Treatment Plant (STP) in front of Village 2, University Technology of Petronas. The MLSS of primary sludge had a range of 2500 mg/L to 4500 mg/L. Five reactors was used with each SRT was varied at 10, 20, 30, 40 and 50 days. The volume of each reactor was measured as 18 L each. Waste water was filled into every reactor and an approximate of 90 L of wastewater was fed into each reactor everyday in a 24 hours continuous operation. The feed flow rate was set at 6.5 ml/min. 5 containers was placed under the reactors to feed the effluent, which was used for sampling later. Each reactor had varied sludge age with different amount of sludge was wasted everyday. Gradually, synthetic hydrogen sulfide was added in the influent and the concentration was increased from 200 mg/L to 300 mg/L and finally 900 mg/L. Present results showed that activated sludge system can be used to nitrify waste water containing high amount of ammonia concentration at SRT of 10 days. In term9 of biomass growth, SRT of 10 days shows the highest biomass growth obtained, and thus proving to be the most optimum SRT in the removal of ammonia in sulphide-rich waste water. More over, the increase of sulphide concentration in the system did not affect the efficiency of each reactor since all the microorganisms had already adapted well with the sulphide-rich environment.

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Biological processes are used to convert the finely divided and dissolved organic matter in wastewater into flocculants settleable biological and inorganic solids that can be removed in sedimentation tanks. In many cases, these processes which also called 'secondary processes' are employed in conjunction with the physical and chemical processes that are used for the preliminary and primary treatment of wastewater.

The most commonly used biological processes are:

1) Activated sludge process

   Liquid wastewater is aerated to allow micro organisms to utilize organic polluting matter with 95% reduction. The microbial biomass and treated effluent are separated by sedimentation with a portion of the biomass (sludge) returned to the aeration tank to seed the incoming wastewater.

2) Biological filtration

   Wastewater is distributed over a bed of inert medium on which micro-organisms develop and utilize the organic matter present. Aeration occurs through natural ventilation and the solids are not returned to the filter.

3) Stabilization ponds

   These are large lagoons where wastewater is stored for long periods to allow a wide range of micro organisms to break down organic matter. Many different types and designs of ponds are available including aerated, non-aerated, and anaerobic ponds. Some designs rely on algae to provide oxygen for bacterial breakdown of organic matter.

4) Anaerobic digestion

This process is used for high strength organic effluents such as in pharmaceutical, food and drink industries. Wastewater is stored in sealed tank which excludes oxygen. Anaerobic bacteria will breakdown organic matter into methane, carbon dioxide and organic acids. But, its final effluent contains high BOD and still requires further treatment.

This study is aimed at exploring the first process, which is activated sludge process to remove ammonia in high sulfide wastewater by varying the solid retention time (SRT).

1.2 Problem Statement

Nitrogen is the principal nutrients of concern in treated wastewater discharges. Discharges containing nitrogen may accelerate the eutrophication of lakes and reservoirs. It also may stimulate the growth of algae and rooted aquatic plants in shallow streams. (Tchobonoglous, 1991). Total nitrogen is comprised of organic nitrogen, ammonia, nitrite and nitrate. Ammonia is toxic to fish, and nitrates at high enough dosages in the drinking water cause methemoglobinemia in infants. When such effluents are discharged into the environment, depletion of receiving-water oxygen resources can occur as the ammonia is oxidised to nitrate (Campos, 2001) Therefore, the removal of these excess nutrients is crucial. This research will evaluate the efficiency of activated sludge process in removing excess nutrients mainly ammonia and the effect of varying SRT on its performance.

1.3 Objectives

The main objective of this project is to study the performance of conventional activated sludge in removing ammonia for the treatment of high sulfide wastewater. The variables in this project are Sludge Retention Time (SRT) and various concentration of sulfide in the wastewater. In order to achieve the main objective, the following sub-objectives were formed:

- To study the effect of Solid Retention Time (SRT) on the activated sludge performance. The SRT in this project was varied from 10 days, 20 day, 30 days, 40 days and 50 days. It is controlled by altering the sludge wastage rate.
- To study the effect of different concentration of sulfide on the activated sludge performance. The concentration in this project was varied from 200 mg/L, 300 mg/L and 900 mg/L. Synthetic hydrogen sulfide was added into the influent and the effect on ammonia removal was studied.
- To maintain optimum conditions for the growth of microorganism in the sludge. The parameters that was monitored carefully were pH, Total Carbon: Nitrogen: Phosphorus (C: N: P) ratio, and temperature.
- To study the behavior of Mixed Liquor Suspended Solid (MLSS), Mixed Liquor Volatile Suspended Solid (MLVSS), Sludge Volume Index (SVI), and Chemical Oxygen Demand (COD) under various sampling days in different SRT.

1.4 Scope of Study

The scope of the research is to study the performance of conventional activated sludge in removing ammonia. Although there are two types of nutrients that are essential to be removed in wastewater effluent which are nitrogen and phosphorus, this project only chose the removal of nitrogen as the scope of study. The concern of the research is narrowed to the removal of nitrogen-ammonia in wastewater. The two variables in this project are Sludge Retention Time (SRT) and the concentration of synthetic hydrogen sulfide added into the influent. The scope of this project is to study the effect of SRT on activated sludge process in removing ammonia under different sulfide concentration.

The scope for the Final Year Project 1 (FYP1) is to study and familiarize with the operation of conventional activated sludge process. The scope for the Final Year Project 2 (FYP2) is specifically allocated for experimental and laboratories work to get the results and findings. Continuous research and literature review was also conducted throughout both semesters for references purposes. Besides that, project work from FYP1 was further continued to meet all the objectives lined up for the project.

CHAPTER 2

THEORY AND LITERATURE REVIEW

2.1 Theory

2.1.1 Conventional Activated Sludge Process

The activated sludge process is the most widely used biological wastewater treatment process today, treating both domestic and industrial wastewater. This process was developed in England in 1914 by Arden and Lockett (Gray, 2004) and was so named because it involved the production of an activated mass of microorganisms capable of stabilizing a waste aerobically. The activated sludge process relies on a dense microbial population being mixed with the wastewater under aerobic conditions. With unlimited food and oxygen, extremely high rates of microbial growth can be achieved, resulting in the utilization of the organic matter present either as oxidized end-products (CO_2, NO_3, SO_4, PO_4) or new micro-organisms (Gray, 2004).

The basic process description of an activated sludge system consists of organic waste which is introduced into a reactor where aerobic bacterial culture is maintained in suspension. The reactor contents are referred to as the "mixed liquor". In the reactor, the bacterial culture carries out conversion in general accordance to the following stoichiometry:

$$\text{Organic\_matter} + O_2 + \text{nutrients} \xrightarrow{\text{bacteria}} CO_2 + NH_3 + \text{new\_bacterial\_cells} + \text{other\_end\_products}$$

The ultimate aerobic environment in the reactor is achieved by the use of diffused or mechanical aeration, which also serves to maintain the mixed liquor in a completely mixed regime. After a specified time, the mixture of new cells and old cells is passed into a settling tank, where the cells are separated from the treated waste water. A portion of

the settled cells is recycled to maintain the desired concentration of organisms in reactor, and a portion is waste.

The activated sludge process consists of two phases, aeration and sludge settlement. The main components of most activated sludge system are:

1) Reactor:

   The main criterion of a reactor is that the contents can be adequately mixed and aerated.

2) Activated sludge:

   This is the microbial biomass within the reactor which is comprised mainly of bacteria and other microfauna and flora. The sludge is a flocculant suspension of these microorganisms and often referred to as the mixed liquor. The normal concentration of mixed liquor expressed as suspended solid is between 2000 – 5000 mg/L (Gray, 2004)

3) Aeration / mixing system:

   Aeration and mixing of the activated sludge and incoming wastewater are essential. It has a dual function. The first function is to supply oxygen to the aerobic microorganisms in the reactor for respiration. The second function is to maintain the microbial flocs in a continuous state of agitated suspension, which ensures maximum contact between the surface of the floc and the wastewater. The aeration are normally done using a single system, either surface aeration or diffused air is used.

4) Sedimentation tank:

   Final settlement of the activated sludge displaced from the aeration tank by the incoming wastewater is required. This separates the microbial biomass from the treated effluent.

5) Returned sludge:

   The settled activated sludge in the sedimentation tank is recycled back to the reactor to maintain the microbial population at a required concentration in order to ensure continuation of treatment.

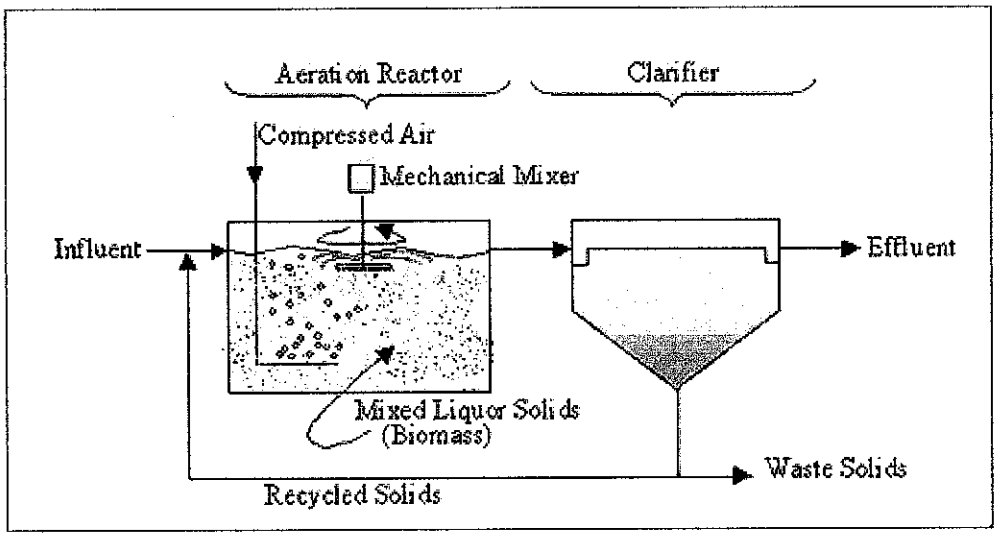


Figure 2.1: Main components of conventional activated sludge

Source: www.iem.bham.ac.uk/images/

2.1.2 Nutrients in wastewater

Domestic sewage is made up largely by organic carbon, either in solution or as particulate matter. About 60% is in particulate form, and of this, slightly under a half is large enough to settle out of suspension (Gallarneau, 1997). These particles are absorbed on to the flocs of the activated sludge during treatment. The bulk of organic matter is easily biodegradable, consisting of proteins, amino acids, carbohydrates and fatty acids. The average carbon to nitrogen to phosphorus ratio (C:N:P) is stated to be 100:5:1 (Suman Raj, 2003) which is the ideal condition for bacterial growth.

Trace component such as S, Na, Ca, Mg, K and Fe are also required and are available in abundance in domestic sewage. Nutrients therefore needed to be added to the mixed liquor to obtain maximum bacterial growth and to optimize carbonaceous treatment. Lack or insufficiency of a critical nutrient may result in incomplete treatment, because the bacteria are unable to grow optimally (Davies, 2005)

However, excess in nutrients is also undesirable since they can contribute to eutrophication, the gradual change of water bodies into marshes, meadows, and forests. It can also contribute to massive algae blooms leading to oxygen depletion in water and its

associated problems. Certain forms of nitrogen can cause specific problems too. Ammonia is toxic to fish, and nitrates at high enough dosages in the drinking water cause methemoglobinemia in infants. Nitrates are converted to nitrites in the stomach and interfere with the oxygen-carrying capacity of the hemoglobin in blood (Reis, 2008)

In the field of wastewater treatment, there are concerns regarding several forms of nitrogen: ammonia, organic, nitrate, and nitrite. Under the right conditions, each of these is biologically convertible to one of the other forms. This creates certain challenges in the treatment of nitrogen in wastewater. Because of these challenges, it is important to properly collect, preserve, and analyzes samples for the specific forms of nitrogen so that the appropriate treatment of these wastes can be made.

2.1.3 Nitrification process

The process in which the nitrogen in the untreated waste water is substantially converted to nitrate is known as 'biological nitrification' (Chen, 2008). It is an autotrophic process, where the energy for bacterial growth is derived from oxidation of nitrogen compounds, primarily ammonia.

Nitrification of ammonia is a two step process involving two genera of microorganisms, Nitrosomonas and Nitrobacter. In the first step, ammonia is converted into nitrite and then the nitrite is converted into nitrate in the second step. The conversion process is as follows:

First step:

$$NH_4 + \frac{3}{2}O_2 \xrightarrow{\text{Nitrosomonas}} NO_2^- + 2H^+ + H_2O$$

Second step:

$$NO_2^- + \frac{1}{2}O_2 \xrightarrow{\text{Nitrobacter}} NO_3^-$$

Nitrosomonas and Nitrobacter used the energy yield from oxidation of ammonia for cell growth and maintenance. If the dissolved oxygen is not replaced, then aerobic growth will eventually stop when the oxygen is exhausted, allowing only the slow anaerobic processes to continue.

The specific growth rate of nitrifying bacteria is affected by a number of environmental factors. In particular, the factors are solid retention times, dissolved concentrations, temperatures, a wide range of inorganic and organic compounds, pH and key nutrients (Gray, 2004).

