AMMONIA-N REMOVAL BY USING SOIL WATER-OPTIMIZATION

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We hereby declare that we have checked this thesis and in our opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Bachelor of Chemical Engineering (Biotechnology).

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STUDENT'S DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledged. The thesis has not been accepted for any degree and is not concurrently submitted for award of other degree.

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ABSTRACT

Ammonia-N occurs naturally and is produced by human activity. It is an important source of nitrogen which is needed by plants and animals. Bacteria found in the intestines can produce ammonia-N. In the poultry farm, ammonia-N can be found the chicken's manure, where in the high concentration of ammonia-N can cause health effect to the poultry. This is because ammonia-N was a strong colorless gas that can poison the respiratory system if exposed to the gas for a long period and in high concentration. Therefore, a solution must be studied to reduce the ammonia-N's concentration in the poultry farm. There are plenty of studies done to reduce the ammonia-N's concentration by the researchers. Soil water culture will be the best treatment since it is simple and the cost to do the treatment is cheaper than other treatments existed. There are several microorganisms that can be found in the soil mixed culture that are *Nitrosomonas* sp., *Nitrobacter sp, Nitrosolobus* sp., *Nitrospira sp, and Nitrovibrio* sp.. These microorganisms are important for nitrification process where ammonia-N's concentration will be reduced. Two parameters will be considered in these treatments, which are soil mixed culture to water ratio and the temperature during the treatment. In this study, it was aiming to optimize the parameters for the reduction of ammonia-N's concentration by using microorganism found in soil mixed culture. This study using optimization method, where a few combination of parameter were used to obtain the best process input values. For soil mixed culture, ratio of 1:4, 1:5, 1:6, 1:7 and 1:8 were used, while for temperature, range between 25°C to 35°C were used in the experiment. Soil mixed culture was mixed with ammonia-N solution at the best condition to reduce the ammonia-N's concentration by using nitrification process. The mixture of ammonia-N and soil mixed culture were incubated for about 3 hours. The concentration of the mixture was measured by using spectrophotometer. Based on 13 experimental runs, the experiment was proceed to the validation run where temperature of 27.5°C and 32.5°C and soil to water ratio of 1:5 and 1:7 were used to know the experiment error to choose the best parameters for ammonia-N removal. The best condition for the highest ammonia-N removal that was 57% were at temperature of 32.5°C and soil mixed culture of ratio 1:5 with the experiment error of 9.195%. Since the result obtained showed that ammonia-N concentrations

were closed to theoretical value using RSM, this can proved that RSM analysis was a useful technique for optimizing the nitrification of ammonia-N. From this study, the research can be furthered by testing the effect of pH on the nitrification process since nitrification also effected by pH value of soil mixed culture.

ABSTRAK

Ammonia-N terjadi secara semula jadi dan juga dihasilkan daripada aktiviti manusia. Ia adalah sumber penting di dalam nitrogen yang diperlukan oleh tumbuh-tumbuhan dan haiwan. Bakteria yang terdapat di dalam usus boleh menghasilkan ammonia-N. Di dalam industri penternakan ayam, ammonia-N boleh didapati di dalam najis ayam, di mana dalam kepekatan yang tinggi ammonia-N boleh mengganggu kesihatan ayam. Ini kerana ammonia-N boleh meracuni sistem pernafasan jika terdedah terlalu lama. Oleh itu, kajian harus dilakukan bagi mengurangkan kepekatan ammonia-N. Terdapat banyak kajian yang telah dijalankan untuk mengurangkan kepekatan ammonia-N oleh para penyelidik. Air campuran tanah adalah rawatan yang terbaik kerana ianya mudah dan kos untuk melakukan rawatan tersebut jauh lebih murah berbanding rawatan lain. Terdapat beberapa mikroorganisma yang boleh didapati di dalam air campuran tanah tersebut iaitu *Nitrosomonas* sp., *Nitrobacter* sp, *Nitrosolobus* sp., *Nitrospira* sp., dan *Nitrovibrio* sp.. Mikroorganisma ini penting untuk proses penitritan dimana kepekatan ammonia-N dapat dikurangkan. Dua parameter digunakan dalam rawatan ini, iaitu nisbah air campuran tanah dan suhu semasa rawatan. Dalam kajian ini, disasarkan dengan menggunakan mikroorganisma yang terdapat di dalam air campuran tanah, parameter untuk pengurangan kepekatan ammonia-N dapat dioptimakan. Kajian ini menggunakan kaedah pengoptimuman, di mana gabungan beberapa parameter akan digunakan untuk mendapatkan nilai masukan proses yang terbaik. Bagi air campuran tanah, nisbah 1:4, 1:5, 1:6, 1:7 dan 1:8 digunakan, manakala bagi suhu, julat di antara 25°C hingga 35°C telah digunakan di dalam eksperimen ini. Air campuran tanah tersebut telah dicampurkan dengan ammonia-N di kondisi terbaik untuk mengurangkan kepekatan ammonia-N dengan menggunakan process nitrifikasi. Campuran ammonia-N dan air campuran tanah tersebut telah disimpan di dalam inkubator selama 3 jam. Kepekatan campuran tersebut telah diukur dengan menggunakan spektrofotometer. Berdasarkan 13 eksperiment yang telah dijalankan, eksperimen diteruskan dengan eksperimen validasi, di mana suhu 27.5°C dan 32.5°C dan nisbah air campuran tanah 1:5 dan 1:7 telah digunakan untuk mengetahui ralat eksperimen, oleh itu, parameter terbaik untuk pengurangan ammonia-