2.1.4 Sludge residence time or sludge age

The sludge residence time (SRT) affects the character and condition of the activated sludge flocs within the aeration basin. It is one of the parameters that can be varied to control the process.

SRT is calculated as the total amount of sludge solids in the system divided by the rate of loss of sludge in the system. The simplified equation is (Gray, 2004):

$$ts = \frac{VX}{[(QwXw)+(QeXe)]}$$

where:

V = volume of the liquid in the aeration tank (m3)

X = MLSS (mg/L)

Qw = sludge wastage rate (m3/day)

Xw = MLSS in the waste sludge stream (mg/L)

Qe = effluent discharge rate (m3/day)

Xe = effluent suspended solid concentration (mg/L)

ts = SRT in days

SRT is an operational factor giving control over sludge activity because it is the net specific growth rate of the sludge. A low SRT (< 0.5 day) indicates sludge with high

growth rate as used in high-rate units for pretreatment. A high SRT (> 5 days) indicates sludge with low growth rate such as in extended aeration system. Conventional activated sludge has a SRT between 3 and 4 days, and has good settling properties (Tchobanoglous, 1991). The SRT is controlled by altering the sludge wastage rate. The process is controlled by daily wasting of quantity of flow equal to the volume of the aeration tank divided by the SRT.

SRT is an operational factor giving control over sludge activity because SRT is the reciprocal of the net growth rate of the sludge and thus can be considered as a measure of sludge activity. The typical design parameter for activated sludge processes is represented in table 1:

Process modification	Sludge residence time θc (day)	MLSS (mg/L)
Conventional	5-15	1500 – 3000
Complete-mix	5-15	2500 – 4000
Step-feed	5-15	2000 – 3500
Modified aeration	0.2 – 0.5	200 – 1000
Single-stage nitrification	8-20	2000 - 3500

Table 2.1: Design parameters for activated-sludge processes (Tchobanoglous, 1991)

2.2 Literature studies

There were many literatures found on the study of nutrients removal using conventional activated sludge system. But no literatures could be found about previous studies that varies sulphide concentration in influent and Sludge Residence Time (SRT) simultaneously. Most of the studies found had either varies only SRT or only sulphide concentration. However, these literatures would be able to help in explaining the results that are obtained in this project.

Campos *et al* (2001) examined the nitrification process in saline waste water with high ammonia concentration using activated sludge system. Ammonia loading rate (ALR) was varied between 1 g to 4 g. When this concentration of salt was increased more than 525 mM, the system started to accumulate ammonia and nitrification efficiency fell sharply. They also found out that high salt concentrations did not have long-term effects on the physical properties of sludge. Results obtained from activity tests showed that adapted biomass is less sensitive to high saline concentrations. They concluded that the results show that activated sludge units can be used to nitrify wastewater containing high ammonia and salt concentrations at high Ammonia Loading Rate (ALR).

The effect of chromium addition on the activated sludge process was studied by Stasinakis *et al* (2002). The concentration of Cr was varied to 0.5 mg/L, 1 mg/L, 3 mg/L and 5 mg/L. They found out that the Cr with concentrations of 0.5 mg/L caused significant inhibition of the nitrification process and up to 74% decrease in ammonia removal efficiency. On the contrary, the effect of Cr on organic substrate removal was minor for concentrations up to 5mg/L. This indicated that nitrifying bacteria are very sensitive to Cr concentration in the system.

In term of Sludge Residence Time, there had been various literatures that study the effect of SRT on the performance of activated sludge system. One of them was by Yong Li *et al* (2008) that examined the effect of SRT as a decisive factor for aerobic granulation in SBR. The varied SRT is 3 days, 6 days, 9 days, 12 days and 40 days. They observed that after 30 days, the effluent concentration become stabilized and constant biomass was obtained. The formation of aerobic granular in SBR does not change significantly in the

range of SRT studied. They concluded that SRT does not have any effect on aerobic granulation.

Another study that examined the effect of SRT on activated sludge system was by Chua *et al* (2003). They used municipal waste water and the parameters observed were pH, SRT and acetate concentration in influent. The SRT was varied at 3 days, 5 days and 10 days. The production of polyhydroxyalkanoates (PHA) under the assigned parameters was observed. SRT of 3 days gave better production of PHA rather than SRT of 10 days. The supply of acetate in the influent had increase the production of PHA rather than influent without addition of acetate.

In 2005, Halalsheh *et al* had conducted a research to study the effect of SRT and temperature on biological conversion and scum forming potential. They had developed a simple test to measure and compare the tendency of different sludge to form a scum layer. The results showed that higher protein concentration at elevated SRT and 25 °C increased the negative surface charge of sludge flocs and also reduced the ability of sludge to attach to gas bubbles and float. Floc average size increased with increasing SRT and temperature, especially for sludge with 75 d SRT at 25 °C. On the other hand, settling properties of sludge were negatively affected by increasing SRT to 75 d at 25 °C.

CHAPTER 3

METHODOLOGY

3.1 Procedure Identification

3.1.1 Calculations

a) Calculation of influent flow rate:

Influent flow rate was set to be constant in each reactor to ensure an even distribution of influent. The flow rate was controlled by two Masterflex console drivers. Before that, the desired flow rate was calculated first before being set up using the drivers:

The calculation was as follows:

$$HRT = \frac{V}{Q \times 1000}$$

Where V = volume of reactor (ml)

Q = influent flow rate (ml/min)

HRT = Hydraulic Retention Time

Hydraulic Retention Time (HRT) is the aeration period or loading rate expresses the rate at which sewage is applied in the aeration tank. HRT for this experiment was set up for 2 days (48 hours). The HRT was set up at a sufficiently long hours to allow the required degree of adsorption, flocculation, and mineralization.

The calculation was as follows:

$$2days = \frac{volume\_of\_aeration}{flow\_rate \times 1000}$$

$$Q = \frac{18000\ ml}{60\ \text{min} \times 48\ hours}$$

$$Q = 6.25\ ml\ /\ \text{min}$$

The influent flow rate was set up at 6.3 ml per minute.

b) Calculation of Mixed Liquor Suspended Solid (MLSS) and Mixed Liquor Volatile Suspended Solid (MLVSS):

MLSS and MLVSS are the terms used for the mixture of solids resulting from combining recycled sludge with influent wastewater in the bioreactor. The solids are comprised of biomass, nonbiodegradable volatile suspended solids, and inert inorganic total suspended solids. MLVSS is used to designate that portion of the MLSS that is active microbes. MLVSS concentration is only an approximate indicator of the actual in a mixture of activated sludge.

Before starting up the reactor, the sludge used is checked for the quantity of their MLSS and MLVSS. The desired value of MLVSS is at range of 3000 – 4000 mg/L. This value is crucial to maintain the biological growth of microorganism in the activated sludge treatment. The sludge is diluted with wastewater if the MLVSS value is too high or the sludge is thickened by adding up more sludge if the MLVSS value is too low.

The calculation for MLSS is as follows:

$$MLSS = \frac{(weight\_of\_filter\_paper\_at\_103°C) - (weight\_of\_filter\_paper)}{\left(\frac{Volume}{1000ml}\right)}$$

The calculation for MLVSS is as follows:

$$MLVSS = \frac{(weight\_of\_filter\_paper\_at\_550°C) - (weight\_of\_filter\_paper)}{\left(\frac{Volume}{1000ml}\right)}$$

c) Calculation of waste sludge:

The solid retention time (SRT) is the variable in this experiment. SRT is the average time the mass of biological solids remains in the system before being wasted. SRT is calculated as the total amount of sludge solids in the system divided by the rate of loss of sludge in the system. The simplified equation is (Gray, 2004)

$$ts = \frac{VX}{[(QwXw) + (QeXe)]}$$

where:

V = volume of the liquid in the aeration tank (m3)

X = MLSS (mg/L)

Qw = sludge wastage rate (m3/day)

Xw = MLSS in the waste sludge stream (mg/L)

Qe = effluent discharge rate (m3/day)

Xe = effluent suspended solid concentration (mg/L)

ts = SRT in days

The SRT is controlled by altering the sludge wastage rate. The process is controlled by daily wasting of quantity of flow equal to the volume of the aeration tank divided by the SRT. For each reactor, the SRT was varied at 10 days, 20 days, 30 days, 40 days and 50 days.

The waste sludge was calculated using the following formula (Gray, 2004)

$$Waste\_sludge = \frac{MLVSS \times Aeration\_volume}{SRT}$$

After calculation, the results were tabulated in the table below:

Reactor	1	2	3	4	5
Sludge age (days)	10	20	30	40	50
Sludge wasted (ml)	1728	864	576	432	346

Table 3.1: Amount of sludge wasted for each reactor with different sludge age.

3.1.2 Reactor Start – Up

For this research, the wastewater used was taken from Sewage Treatment Plant (STP) in front of Village 2, University Technology of Petronas. Primary sludge used was also taken from STP. The MLSS of primary sludge had a range of 2500 mg/L to 4500 mg/L. Five reactors was used with each SRT was varied at 10, 20, 30, 40 and 50 days. The volume of each reactor was measured as 18 L each. Waste water was filled into every reactor and an approximate of 90 L of wastewater was fed into each reactor everyday in a 24 hours continuous operation. The feed flow rate was set at 6.5 ml/min. Five containers were placed under the reactors to feed the effluent, which was used for sampling later. Each reactor had varied sludge age with different amount of sludge was wasted everyday. After the reactor was started up, the acclimatization of the sludge to the wastewater was carried out by means of gradual introduction. The sludge was acclimatized with 2 days of HRT in continuous reactors. Submerged aeration was given to the mixed liquor in the reactors using three air diffusers for each reactor. The feed flow rate was set using console drivers at 6-6.5 ml/min. Synthetic hydrogen sulfide was added gradually in the influent and the concentration was increased from 200 mg/L to 300 mg/L and finally 900 mg/L.

3.1.3 Maintaining of Parameters for Optimum Reactor Performance

Activated sludge process depends greatly on some parameters for it to have an optimum performance. The microbial of an activated sludge process were very sensitive to any changes and needed careful supervision and constant monitoring. Therefore, these parameters needed to be maintained and monitored regularly to ensure an optimum condition for the microbial, and thus improving the reactor performance.

The parameters are:

a) pH

pH of the aerated sludge was maintained at 7 – 8.5. Sulfuric acid was added if the pH is too alkaline or sodium hydroxide was used if the pH is too acidic. This procedure was done on daily basis.

b) Temperature

The water temperature was maintained at 25°C – 35°C. This was measured by using thermometer. This procedure was done on daily basis.

c) Total carbon, nitrogen and phosphorus ratio

C:N:P ratio was maintained at 100:5:1. The ratio was measured by using spectrophotometer and the provided TOC, TN and TP vials. Lack in carbon was adjusted by adding methanol while lack in nitrogen was adjusted by adding urea and finally. Lack in phosphorus was adjusted by adding phosphoric acid. This procedure was done once in two weeks.

d) MLSS and MLVSS

The values of MLSS and MLVSS were monitored daily to understand the ongoing biochemical activities depending on the variation in SRT. Three samples were taken from each reactor for more accurate results.

3.1.4 Measuring Ammonia Content in Wastewater

1. The method used was Nessler Method based on the procedure from the HACH system.
2. 25 ml of mixing graduated cylinder was filled with sample. The sample was taken from influent and effluent of each reactor.
3. Another 25 ml of mixing graduated cylinder was filled with demonized water as blank sample.
4. Three drops of Mineral Stabilizer was added to each mixing cylinder and the cylinders were inverted a few times to ensure maximum mixing.
5. After that, three drops of Polyvinyl Alcohol was added to each mixing cylinder and the cylinders were inverted a few times to ensure maximum mixing. The Polyvinyl Alcohol Dispersing Agent aids the color formation in the reaction of Nessler Reagent with ammonium ions. A yellow color is formed proportional to the ammonia concentration.
6. 1.0 ml of Nessler Reagent was added into the cylinder using a pipette and the cylinders were again inverted a few times to ensure maximum mixing.
7. The cylinders were left for 1 minute of reaction period.
8. After 1 minute, the sample from each cylinder was poured into 10 ml square sample cells and wiped with clean cloth to prevent any interference while reading.
9. The square cell that contains blank sample was inserted into the cell holder of a spectrophotometer with the fill line facing the user.
10. Button 'ZERO' was pressed and the reading showed 0.00 mg/L NH_3-N
11. Finally, the square cell that contains sample was inserted and the reading was taken.
12. The unit of ammonia content in the sample was in mg/L
13. For each reactor, three samples were tested to obtain more accurate results.

Figure 3.1 shows the basic procedures to measure ammonia content in waste water.

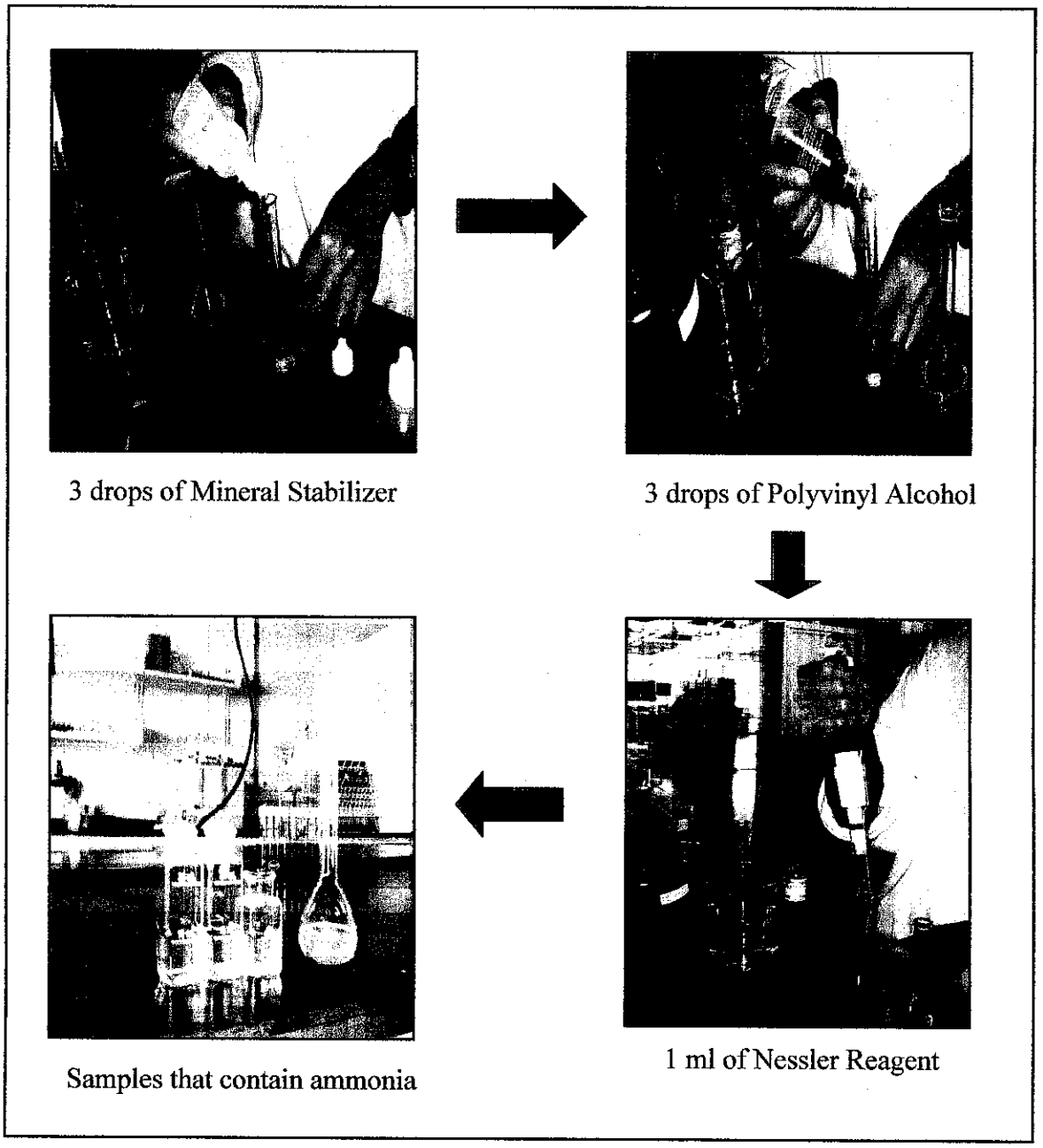


Figure 3.1: Ammonia sampling procedures.

3.1.5 Measuring Nitrate Content in Wastewater

1. High range Cadmium Reduction Method was used to measure nitrate content in waste water. This method could detect nitrate content at higher range from 0.3 mg/L to 30 mg/L.
2. 10 ml of sample was filled in a square sample cell. The sample was taken from influent and effluent of each reactor.
3. The content of one Nitra Ver 5 Powder Pillow was added into the square cell and was mixed vigorously for approximately 1 minute. Stopper was used to avoid any sample from leaking out.
4. After that, the sample was left for 5 minutes of reaction period.
5. Sample that contained nitrate will become brownish in color.
6. When the timer expires, a second square sample cells was filled with sample as blank sample.
7. The square cell that contained blank sample was wiped with clean cloth and was inserted into the cell holder of a spectrophotometer with the fill line facing the user.
8. Button 'ZERO' was pressed and the reading showed 0.00 mg/L NO_3^- - N
9. Finally, the square cell that contains sample was wiped with clean cloth and inserted into the cell holder.
10. The reading was take and the unit of nitrate content in the sample was in mg/L
11. For each reactor, three samples were tested to obtain more accurate results.

Figure 3.2 shows the basic procedures to measure nitrate content in waste water.

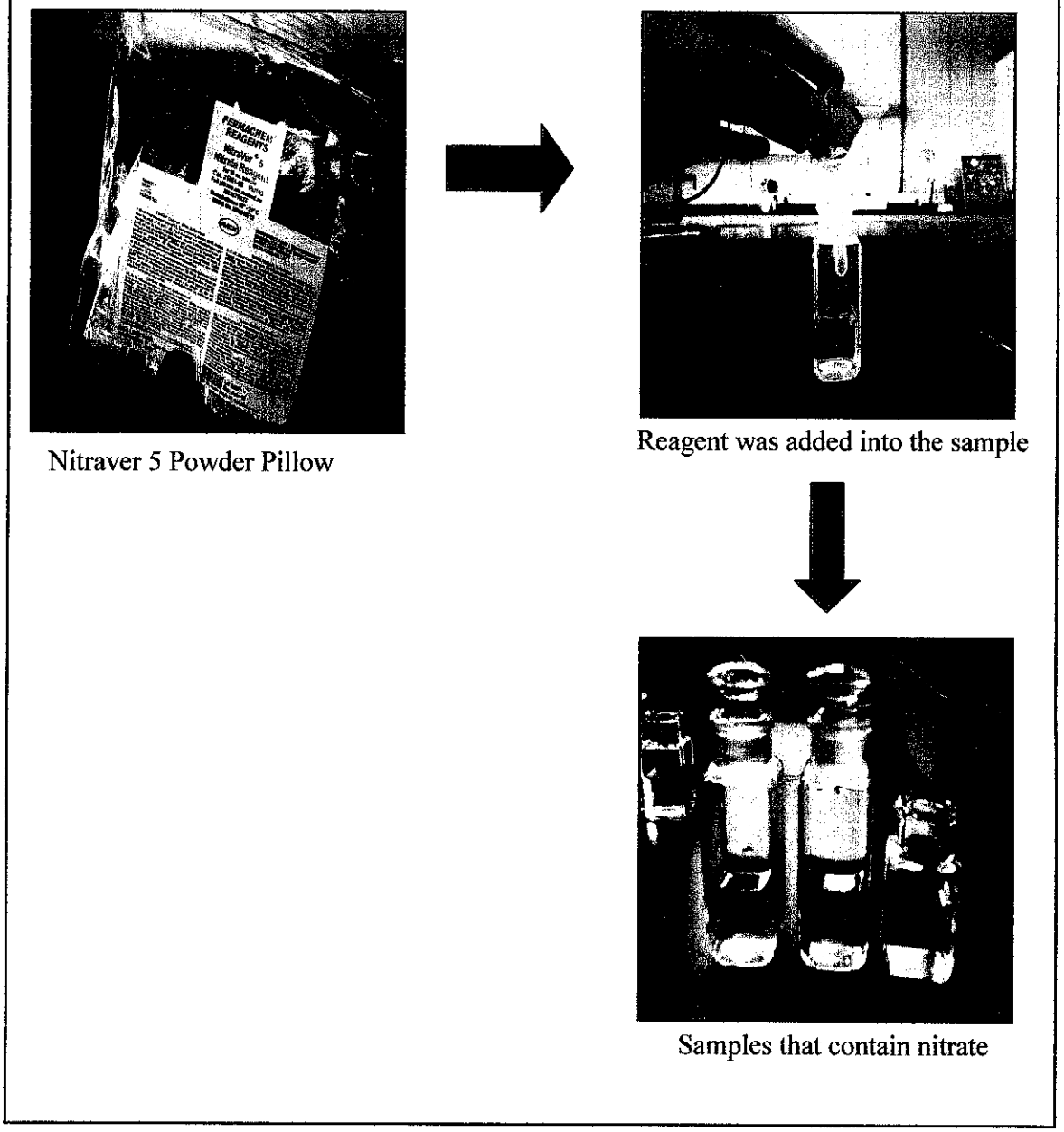


Figure 3.2: Nitrate sampling procedure

3.2 Tools and Techniques

3.2.1 Spectrophotometer

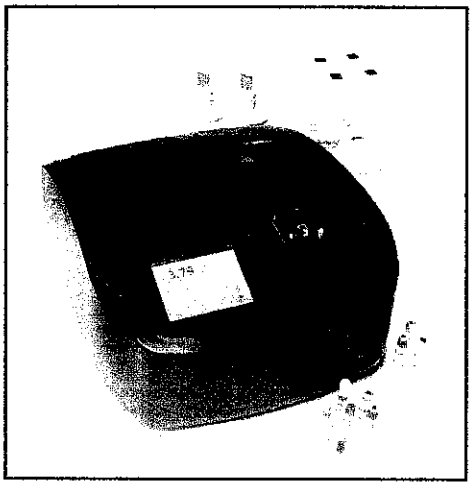


Figure 3.3: HACH Spectrophotometer

Every chemical analysis in this project was done using HACH spectrophotometer. A spectrophotometer is a photometer which is a device for measuring light intensity that can measure intensity as a function of the color, or more specifically, the wavelength of light. The spectrophotometer measures quantitatively the fraction of light that passes through a given solution. In a spectrophotometer, a light from a lamp in a near-IR/VIS/UV spectrophotometer is guided through a monochromator, which picks light of one particular wavelength out of the continuous spectrum. This light passes through the sample that is being measured. After the sample, the intensity of the remaining light is measured with a photodiode or other light sensor, and the transmittance for this wavelength is then calculated. For this project, the samples were inserted into the cell holder and a detection value was taken as to identify the nutrient content in these samples.

3.2.2 pH Meter

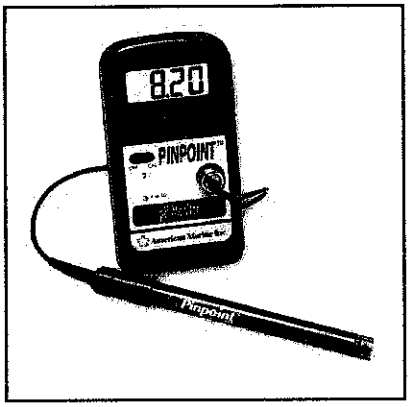


Figure 3.4: pH meter

A pH meter is an electronic instrument used to measure the pH , either alkalinity or acidity of a liquid. The pH probe measures pH as the activity of hydrogen ions surrounding a thin-walled glass bulb at its tip. The probe produces a small voltage that is measured and displayed as pH units by the meter. The small voltage is about 0.06 volt per pH unit. In this project, pH meter was used to monitor the pH of water so that it can be maintained at desired value which was at the range of 7 to 8.5.

3.3 Chemical Reagent

In this project, there are several chemical reagent used to complete the experiments. Some of the reagents are:

3.3.1 Nitraver 5 Nitrate Reagent Powder Pillow

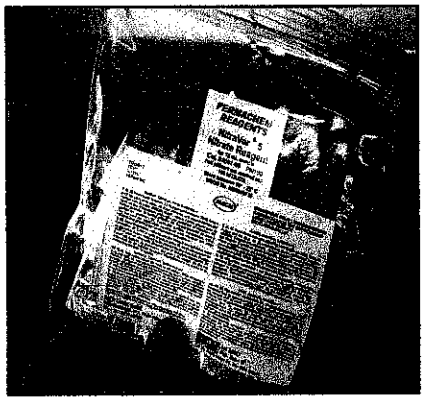


Figure 3.5

This is a hazardous reagent that should be handled with care. The apparatus that contained this reagent should be washed carefully after using. The complete MSDS of this reagent is attached in the appendix.

3.3.2 Sodium Sulfide Hydrate

Figure 3.6

This reagent contains yellow crystals with strong sulfur dioxide odor. It gives off flammable vapors. Vapors may form explosive mixture with air. It is corrosive and can cause severe burns. The complete MSDS of this reagent is attached in the appendix.

3.3.3 Nessler Reagent

Figure 3.7

Neßler's reagent is used to detect small amounts of ammonia. A yellow coloration indicates the presence of ammonia: at higher concentrations, a brown precipitate may form. It is toxic if swallowed, inhaled or absorbed through the skin. It presents a neurological hazard and may act as a carcinogen and be a reproductive hazard. It is corrosive and causes burns. The complete MSDS of this reagent is attached in the appendix.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Results and discussion

After the experiments, all the results were tabulated as shown in Appendix 1. The results taken were an average from three samples that had been tested.

In this section, the findings would be explained generally based on the trends that can be observed. The findings would be arranged according to the type of experiments done, i.e. ammonia content, nitrate content, Mixed Liquor Suspended Solis, and Chemical Oxygen Demand (COD).

4.1.1 Observation of bacterial growth

The bacterial growth in the activated sludge was observed by using high frequency microscope. The bacterial and microbes were studied to determine the efficiency of the activated sludge used. From the observation, the microbes moved rapidly and some even undergo cell division. But, after some time, the microbes started to decrease along with the declined in sludge volume. This is because; the bacteria had entered the *declining growth phase* where the bacterial mass decreases because of limitation in the food supply.