N dapat ditentukan. Kondisi terbaik untuk mengurangan ammonia-N paling tinggi adalah sebanyak 57% adalah apabila suhu 32.5°C dan nisbah air campuran tanah 1:5 dengan ralat eksperiment sebanyak 9.195%. Disebabkan hasil eksperimen menunjukkan kepekatan ammonia-N menghampiri nilai teori menggunakan RSM, ia dapat membuktikan bahawa analisis mengunakan RSM merupakan teknik yang berkesan untuk mengoptimumkan nitrifikasi ammonia-N. Berdasarkan kajian ini, penyelidikan dapat diteruskan dengan menguji kesan pH ke atas proses nitrifikasi. Ini kerana proses tersebut juga dipengaruhi oleh pH air campuran tanah.

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LIST OF ABBREVIATIONS

AFDM	Ash Free Dry Mass
AMO	Ammonia Monooxygenase
ANFO	Ammonium Nitrate/Fuel Oil
ANAMMOX	Anaerobic Ammonium Oxidation
ANOVA	Analysis of variance
AOB	Ammonia Oxidizing Bacteria
BAFs	Biofilter Aerated Filters
CCD	Central Composite Design
Cd	Cadmium
CO_2	Carbon dioxide
DF	Degree of Freedom
DO	Dissolved Oxygen
DOC	Dissolved organic carbon
DOE	Design of experiment
EC	Electrical Conductivity
EPA	Environmental Protection Agency
GPS	Global Product Strategy

$H_2N_2O_2$	Hyponitrous acid
MBR	Membrane bioreactor
MLSS	Mixed Liquor Suspended Solids
MS	Mean Squares
NH_2OH	Hydroxylamine
NH_3	Ammonia
NH^{4+}	Ammonium
NH_4NO_3	Ammonium nitrate
NO_{2-}	Nitrites
NO_{3-}	Nitrates
O_2	Oxygen
ppm	parts per million
R.CO.NHOH	Hydroxamic Acid
RSM	Response Surface Methodology
SRP	Soluble Reactive Phosphorus
SS	Sum of Squares
SUVA	Specific Ultraviolet Absorbance
TOC	Total Organic Carbon
UMP	Universiti Malaysia Pahang
WWTP	Water Treatment Process

CHAPTER 1

INTRODUCTION

1.1 Motivation, Problem Statement and Brief Review

Ammonia-N is produced by microbiological decomposition of organic nitrogen compounds in manure (Ritz, Fairchild & Lacy, 2004). Ammonia-N, known to have very pungent odor will harm the chicken's respiratory system and eyes. The mucous membranes of chicken's respiratory system will irritated at the high concentration of ammonia-N in the air inside the chicken barn. And consequently, this situation enables the *E.coli* sp. to infect the respiratory system since there is no more protection in the respiratory tract. At the concentration of 10 ppm that happens over several weeks will cause the chicken's respiratory tract to damage because its ability to remove bacteria from their lungs is interfered. This is because the cells that produce mucus along the respiratory tract that enable it to trap bacteria are damaged by the high concentration of Ammonia-N. At 100 ppm of concentration of ammonia-N will decrease shell thickness and egg size and may cause some mortality (Aziz & Barnes, 2010). Ammonia-N also can affect the environment causing eutrophication and soil acidification. Eutrophication can cause lack of oxygen content in the lake, where can affect fish and other aquatic life, while soil acidification will affect the environment if there is no treatment carried out ("Impacts of ammonia," 2008). Ammonia-N can also pollute environment by cause smog and decreased visibility. When ammonia combines with NOx and SOx emissions from industrial and vehicle combustion processes it forms fine particulates. These fine particulates which are also known as PM2.5 are a contributor to haze or smog in cites and haze decreased visibility in pristine areas ("Impacts of ammonia," 2008).