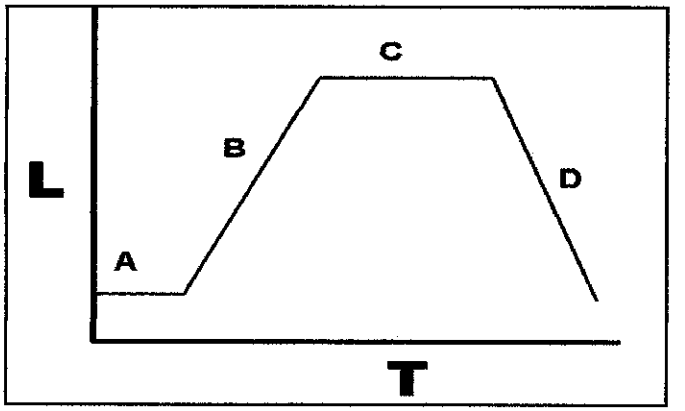


Figure 4.1: Bacterial growth curve log number (L) vs time (T)

Source : Wikipedia.org.com

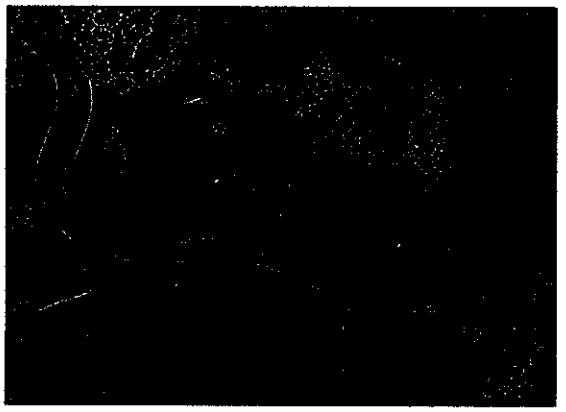
A) Lag phase – bacteria adapt themselves to growth conditions. It is the period where the individual bacteria are maturing and not yet able to divide.

B) Exponential phase – a period characterized by cell doubling. The number of new bacteria appearing per unit time is proportional to the present population. The slope of this line is the specific growth rate of the organism, which is a measure of the number of divisions per cell per unit time.

C) Stationary phase – the growth rate slows as a result of nutrient depletion and accumulation of toxic products.

D) Death phase – bacteria run out of nutrient and died.

Some of the bacteria observed were:



Nematodes

This is an example of metazoa, which play important roles as predators, consuming bacterial cells. These nematodes have long, thin bodies often 500 μm in length, multicelled and can be seen feeding on large suspended particles.

Filamentous

These organisms will always present in small numbers in healthy activated sludge plants which operate normally. But too many of filamentous bacterias will cause bulking and foaming which can be a problem to the activated sludge system.

Arcella

This is a type of amoeboid protozoa. It contained within a porous chitinous shell through which the pseudopodia can extend. The shell is spherical.

Trachelophyllum

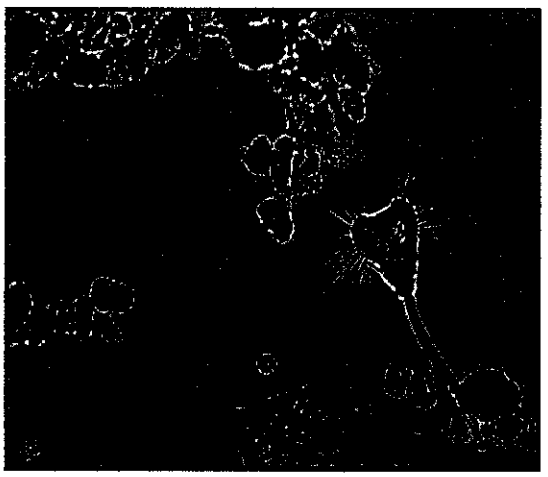
Trachelophyllum is a type of ciliate protozoa. It has flat long body completely covered with cilia. This organism is usually associated closely with the flocs, through which it moves.

Oligochaete

The most common oligochaete is the bristle worm. The oligochaete has tufts of bristles on each segment of its body and moves by a smooth action. It is very large in size (3000 μm in length)

Rotifers

This is also in protozoa class like oligochaete and nematodes. It consumed bacterial cells and play as predators in the system. These are large, multicelled organisms with highly specialized ciliate mouthparts and often a branched tail. The body moves by contracted and flexed itself.



Suctorian

Suctorian is a type of ciliate protozoa. They produce ciliate larvae and are likely to be holotrichous, feeding largely upon other ciliate protozoa.

Euplotes

Euplotes is type of crawling and free-swimming protozoa. It is ciliate and has ridges on the back and cirri at the front and back.

Figure 4.2: Microscope observation of microbial cells

4.1.2 Ammonia content in effluent

Ammonia is a form of nitrogen in untreated wastewater. During biological treatment, most of the particulate organic nitrogen was transformed to ammonium and other inorganic forms. Most of nitrogen in treated effluent is in ammonia form. The removal of ammonia was measured using Nessler Method and the sample used was in the influent and effluent from each reactor.

	AVERAGE					
Date	INFLUENT	[illegible]	EFFLUENT 2	EFFLUENT 3	EFFLUENT 4	EFFLUENT 5
20.2.2009		[illegible]	0.3600	0.3000	0.3633	0.2767
23.2.2009		[illegible]	0.0750	0.4750	0.3600	0.0000
2.3.2009		[illegible]	2.5633	3.9933	5.0900	3.2900
4.3.2009		[illegible]	3.4200	4.2500	4.9867	3.9800
6.3.2009		[illegible]	1.6733	0.8400	3.3667	0.6400
13.3.2009	16.4000	[illegible]	3.5000	2.4533	8.5400	9.6400
16.3.2009	13.3200	[illegible]	2.9067	4.6200	6.4400	2.5833
18.3.2009	16.3200	[illegible]	3.0333	5.7400	3.2167	2.1167
19.3.2009	16.0200	[illegible]	2.9267	4.7800	3.2267	2.2533
20.3.2009	12.8000	[illegible]	2.7967	4.5600	3.2500	2.0333
21.3.2009	13.8800	[illegible]	0.6367	5.6800	5.1600	3.4000
22.3.2009	12.2400	[illegible]	0.4650	3.5100	3.7800	2.2267
23.3.2009	11.3800	[illegible]	0.6467	2.0200	2.1200	2.4733
25.3.2009	15.0000	[illegible]	0.8800	3.6200	0.5800	0.8433
27.3.2009	12.9200	[illegible]	2.4733	3.4533	1.2567	1.0767
28.3.2009	7.9200	[illegible]	2.5200	2.3033	1.3900	1.1500
29.3.2009	5.0000	[illegible]	2.4967	2.2267	1.1300	1.0533
30.3.2009	11.3600	[illegible]	2.9500	2.7600	1.7567	1.9533
1.4.2009	6.9800	[illegible]	1.6533	1.5000	1.2100	1.0367
3.4.2009	13.2200	4.7500	3.0400	2.8400	3.5733	2.0500
7.4.2009	29.4433	[illegible]	4.7600	4.7500	0.9400	0.8067
8.4.2009	32.5233	[illegible]	2.5400	0.7833	0.2367	0.1300
10.4.2009	26.6933	[illegible]	0.2900	0.5933	0.1800	0.1100
13.4.2009	22.9533	[illegible]	0.2867	0.1500	0.0867	0.0400
15.4.2009	62.6667	0.9100	0.1800	0.1467	0.1433	0.1400
17.4.2009	68.6667	[illegible]	0.6900	0.5167	0.1500	0.1333
20.4.2009	62.4500	[illegible]	1.1133	1.8067	0.9333	0.4667
22.4.2009	101.6000	0.0633	1.7767	0.0167	0.1000	0.0633
24.4.2009	121.5000	[illegible]	1.1900	0.5133	0.3600	0.2367
27.4.2009	136.1667	[illegible]	0.5600	1.5867	0.6400	0.4967
29.4.2009	135.5000	[illegible]	0.3167	0.5867	0.8433	0.3667

Table 4.1: Average data for ammonia content in effluent and influent

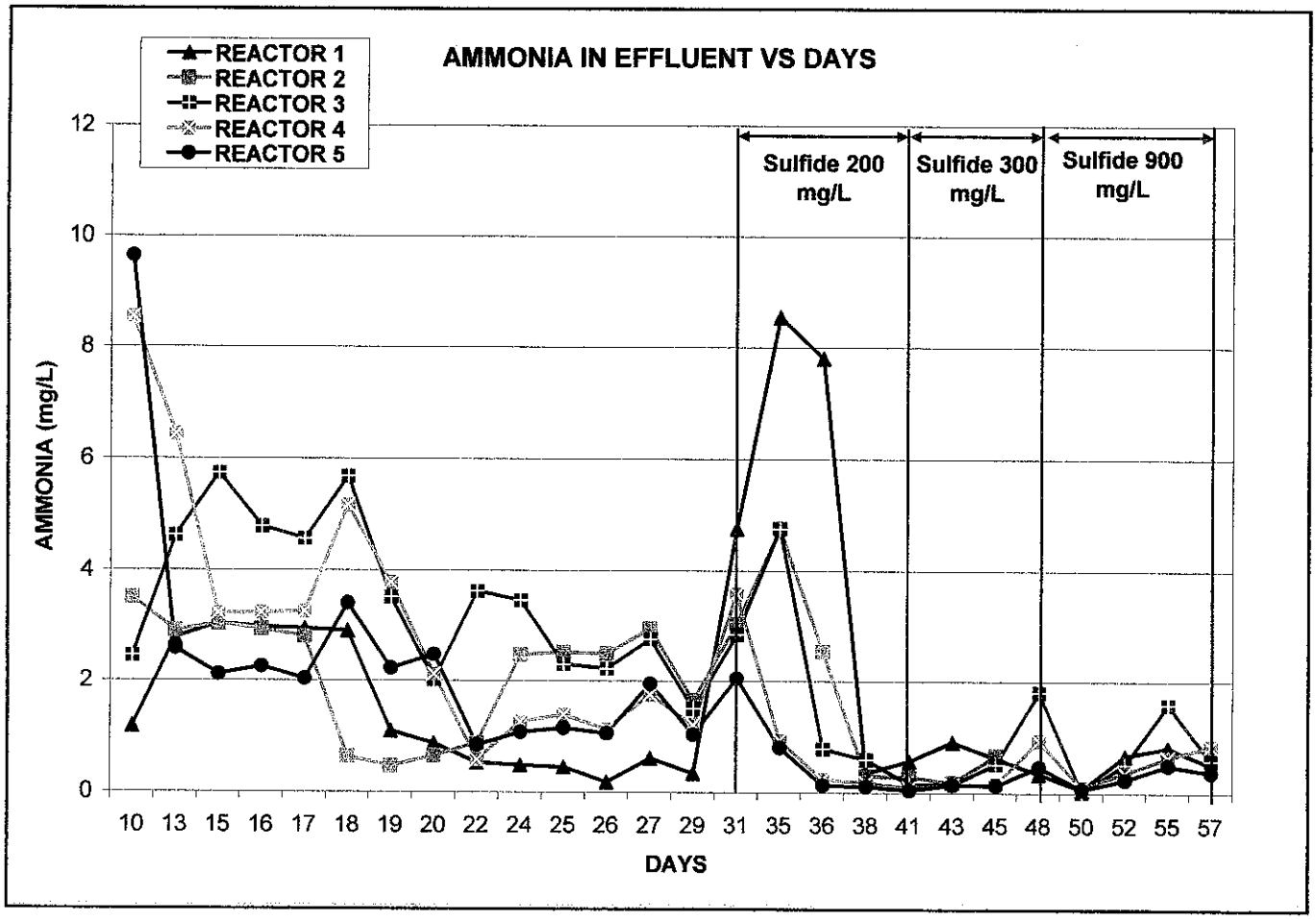


Figure 4.3: Graph of Ammonia in Effluent vs Days

From the graph, it was observed that during acclimatization phase, effluent in reactor 1 contains the least ammonia compared to other reactors. Even though the sludge wasted from Reactor 1 has the highest volume, but the microorganism in the reactor was proven to be the most efficient than the other reactors. It was believed that the condition in reactor 1 is the most suitable for microorganism to do their work in removing ammonia.

However, on the 31st day, after synthetic sulfide is added into each reactor with concentration of 200 mg/L, it was observed that the content of ammonia in Reactor 1 has increased dramatically along with other reactors. This is because of the effect of sulfide that 'shocked' the microorganisms. Metcalf and Eddy stated in their book "Wastewater Engineering, Treatment, Disposal and Reuse" that sulfates are reduced to sulfides in sludge digesters and may upset the biological process if the sulfide concentration exceeds 200 mg/L.

Nevertheless, after a week, more sulfides synthetic was added with higher concentration of 300 mg/L. From the graph, it shows that the ammonia content gradually decrease and even though the new sulfide added has higher concentration, it does not give significant impact to the content of ammonia in effluent. It was believed that the microorganisms in each reactor have become resistant to the amount of sulfide, and thus the sulfide does not affect them anymore. The microorganisms finally had adapted well in the reactor regardless of what amount of sulfide concentration was added in the system.

During this experiment also, it was observed that microorganisms in reactor 5 have the highest working efficiency since they removed the highest amount of ammonia. It is because of the sludge wasted from the reactors is the lowest, thus more bacteria was left in the reactor to cope with the sudden amount of sulfide in the reactor.

4.1.3 Ammonia content in influent

The waste water used from Sewage Treatment Plant had been tested to contain very high content of ammonia. After being treated, the content decrease drastically. This proved that the reactors used were very efficient and effective in removing ammonia from waste water.

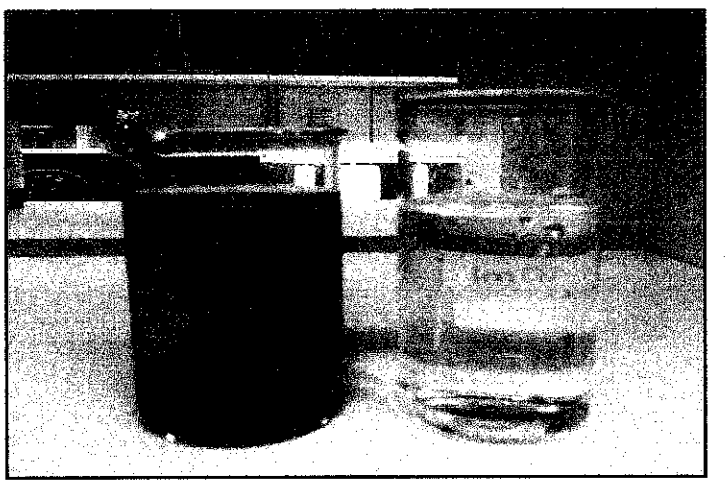


Figure 4.4

Left: Influent with high amount of sulfide

Right: Treated effluent (very clear liquid)

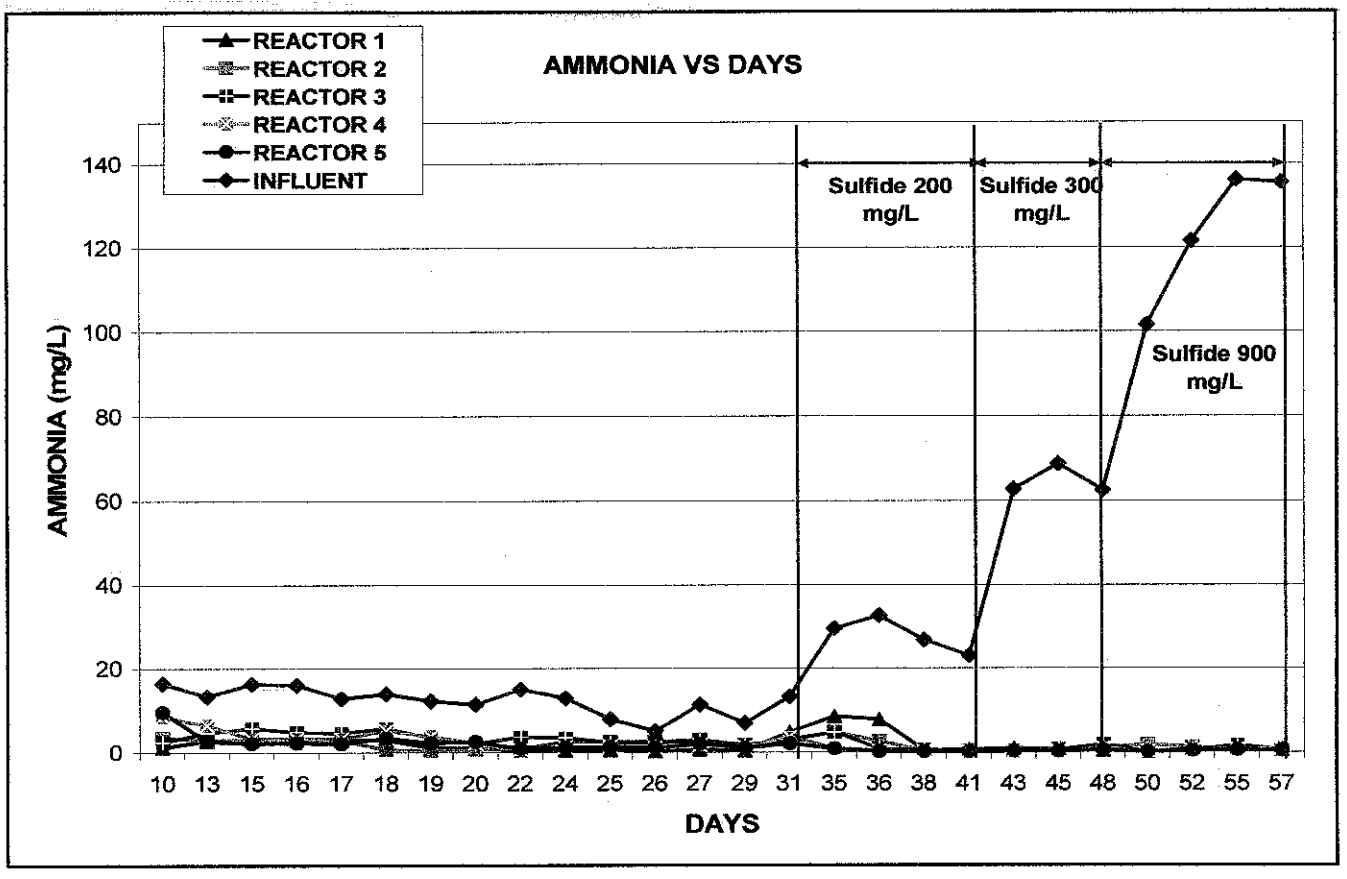


Figure 4.5: Graph of ammonia in the influent vs days

From the graph, it was observed that the addition of sulfide has a drastic effect on ammonia content in the influent. Ammonia content in influent increased significantly with the increase of sulfide concentration. However, the ammonia in effluent still remains at considerably lower amount since it was being treated by the microorganisms and most of ammonia had been oxidized to nitrate.

4.1.4 Nitrate content in Effluent

The process in which the nitrogen in the untreated waste water is substantially converted to nitrate is known as "biological nitrification". It is an autotrophic process, where the energy for bacterial growth is derived by the oxidation of nitrogen compounds, primarily ammonia. During this experiment, the amount of nitrate is tested. The sample taken is from effluent and influent with 10 ml samples for each reactor and three times reading to obtain more accurate results. The oxidation of nitrogenous matter is proceeds as follows:

$$NH_3 \rightarrow NO_2^- \rightarrow NO_3^-$$

	AVERAGE					
Date	INFLUENT	[illegible]	EFFLUENT 2	EFFLUENT 3	EFFLUENT 4	EFFLUENT 5
13.3.09	0.7500	[illegible]	27.8667	12.9333	31.8000	20.1667
16.3.09	0.4333	[illegible]	22.7667	19.3667	20.3000	16.9000
18.3.09	0.8667	[illegible]	19.1000	15.9333	20.8333	19.4333
19.3.09	0.8333	[illegible]	14.5000	15.7667	19.9000	18.3000
20.3.09	0.3667	[illegible]	16.9000	15.6667	18.3667	18.0000
21.3.09	1.5667	[illegible]	13.8667	13.7333	10.5333	15.4333
22.3.09	0.9667	[illegible]	25.2000	15.4333	12.2000	18.2333
23.3.09	0.6667	[illegible]	23.4667	15.9333	19.4000	17.8333
25.3.09	0.4667	[illegible]	21.0333	16.7500	27.0500	16.2667
27.3.09	0.5000	[illegible]	14.9333	16.2333	25.1667	22.3333
28.3.09	0.2333	[illegible]	13.5667	15.7000	21.5667	22.0000
29.3.09	0.2000	[illegible]	14.6333	14.5667	19.7333	17.9667
30.3.09	0.3000	[illegible]	16.8000	15.0000	22.2000	18.8333
1.4.09	0.4000	[illegible]	15.4333	15.6333	20.8667	18.8667
3.4.09	0.5333	12.3000	17.0333	13.2000	17.9000	15.3000
7.4.09	0.3000	[illegible]	13.3667	18.0333	29.8333	34.5000
8.4.09	0.2000	[illegible]	32.5667	32.3333	29.5333	33.8000
10.4.09	0.2000	[illegible]	28.6667	27.1667	26.4333	32.5667
13.4.09	0.2333	[illegible]	18.3333	14.2000	11.6667	13.5000
15.4.09	0.2333	16.2000	10.6333	6.9000	6.5333	9.8333
17.4.09	0.1333	[illegible]	9.1333	3.6000	4.7667	4.7333
0.4.2009	0.1333	[illegible]	4.3667	3.5667	2.3000	2.9000
2.4.2009	0.2333	6.3667	2.6000	2.8333	2.8333	2.7000
4.4.2009	0.2000	[illegible]	3.1667	2.7667	3.7333	3.6000
7.4.2009	0.5000	[illegible]	4.6000	2.7000	4.7667	4.4667
9.4.2009	0.2000	[illegible]	1.8000	0.6333	2.6667	1.5667

Table 4.2: Average data for nitrate content in effluent and influent

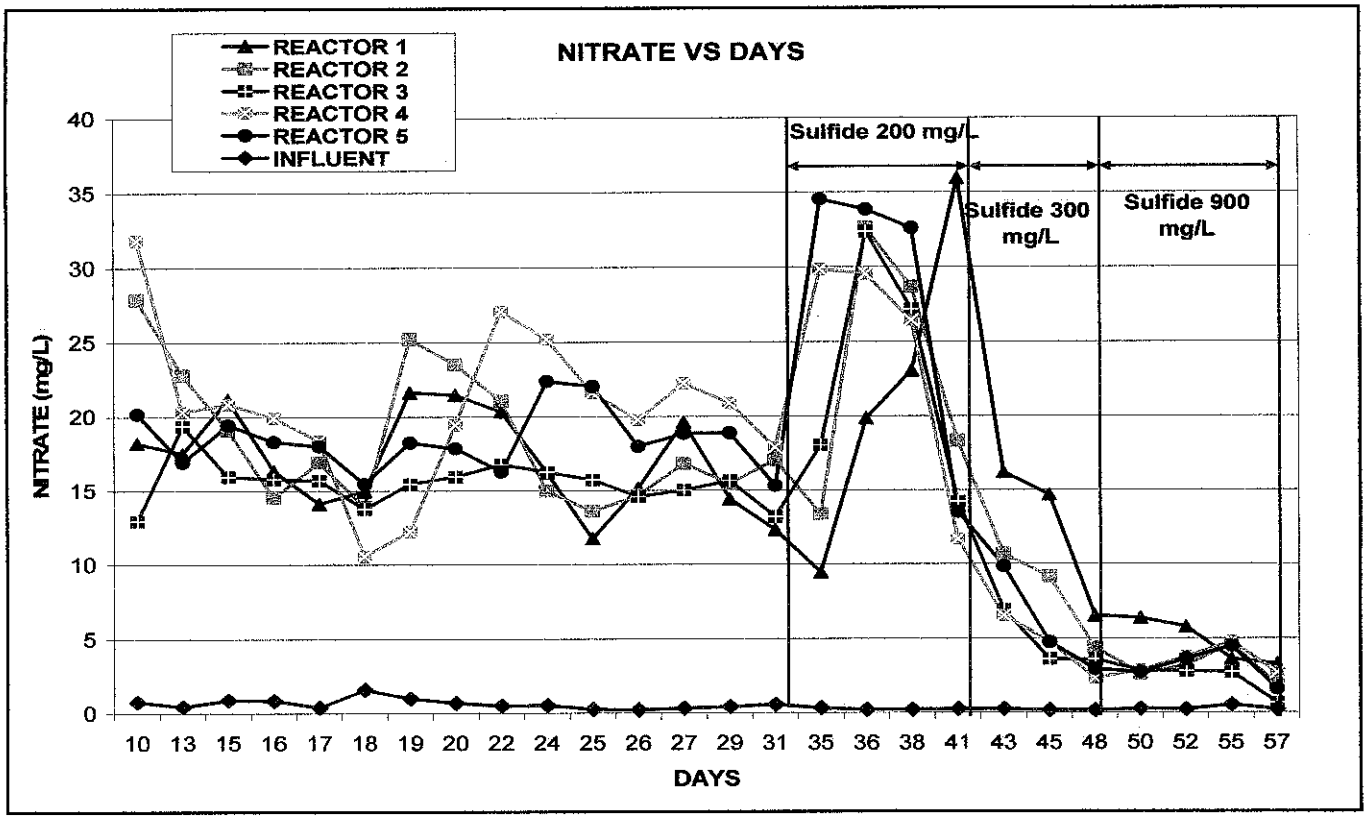


Figure 4.6: Graph of nitrate content vs days

From the graph, it was observed that the addition of sulfide also increase the amount of nitrate quite significantly. But after 41 days, the addition of higher concentration of sulfide has decrease the nitrate content in each reactor. From this graph, Reactor 1 has the highest content of nitrate compared to the others. This is because Reactor 1 has the highest content of ammonia. Therefore, more ammonia is converted to nitrate. In other words, high ammonia will yield to high nitrate content in a reactor. It was observed that the addition of sulfide had become a reducing agent for nitrate content in waste water.

It is also shows that there was very low nitrate content in influent. This is because nitrification process, where ammonia is converted into nitrate, will only take place in aerobic (require oxygen) condition. The present of oxygen is crucial to oxidize the ammonia compounds so that the energy derived can be used by Nitrobacter and Nitrosomanus to grow. Therefore, low nitrate content is because there is no oxidation process taking place since there is no or low oxygen in the influent.