By reduce the ammonia-N's concentration, the productivity of poultry farm can be maintained, thus, increase the income for the farmers. This study also will help to maintain the environment from pollution that will cause another effect such as extinction of certain aquatic life and reduce air pollution. There are few studies done by the researchers to reduce the amount of ammonia-N in the environment by using microorganisms. One of the studies is the quantification of viable ammonia oxidizing bacteria (AOB) extracted from soil. This technique was used to enumerate a *Nitrosomonas europaea* population after inoculation to soil and the indigenous AOB populations in native and N-enriched soils (Wang et al, 2012). The second study done by using ammonia inhibition of microbial activity in biological wastewater treatment process, studied by using a dehydrogenase assay with specific inhibitor of nitrification, strongly correlated with the total amount of ammonia and the pH of wastewater. Nitrifying bacteria were more sensitive than heterotrophic bacteria when the ammonia concentration went over above 3000 mg l^{-1} (Lee, Jung & Chung, 2000).

1.2 Objective

To optimize the parameter for the reduction of ammonia-N's concentration by using microorganism found in soil mixed culture.

1.3 Scope

The study covers the objective, which is the scope of study, is to obtain the highest amount of concentration reduced by using optimization. Two parameters are used in the interaction between ammonia-N and soil mixed culture and also the limitation of the experiments. The first parameter is temperature, where it is ranged between 25°C to 35°C. The second parameter is the ratio of soil to water, where it is 1:4, 1:5, 1:6, 1:7 and 1:8. At first, soil mixed culture will be prepared by using the ratio that is specified. Each soil mixed culture with the specified ratio will be prepared at the same time and will be stored in the refrigerator to stop the microorganism's activity. Ammonia-N solution is prepared by dissolving ammonium nitrate salt with deionized water.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Ammonia-N is the nitrate of ammonia that has the structure of white crystalline solid at room temperature and standard pressure. Its chemical formula is NH_4NO_3. Ammonia-N can be found in water where it is dissolved form of nitrogen inorganic compound and is the preferred form for algae and plant growth (Shifflett). Ammonia is found in water where dissolved oxygen is lacking since it is the most reduced form of nitrogen (Shifflett). Commonly used as chemical intermediate, Ammonia-N is also used as fertilizer at concentrations up to 27.5% ("GPS product safety," 2013). Not just known as high-nitrogen fertilizer, Ammonia-N also can be used as oxidizing agent in explosive devices such as ANFO[1], a very popular explosive. It is also used as hydrating salt, commonly known as instant cold packs in endothermic process ("Ammonium nitrate,").

Ammonia is anthropogenic point sources that is include household chemicals, the plastics industry, textile industry, oil refineries, iron and steel mills, meat processing plants, and sewage treatment plants (Hugdahl, 2012). Bacteria will quickly oxidize ammonia to nitrate when dissolved oxygen is readily available through nitrification process. Decomposition of dead plant and animal will produce ammonia by bacteria (Shifflett). Global nitrogen cycle played by ammonia as a key role since it is decomposed by nitrogen-rich organic matter (Hugdahl, 2012). High levels of ammonia can be toxic to life beneath the water depend on the temperature and measurement of its acidity of its pH (Shifflett). This is because fish and amphibians are lack of specific enzymes that detoxify ammonia (Hugdahl, 2012). A standard for ammonia

concentration at 0.019 mg/L is established by a Canadian Water Quality Guideline for Protection of Freshwater Aquatic Life. Ionized ammonia's toxicity is much less compared to un-ionized ammonia, therefore safer for aquatic life (Hugdahl, 2012). However, ammonia in drinking water is not considered toxic to human if in low concentration. This is because it naturally is produces in the human body, therefore efficiently detoxified and targeted by specific enzymes (Hugdahl, 2012). The toxicity of a given ammonia concentration can be increases in higher pH and temperatures. Pollution can be occurred when excessive aquatic production stimulated by high ammonia concentration. In 1990, ammonia pollution was listed as the top priority on Environment Canada's Canadian Chemical Spill Priority List where it is known as a very common environmental pollutant (Hugdahl, 2012). Human and animal wastes, fertilizers and byproducts from industrial manufacturing processes are important sources of ammonia to lakes and streams. To reduce ammonia concentration, a few techniques can be done that are filtration of runoff water from barnyards and other areas where animals kept in high amount, controlled fertilizing yards and proper septic system maintenance (Shifflett).