4.1.5 Percentage of Ammonia Removal

Percentage of ammonia removal was calculated using the following formula:

$$\frac{\text{Ammonia content in influent (mg/L)} - \text{Ammonia content in effluent (mg/L)}}{\text{Ammonia content in influent (mg/L)}} \times 100$$

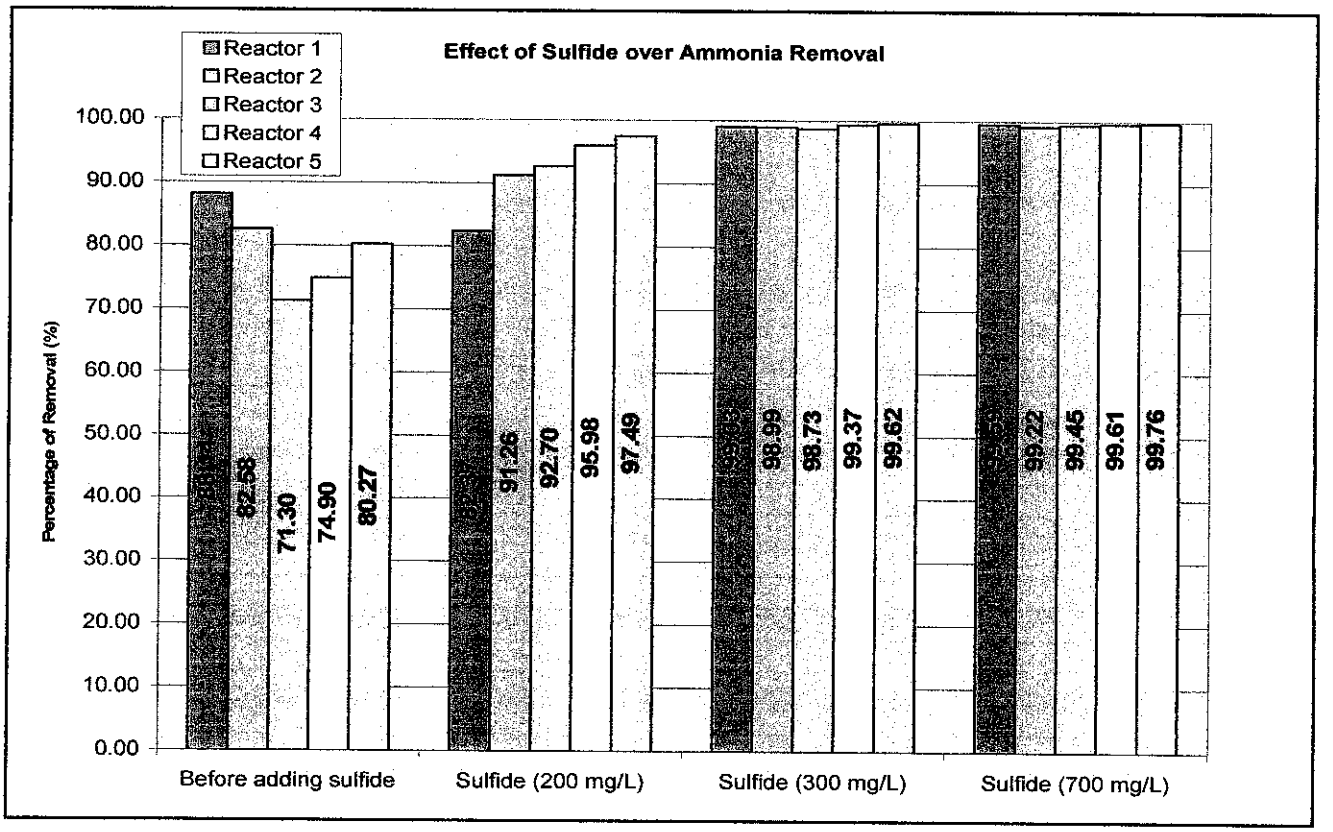


Figure 4.7: Percentage of ammonia removal

From the graph, it was observed that sulfide had a significant effect of the biomass. Before sulfide addition, reactor 1 with SRT of 10 days had the highest percentage removal. But, after 200 mg/L of sulfide concentration was added in the influent, the removal percentage in Reactor 1 had decrease drastically.

This is due to the amount of bacteria that cannot adapt with the sudden addition of sulfide and thus had drastically decreased the performance of the reactor. From the graph, it was concluded that nitrifying bacteria were sensitive to the addition of sulfide but gradually

become adapted to the sulfide-rich environment after more concentration of sulfide was added. The high sensitivity of nitrifiers is mostly attributed to the fact that nitrification is performed exclusively by two species of chemo-autotrophic nitrifying bacteria, Nitrosomonas and Nitrobacter. As nitrifiers have slow growth rates and are only present in the mixed liquor in very small numbers, even a small reduction in their growth rate, caused by the presence of sulfide may result in their washout and in nitrification inhibition. However, over time, the nitrifying bacteria become resistance to the sulfide concentration and thus, resulting to almost constant ammonia removal percentage in each reactor.

The graph also clearly shows the most optimum ammonia removal in rich-sulfide waste water can be achieved in Reactor 5 with SRT of 50 days with the removal percentage of 99.76%. This is due to the nature of nitrifying bacteria which are very slow grower. Therefore, nitrification proceeds at much slower rate and thus, longer SRT is required. This provides sufficient contact between wastewater and the bacteria to ensure maximum nitrification.

4.1.6 Biomass Growth

Biomass growth is crucial to study the amount of ammonia degraded for each milligram biomass per day. Biomass growth was calculated by using the following formula:

$$\text{Biomass Growth} = \frac{Q(NH_3\ \text{in}) - Q(NH_3\text{Out})}{\text{MLVSS}}$$

The plotted graph was as follows:

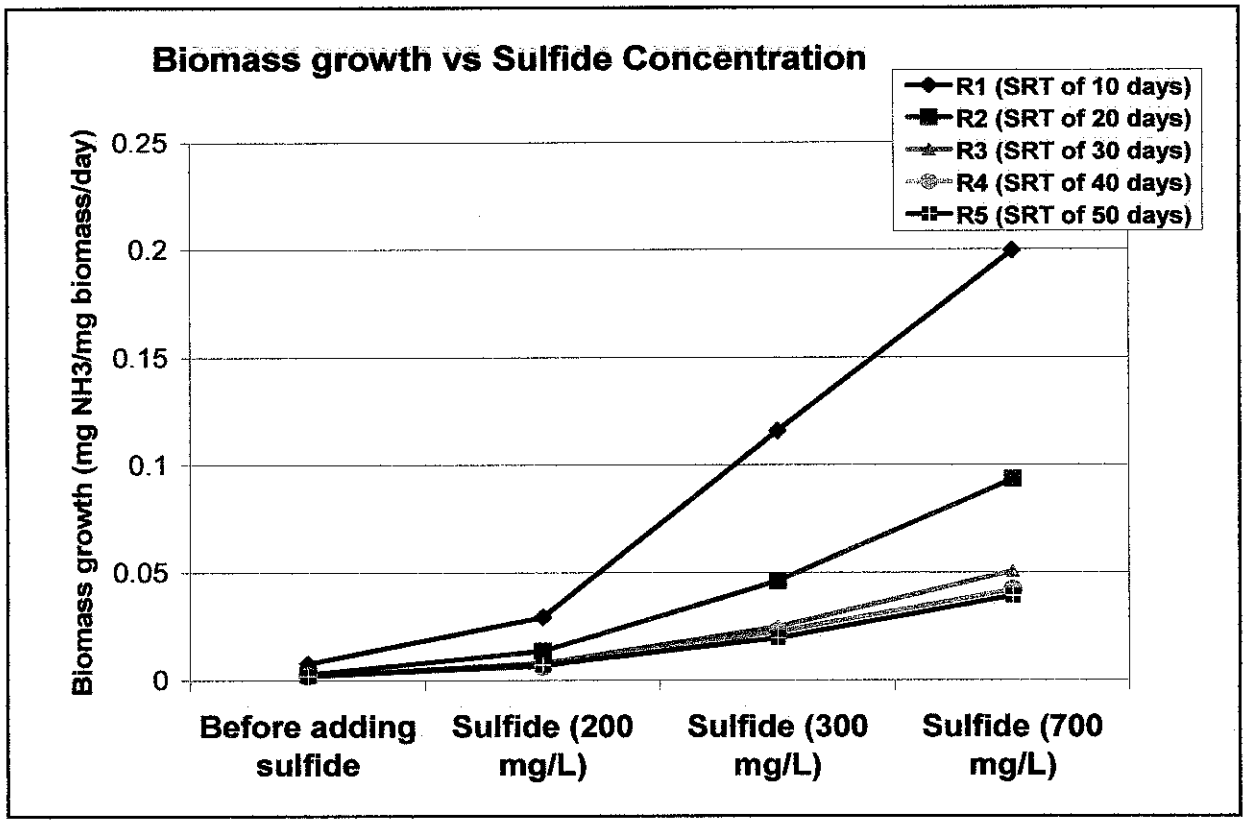


Figure 4.8: Biomass Growth vs Sulfide Concentration

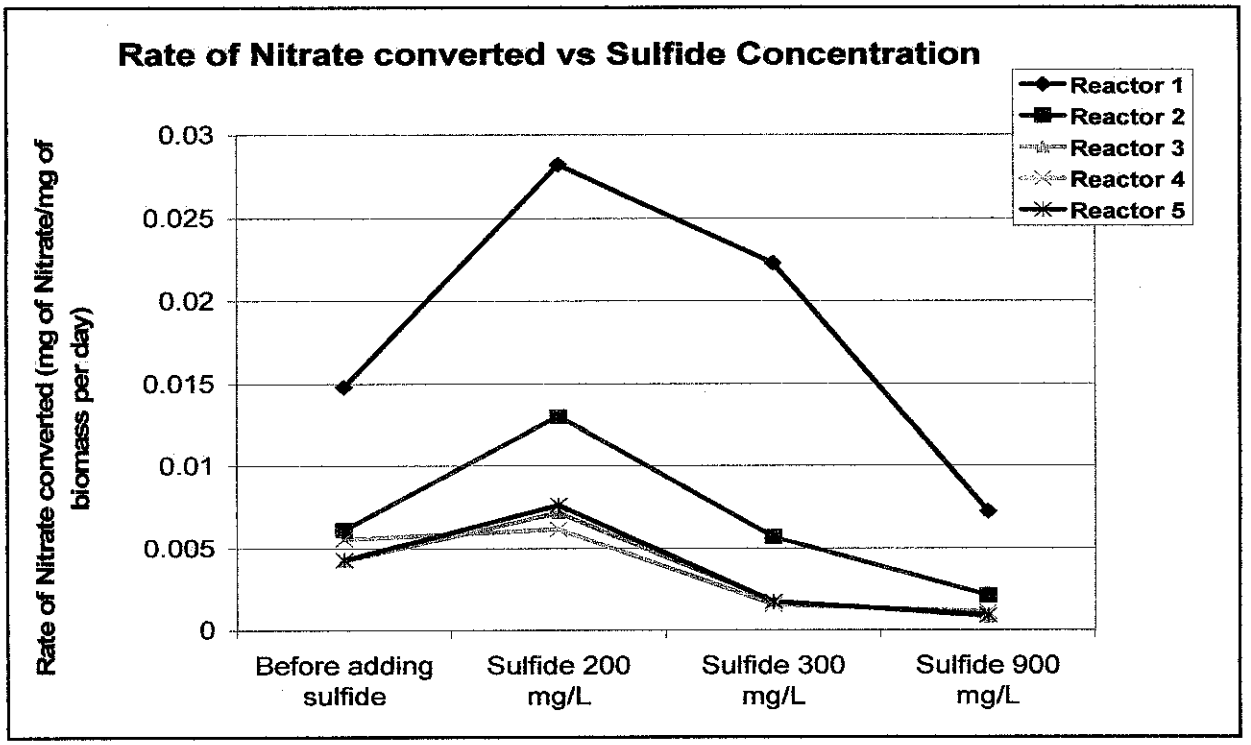


Figure 4.9: Rate of Nitrate converted vs Sulfide Concentration

From the above graphs, it was observed that biomass in reactor 1 with SRT of 10 days had the highest growth rate compared to other reactors. Although the sludge wasted from this reactor was the highest, but the microorganisms in this reactor was found to be the most active and degraded the most ammonia per day. There was no significant difference between SRT 30, SRT 40 and SRT 50 days. From the graph, it was concluded that SRT of 10 days is the most optimum SRT for biomass growth.

4.1.7 Performance on COD removal

The organic waste in wastewater caused the reduction in the dissolved oxygen concentration, which is normally due to the microbial breakdown of the organic matter present. Chemical Oxygen Demand (COD) measures the organic content in terms of biodegradable and non-biodegradable compounds. During this project, COD test was also conducted to examine the overall performance of each reactor. The oxygen equivalent of the organic matter that can be oxidized is measured by using a strong chemical oxidizing agent in an acidic medium. Lower COD content in waste water is desirable because it indicates lower organic content in the wastewater.

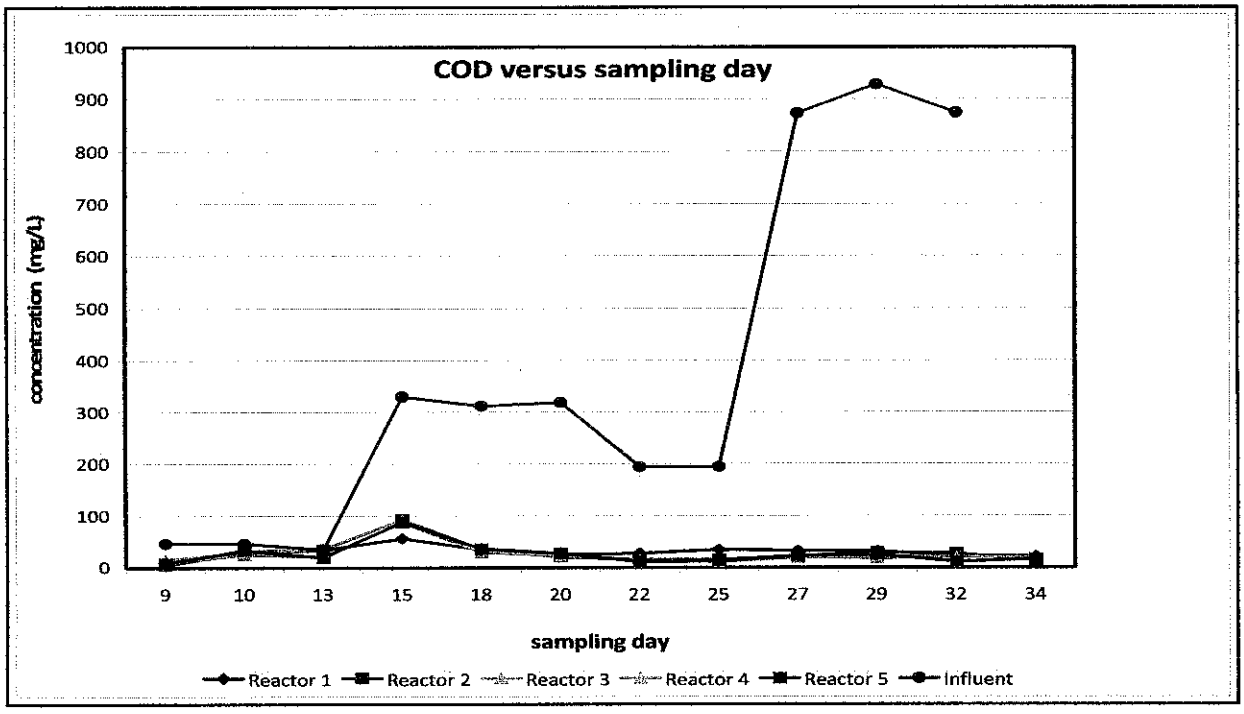


Figure 4.10: COD concentration in influent and effluent

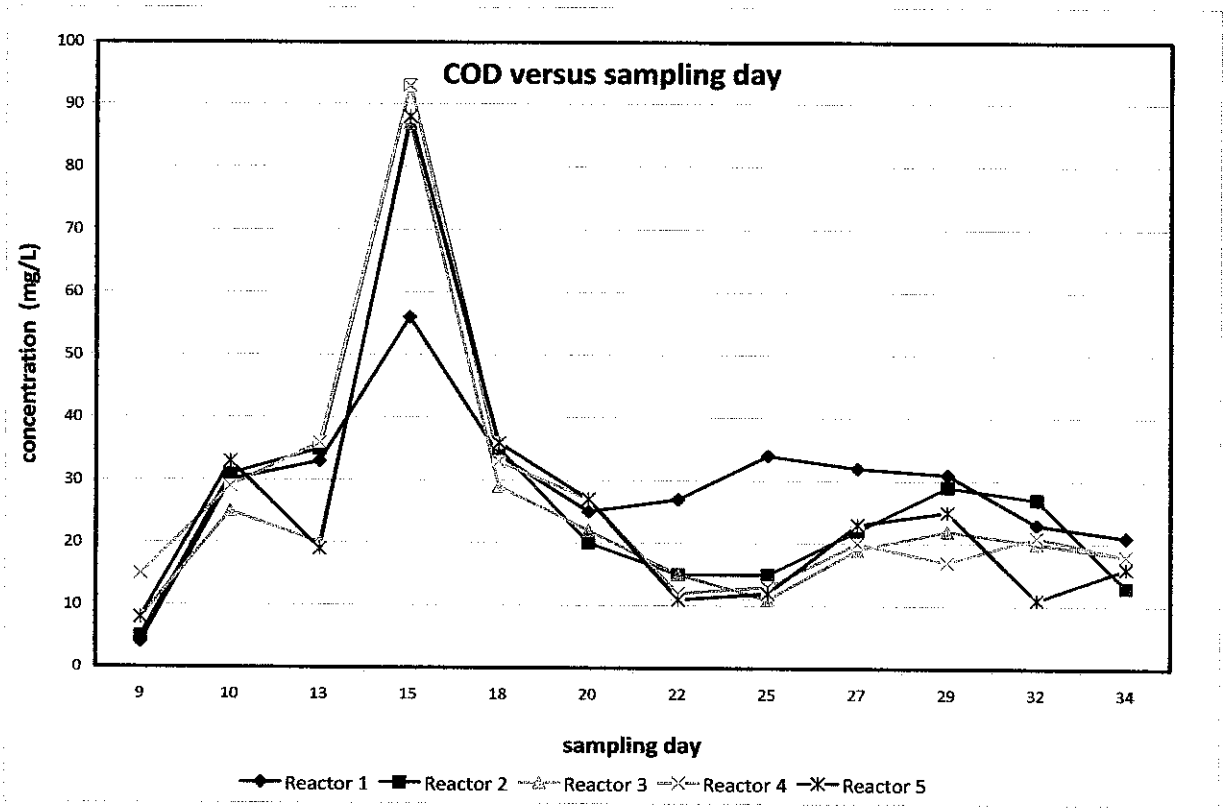


Figure 4.11: COD concentration in effluent

From the graph, the effect of sulfide addition to COD content was examined. The content shows significant increase of COD concentration in the influent. However, the concentration of COD in effluent was much lower after being treated by the microorganisms.

Similar patterns with ammonia removal were found in COD removal. Before addition of sulfide, Reactor 1 was observed to contain lower COD and thus, having the highest removal efficiencies than any other reactors. But after sulfide was added, it was observed that Reactor 5 performance improved and thus, having the highest removal efficiency.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

From the studies done, it was found that activated sludge system was very efficient in removing ammonia from wastewater. The growth of microbes in all five reactors has proven to be satisfactory. Activated sludge system is very efficient to remove ammonia in high-sulfide waste water.

From this experiment also, it can be concluded that all five reactors with different SRT have percentage removal of > 99% in high sulfide. However, SRT of 10 days is proved to be the most optimum for bacterial growth since it has the highest Ammonia degraded per 1 mg of biomass per day.

Before addition of sulphide, microbes in Reactor 1 had shown a significant increase in performance. The ammonia content was the lowest compared to the other reactors. This proved that SRT of 10 days was the most optimum SRT for bacterial growth.

However, after the addition of sulphide, reactor 5 with SRT of 50 days showed the lowest amount of ammonia. After a certain amount of time, the increase of sulphide concentration did not affect the biomass anymore as all the micro organisms had already adapted with the sulphide-rich environment.

Present results shows that activated sludge system can be used to nitrify waste water containing high amount of ammonia concentration at SRT of 10 days. In sulphide-rich waste water, SRT of 50 days shows the lowest ammonia content. But in term of biomass growth, SRT of 10 days has the highest biomass growth, and thus proving to be the most optimum SRT.

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APPENDICES

MATERIAL SAFETY DATA SHEET

PRODUCT IDENTIFICATION

rade Name:	Sodium Sulfide, 9-hydrate	**CAS #:**	1313-84-4
hemical Family:	Inorganic Sodium Compounds	**Formula:**	Na_2S*9H_2O
ormula Weight:	240.18	**NIOSH/TECS No.:**	WE1925000
roduct Use:	Laboratory reagent	**Product Codes:**	3910
ommon Synonyms:	Sodium Sulfide, Nonahydrate		

PRECAUTIONARY LABELING

afety Data: Health: 2 **Flammability**: 2 **Reactivity**: 1 **Contact**: 4
aboratory Protective Equipment: Goggles, lab coat, vent hood, proper gloves, and extinguisher

.S. Precautionary Labeling: DANGER! CORROSIVE. CAUSES SEVERE BURNS. HARMFUL IF SWALLOWED OR HALED. CONTACT WITH ACID LIBERATES POISONOUS GAS. KEEP REFRIGERATED. Keep away from heat, sparks, ame. Do not get in eyes, on skin, on clothing. Avoid breathing dust. Keep in tightly closed container. Use with adequate ventila- on. Wash thoroughly after handling. In case of fire, soak with water. In case of spill, sweep up and remove. Flush spill area with ater.

torage Color Code: Red stripe (store separately)
ternational Labeling: Avoid contact with eyes. After contact with skin, wash immediately with plenty of water. Keep container ghtly closed.

I COMPONENTS

CGIH TLV:	N/E	**OSHA PEL**:	N/E	**Percentage**:	98-100

/ PHYSICAL DATA

oiling Point (760 mm Hg):	174 °C (345 °F)	**Melting Point (760 mm Hg)**:	174 °C (122 °F)
ecific Gravity (H_2O=1):	1.86	**Vapor pressure (mm Hg)**:	N/A
apor density (air=1):	N/A	**Evaporation Rate**:	N/A
Volatile by Volume:	0 (21 °C)	**Solubility in H_2O (%)**:	Appreciable (10%)
H:	13.5 (10% solution)	**Physical State**:	Solid
efficient Water Distribution:	N/A	**Odor threshold (ppm)**:	N/A
ppearance and Odor:	White to yellow crystals. Sulfur dioxide odor		

FIRE AND EXPLOSION HAZARDS DATA

ash Point (Closed Cup): N/A
ammable Limits: **Upper**: N/A **Lower**: N/A
Autoignition Temperature: NA
Extinguishing Media: Use water spray

nusual Fire & Explosion Hazards: Gives off flammable vapors. Vapors may form explosive mixture with air. Closed containers posed to heat may explode.

ecial Firefighting Procedures: Firefighters should wear proper protective equipment and self-contained breathing apparatus with ll face piece operated in positive pressure mode. Move containers from fire area if it can be done without risk. Use water to keep e-exposed containers cool.

oxic Gasses Produced: Hydrogen sulfide
xplosive Data-sensitivity to Mechanical Impact: None identified.
xplosion Data-sensitivity to Static Discharge: None identified.

I HEALTH HAZARD INFORMATION

LV/TWA: Not established
hort-term Exposure Limit: Not established
ermissible Exposure Limit: Not established
oxicity of Components: Intraperitoneal Mouse LD50 for sodium sulfide, 9-hydrate 53 mg/kg
arcinogenicity: **NTP**: No **IARC**: No **Z List**: No **OSHA Reg**: No
eproductive Effects: None identified

ffects of Overexposure:
ıhalation: Irritation of upper respiratory tract.
kin Contact: Severe burns
ye Contact: Severe burns
kin Absorption: May be harmful
ıgestion: Severe burns to mouth, throat, and stomach, nausea, vomiting, diarrhea

hronic Effects: None identified
arget Organs: Eyes, skin
[edical Conditions Generally Aggravated by Exposure: Respiratory system disease, central nervous system disorders
rimary Routes of Entry: Inhalation, ingestion, absorption, skin contact, eye contact

MERGENCY AND FIRST AID PROCEDURES:

NGESTION: CALL A PHYSICIAN. If swallowed do not induce vomiting. If conscious, give water, milk, or milk of magnesia.
NHALATION: If inhaled, remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.
ompt action is essential.
KIN CONTACT: In case of contact, immediately flush skin with plenty of water for at least 15 minutes while removing contami-
ıted clothing and shoes. Wash clothing before re-use.
YE CONTACT: In case of eye contact, immediately flush with plenty of water for at least 15 minutes.

I REACTIVITY DATA

ability: Stable
compatibility (Material to avoid): Strong oxidizing agents, strong acids, most common metals.

ıazardous Polymerization: Will not occur
ɔnditions to Avoid: Heat, flame, other sources of ignition.
ɛcomposition Products: Hydrogen sulfide

II SPILL OR LEAK PROCEDURES

eps to be Taken in Case Material is Released or Spilled: Wear self-contained breathing apparatus and full protective clothing.
ıt off ignition sources; no flares, smoking, or flame in area. Carefully place material into clean, dry container and cover; remove
ɔm area. Flush spill area with water.

aste Disposal Method: Disposal must be made in accordance with Federal, State and Local regulations.
'A Hazardous Waste Number: DOO2 (Corrosive waste)

VIII SPECIAL PROTECTION INFORMATION

Respiratory Protection: None required where adequate ventilation conditions exist. If airborne concentration is high, a dust/mist respirator is recommended. If concentration exceeds capacity of respirator, a self-contained breathing apparatus is advised.

Ventilation: Use adequate general or local exhaust ventilation to keep fume or dust levels as low as possible.
Eye/skin Protection: Safety goggles, uniform, apron, neoprene gloves are recommended.
Storage Requirements: Product must be refrigerated at 2-8 °C (36-46 °F). Keep container tightly closed. Store in a cool, dry, well ventilated area away from heat, sparks or flame. Isolate from incompatible materials.