ANFO[1]-AN/FO, for ammonium nitrate/fuel oil

2.2 Effect of Ammonia-N to Environment

In UK, the significant areas of its valuable habitats are treated by ammonia that is an air pollutant largely emitted from agriculture (Webb et al., 2002). Fertilizers are the major sources of ammonium nitrate in the environment ("GPS product safety," 2013). Urine of cattle, pigs, sheep and other mammals that contain urea is the main source of ammonia (Webb et al., 2002).In streams, rivers and secondary sewage treatment processes, nitrification and denitrification occurred naturally ("GPS product safety," 2013). Ammonia gas is released during naturally occurring processes, such as farm livestock and other mammals excrete the breakdown of urea and birds excrete uric acid (Webb et al., 2002). The absence of bioaccumulation is very limited potential for secondary poisoning due to high water solubility and the ionic nature of the substance ("GPS product safety," 2013).

When reacted with other substances in the atmosphere, ammonia is easily forming ammonium (NH^{4+}) compounds such as ammonium sulphate and ammonium nitrate. Ammonia can effect natural ecosystem by deposited to land either as ammonium-N compound in rainfall or as a gas (Webb et al., 2002). Acidification and nitrogen enrichment of ammonia may damage sensitive habitats. Over the last 50 years, increasing of N input to livestock farming has increase ammonia emission. Wildlife habitats have been greatly reduced over recent years because of the emissions of other air pollutants such as sulphur dioxide (Webb et al., 2002).

Ammonia contains nitrogen, that is this deposition from the atmosphere onto soils and plants may damage plant communities. Removal of ammonium formed from combination of ammonia and others chemical, is from the atmosphere by rain while ammonia itself will be absorbed by land surfaces such as soil and water (Webb et al., 2002). This is because nutrient-poor habitats have been evolved by increasing amount of nitrogen in the soil. Although this extra nitrogen is beneficial to for growth of plants, this enrichment of nitrogen will cause phenomena called eutrophication (Webb et al., 2002). Eutrophication will cause the existing species that not able to cope with extra nitrogen to be replace by fast growing grass species that is undesirable growth. For example, in UK, it has being concerned since eutrophication occurred on many valuable conservation areas (Webb et al., 2002).

Furthermore, Ammonia will also cause soil to become acidic. This is because the ammonia is oxidized to nitrate once deposited on soil. The acidification can be neutralizing by soil, but when the increasing amount of acid deposited is too high, the soil will not be able to cope with it. Pain & Jarvis (1999) claimed that increase in soil acidity can result from nitrification to nitrates. This affects the solubility of both essential and toxic elements which can be particularly damaging to woodlands on weakly buffered soils.

Fish and other aquatic life may be poisoned when toxic elements leached into surface water. Acidified lakes and streams in many upland areas of UK are the most obvious examples of acidification. Most of ammonia deposition close to where it was emitted since ammonia is a very reactive gas (Webb et al., 2002). However, ammonia

can also be deposited as a rainfall at long distance if it reached higher levels in the atmosphere and being blown away. Combination of ammonia, nitrogen and sulphur will form particles containing ammonium-N that can travel for hundreds miles. This will cause others country emission to affect other country (Webb et al., 2002). The most toxic form of the ammonium ion is when it is in the un-ionized form ("Ammonia,"). There are a few physico-chemical parameters that caused by toxicity of ammonia to aquatic life, that are pH, dissolved oxygen, temperature and salinity. When the high temperature and pH, and lower levels of dissolved oxygen and oxygen salinity, it will cause greater ammonia toxicity ("Ammonia,"). When there are low dissolved oxygen concentrations, acute toxicity if ammonia is increases ("Ammonia,"). At low dissolved oxygen concentration toxicity of ammonia to fish increases ("Ammonia,").