IX TRANSPORTATION DATA AND ADDITIONAL INFORMATION

Domestic (D.O.T):
Proper Shipping Name: Sodium sulfide, hydrated (30% water min)
ID/NA: UN1849
Labels: 8 Corrosive
Hazard Class: 8
Packaging Group: II
Regulatory References: 49CFR 172.101

International (I.M.O):
Proper Shipping Name: Sodium sulfide, hydrated (30% water min)
ID: UN1849
Labels: 8 CORROSIVE
Regulatory References: 49CFR PART 176; IMDG Code
Hazard Class: 8
Marine Pollutants: No
I.M.O. Page: 8217
Packaging Group: II

AIR (I.C.A.O.):
Proper Shipping Name: Sodium sulfide, hydrated (30% water min)
ID: UN1849
Labels: 8 CORROSIVE
Hazard Class: 8
Packaging Group: II
U.S. Customs Number: 28301000000
NOTE: When handling liquid products, secondary protective containers must be used for carrying.

Regulatory References: 49CFR PART 175; ICAO. We believe the transportation data and references contained herein to be factual and the opinion of qualified experts. The data is meant as a guide to the overall classification of the product and is not package size specific, nor should it be taken as a warranty or representation for which the company assumes legal responsibility. The information offered solely for your consideration, investigation, and verification. Any use of the information must be determined by the user to be in accordance with applicable Federal, State, and Local laws and regulations. See shipper requirements 49CFR 171.2, Certification 172.204, and employee training 49CFR 173.1(b).

ADDITIONAL INFORMATION

The information in this Material Safety Data Sheet meets the requirements of the United States OCCUPATIONAL SAFETY AND HEALTH ACT and regulations promulgated thereunder (29 CFR 1910.1200 et. seq.) and the Canadian WORKPLACE HAZARDOUS MATERIALS INFORMATION SYSTEM. This document is intended only as a guide to the appropriate precautionary handling of the material by a person trained in, or supervised by a person trained in, or supervised by a person trained in, chemical handling. The user is responsible for determining the application. Depending on usage, protective clothing including eye and face shields and respirators must be used to avoid contact with material or breathing chemical vapors/fumes. Exposure to this product may have serious adverse health effects. This chemical may interact with other substances. Since the potential uses are so varied, ESPI cannot warn all of the potential dangers of use or interaction with other chemicals or materials. ESPI warrants that the chemical meets the specifications set forth on the label. ESPI DISCLAIMS ANY OTHER WARRANTIES, EXPRESSED OR IMPLIED WITH REGARD TO THE PRODUCT SUPPLIED HEREUNDER, ITS MERCHANTABILITY OR OUTER FITNESS FOR A PARTICULAR PURPOSE. The user should recognize that this product can cause severe injury and even death, especially if improperly handled or the known dangers of use are not heeded. READ ALL PRECAUTIONARY INFORMATION. As new documented general safety information becomes available, ESPI will periodically revise this Material Safety Data Sheet.

Prepared By: S. Dierks
Dated: March 1994

Material Safety Data Sheet

Nessler's Reagent Solution

ACC# 40178

Section 1 - Chemical Product and Company Identification

MSDS Name: Nessler's Reagent Solution
Catalog Numbers: S80100, S801001MF, NC9650200, SN16I-500
Synonyms: None
Company Identification:
Fisher Scientific
1 Reagent Lane
Fair Lawn, NJ 07410
For information, call: 201-796-7100
Emergency Number: 201-796-7100
For CHEMTREC assistance, call: 800-424-9300
For International CHEMTREC assistance, call: 703-527-3887

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
7732-18-5	Water	70.94	231-791-2
1310-58-3	Potassium hydroxide	15.99	215-181-3
7774-29-0	Mercuric iodide	7.47	231-873-8
7681-11-0	Potassium iodide	5.60	231-659-4

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: yellow liquid.
Danger! Toxic. Causes eye burns. Causes digestive tract burns. Corrosive. Harmful if swallowed. May cause central nervous system effects. Causes skin burns. Causes respiratory tract burns. This substance has caused adverse reproductive and fetal effects in animals. May cause kidney damage.
Target Organs: Kidneys, central nervous system.

Potential Health Effects

Eye: Causes severe eye burns. May cause irreversible eye injury. Contact may cause ulceration of the conjunctiva and cornea. Eye damage may be delayed.
Skin: Causes skin burns. May cause deep, penetrating ulcers of the skin.
Ingestion: May cause kidney damage. May cause circulatory system failure. May cause perforation of the digestive tract. Causes severe digestive tract burns with abdominal pain, vomiting, and possible death.
Inhalation: Irritation may lead to chemical pneumonitis and pulmonary edema. Causes severe irritation of upper respiratory tract with coughing, burns, breathing difficulty, and possible coma.
Chronic: Prolonged or repeated skin contact may cause dermatitis. Prolonged or repeated eye contact may cause conjunctivitis. Prolonged or repeated exposure may cause adverse reproductive effects. Chronic exposure can lead to iodism characterized by salivation, nasal discharge, sneezing, conjunctivitis, fever, laryngitis, bronchitis, stomatitis, and skin rashes. May cause fetal effects.

Section 4 - First Aid Measures

Eyes: Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid imme diately.
Skin: Get medical aid immediately. Immediately flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes. Discard contaminated clothing in a manner which limits further exposure. Destroy contaminated shoes.
Ingestion: Do not induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Never give anything by mouth to an unconscious person. Get medical aid immediately.
Inhalation: Get medical aid immediately. Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.
Notes to Physician: Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information: Wear appropriate protective clothing to prevent contact with skin and eyes. Wear a self-contained breathing apparatus (SCBA) to prevent contact with thermal decomposition products.
Extinguishing Media: For small fires, use dry chemical, carbon dioxide, water spray or alcohol-resistant foam.
Flash Point: Not applicable.
Autoignition Temperature: Not applicable.
Explosion Limits, Lower: Not available.
Upper: Not available.
NFPA Rating: (estimated) Health: 3; Flammability: 0; Instability: 0

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks: Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container.

Section 7 - Handling and Storage

Handling: Wash thoroughly after handling. Wash hands before eating. Use with adequate ventilation. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale.
Storage: Store in a tightly closed container. Store in a cool, dry, well-ventilated area away from incompatible substances. Keep away from strong acids. Keep away from metals. Keep away from flammable liquids.

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Use adequate general or local exhaust ventilation to keep airborne concentrations below the permissible exposure limits.
Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Water	none listed	none listed	none listed
Potassium hydroxide	2 mg/m3 Ceiling	none listed	none listed
Mercuric iodide	0.025 mg/m3 TWA (as Hg) (listed under Mercury inorganic compounds).Skin - potential significant contribution to overall exposure by the cutaneous r oute (listed under Mercury inorganic compounds).	0.05 mg/m3 TWA (vapor, except organo alkyls, as Hg) (listed under Mercury compounds).10 mg/m3 IDLH (as Hg, not including organo(alkyl) compounds) (listed under Mercury compounds).	0.1 mg/m3 Ceiling (listed under Mercury, aryl and inorganic compounds).
Potassium iodide	none listed	none listed	none listed

OSHA Vacated PELs: Water: No OSHA Vacated PELs are listed for this chemical. Potassium hydroxide: No OSHA Vacated PELs are listed for this chemical. Mercuric

iodide: No OSHA Vacated PELs are listed for this chemical. Potassium iodide: No OSHA Vacated PELs are listed for this chemical.

Personal Protective Equipment

Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin: Wear appropriate gloves to prevent skin exposure.

Clothing: Wear appropriate protective clothing to prevent skin exposure.

Respirators: Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

Section 9 - Physical and Chemical Properties

Physical State: Liquid

Appearance: yellow

Odor: none reported

pH: Not available.

Vapor Pressure: Not available.

Vapor Density: Not available.

Evaporation Rate:>1 (ether=1)

Viscosity: Not available.

Boiling Point: Not available.

Freezing/Melting Point: Not available.

Decomposition Temperature: Not available.

Solubility: Completely soluble in water.

Specific Gravity/Density:1.1-1.3

Molecular Formula: Mixture

Molecular Weight: Not available.

Section 10 - Stability and Reactivity

Chemical Stability: Stable.

Conditions to Avoid: High temperatures, incompatible materials, light.

Incompatibilities with Other Materials: Potassium hydroxide reacts with chlorine dioxide, nitrobenzene, nitromethane, nitrogen trichloride, peroxidized tetrahydrofuran, 2,4,6-trinitrotoluene, bromoform+ crown ethers, acids alcohols, sugars, germanium cyclopentadiene, maleic dicarbide. Corrosive to metals such as aluminum, tin, and zinc causing formation of flammable hydrogen gas. Mercuric iodide is incompatible with strong oxidizing agents, chlorine trifluoride, potassium, sodium and light. Potassium iodide is incompatible with salts of alkaloids, chloral hydrate, mercurials, quinine, potassium chlorate, metallic salts, tannic and other acids, bromine trifluoride, and reactive powdered

Hazardous Decomposition Products: Hydrogen iodide, mercury/mercury oxides,

oxides of potassium.
Hazardous Polymerization: Has not been reported.

Section 11 - Toxicological Information

RTECS#:
CAS# 7732-18-5: ZC0110000
CAS# 1310-58-3: TT2100000
CAS# 7774-29-0: OW5250000
CAS# 7681-11-0: TT2975000
LD50/LC50:
CAS# 7732-18-5:
Oral, rat: LD50 = >90 mL/kg;
.

CAS# 1310-58-3:
Draize test, rabbit, skin: 50 mg/24H Severe;
Oral, rat: LD50 = 273 mg/kg;
.

CAS# 7774-29-0:
Oral, mouse: LD50 = 17 mg/kg;
Oral, rat: LD50 = 18 mg/kg;
Skin, rat: LD50 = 75 mg/kg;
.

CAS# 7681-11-0:
.

Carcinogenicity:
CAS# 7732-18-5: Not listed by ACGIH, IARC, NTP, or CA Prop 65.
CAS# 1310-58-3: Not listed by ACGIH, IARC, NTP, or CA Prop 65.
CAS# 7774-29-0: Not listed by ACGIH, IARC, NTP, or CA Prop 65.
CAS# 7681-11-0: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

Epidemiology: No data available.
Teratogenicity: Iodine salts can cause deformity, illness, and death of fetus. No information reported.
Reproductive Effects: Mercuric iodide can cause reproductive effects based on animal studies.
Mutagenicity: No data available.
Neurotoxicity: No data available.
Other Studies:

Section 12 - Ecological Information

No information available.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.
RCRA P-Series: None listed.
RCRA U-Series: None listed.

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name:	CORROSIVE LIQUIDS, TOXIC, N.O.S.	No information available.
Hazard Class:	8	
UN Number:	UN2922	
Packing Group:	II	

Section 15 - Regulatory Information

US FEDERAL

TSCA
CAS# 7732-18-5 is listed on the TSCA inventory.
CAS# 1310-58-3 is listed on the TSCA inventory.
CAS# 7774-29-0 is listed on the TSCA inventory.
CAS# 7681-11-0 is listed on the TSCA inventory.
Health & Safety Reporting List
None of the chemicals are on the Health & Safety Reporting List.
Chemical Test Rules
None of the chemicals in this product are under a Chemical Test Rule.
Section 12b
None of the chemicals are listed under TSCA Section 12b
TSCA Significant New Use Rule
None of the chemicals in this material have a SNUR under TSCA.
CERCLA Hazardous Substances and corresponding RQs

CAS# 1310-58-3: 1000 lb final RQ; 454 kg final RQ

SARA Section 302 Extremely Hazardous Substances

None of the chemicals in this product have a TPQ.

SARA Codes

CAS # 1310-58-3: immediate, reactive.
CAS # 7774-29-0: immediate, delayed.
CAS # 7681-11-0: immediate, delayed.

Section 313

This material contains Mercuric iodide (listed as Mercury compounds), 7.47%, (CAS# 7774-29-0) which is subject to the reporting requirements of Section 313 of

Clean Air Act:

CAS# 7774-29-0 (listed as Mercury compounds) is listed as a hazardous air pollutant (HAP).

This material does not contain any Class 1 Ozone depletors.
This material does not contain any Class 2 Ozone depletors.

CAS# 1310-58-3 is listed as a Hazardous Substance under the CWA.

None of the chemicals in this product are listed as Priority Pollutants under the

OSHA:

None of the chemicals in this product are considered highly hazardous by OSHA.

CAS# 7732-18-5 is not present on state lists from CA, PA, MN, MA, FL, or NJ.
CAS# 1310-58-3 can be found on the following state right to know lists:

CAS# 7774-29-0 can be found on the following state right to know lists: California, (listed as Mercury compounds), New Jersey, Pennsylvania, (listed as

compounds).

CAS# 7681-11-0 is not present on state lists from CA, PA, MN, MA, FL, or NJ.

California Prop 65

WARNING: This product contains Mercuric iodide, listed as `Mercury compounds', a chemical known to the state of California to cause developmental reproductive

California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations

European Labeling in Accordance with EC Directives

Hazard Symbols:

T

Risk Phrases:

R 20/21/22
swallowed.
R 33 Danger of cumulative effects.
R 35 Causes severe burns.

S 28 After contact with skin, wash immediately with...
S 45 In case of accident or if you feel unwell, seek medical advice