Volatilization are the process where ammonia moves from an area of high concentration such as urine in form of liquid to an area of lower concentration that is atmosphere in form of gas (Webb et al., 2002). The major sources of ammonia emission are the livestock manures. The emission of ammonia depends in the surface area covered with urine or manure, therefore, will cause the emission are large from floors of livestock houses and after manure has been spread. Actually, since animals were first domesticated, ammonia emission from mammal manure and urine is a natural process. The use of nitrogen in fertilizers for grassland and in animal feeds has greatly increased since increases of livestock production. Greater emissions of ammonia and nitrogen in dung and urine increases caused by inefficient nitrogen conversion in the livestock diet's milk and meat. Most type of nitrogen fertilizers produced ammonia emission, but urea based fertilizers produce the greatest emissions (Webb et al., 2002). 80% of ammonia emissions in the UK come from agriculture particularly manure management. This huge amount not only represents the effect of wider environment but also represent an inefficient usage of nitrogen as a fertilizer (Webb et al., 2002). Meisinger & Jokela (2000) claimed that ammonia volatilization occurs because ammonium-N in manure or solution is converted to dissolve ammonia gas, by the reaction:

$$NH_4^+\text{-}N \rightleftarrows NH_{3g} + H^+$$

Ammonia also affects the life of invertebrates due to its toxicity. There are a few studies done to investigate the effects of ammonia on invertebrates. Allan et al (1990) stated that 96 hour LC50s for juvenile school prawns *Metapenaeus macleayi* and leader prawns *Penaeus monodon* to be 1.39 and 1.69 mg un-ionized ammonia NH3-N/l (26.3 mg and 37.4 mg total ammonia-N/l respectively) ("Ammonia,"). As salinity decreases, toxicity of ammonia appears to increases as stated by Miller et al (1990) and Chen and Lin (1991). Ammonia toxicity is indicated by several studies is greatest to early stages of invertebrates ("Ammonia,"). Malet et al (1992) stated that the majority of ammonium toxicity data relates to fish, although most of the species tested is freshwater species, with many coarse fish appearing to be as sensitive to ammonia as salmons. Eddy and Twitchen (1990) suggested that high environmental sodium concentrations can decrease toxicity to fish ("Ammonia,"). Seager et al (1998) and Miller et al (1990) states that ammonium toxicity appears to be less at lower salinity levels for fish, and will gradually decreasing until it reaches a point similar to that found in freshwaters

2.3 Nitrification

The process by which ammonia is converted to nitrites (NO_{2-}) and then nitrates (NO_{3-}) is known as nitrification. This process is carried out by specialized bacteria, where it naturally occurs in the environment. Nitrification in soils and other matrices is initiated by ammonia-oxidizing bacteria where the chemolithotroph *Nitrosomonas europaea* is the most extensively studied. Conversion of ammonia to nitrite is aided with two enzymes that are ammonia monooxygenase (AMO), which oxidizes ammonia to hydroxylamine in a reductant-dependent reaction, and hydroxylamine oxidoreductase, which oxidizes hydroxylamine to nitrite with the release of four electrons. To provide reductant for subsequent oxidations, two electrons are returned to AMO while the remaining reductant needs of the cell are fulfilled by the other two electrons (Hommes, Russell, Bottomley & Arp, 1998). The context of nitrogen cycling in terrestrial ecosystems is already study about nitrification in soils. Nitrification rates are affected by a number of physical and chemical factors. Oxygen, O_2 is typically required for nitrification, and soil conditions which limit O_2 availability will inhibit nitrification. Hommes et al. (1998) claimed that ammonia oxidation is most rapid in

neutral to alkaline soils and rates are inhibited under acidic conditions where the NH^{4+}-NH_3 equilibrium is driven toward NH^{4+} and the availability of NH_3 becomes limiting. NH_3 and not NH^{4+} are the substrate for AMO.

The most common cause of fish mortality in aquariums is an imbalance in the nitrogen cycle. Degradation of nitrogen-containing organic matter such as fish waste and uneaten food will produce ammonia rapidly in aquariums. To eliminate ammonia, healthy, established aquariums contain at least two bacterial strains that work together in a 2-step process known as nitrification. Hugdahl (2012) stated that *Nitrosomonas* bacteria first oxidize ammonia into nitrite (NO_2), which is also highly toxic to fish. In the 2nd step, *Nitrobacter* bacteria convert nitrite into nitrate (NO_{3-}), which is tolerated by fish at much higher levels. In newly aquariums that don't have established colonies of these two necessary bacterial strains will usually build up toxic levels of ammonia quickly (Hugdahl, 2012).

Nitrification is about reduction forms of N which is chiefly an ammonium are converted into nitrites or nitrates in soil and available data on physiology, biochemistry, classification, and energy expenditure of the nitrifying bacteria are summarized ("Nitrification,"). The relative contribution of autotrophs and heterotrophs is impossible to gauge to overall nitrification in soil. Autotrophs appear to be 2-10 times more active in laboratory while heterotrophs capable of oxidizing ammonia. it is possible that the apparently greater nitrifying efficiency of *Nitrosomonas* and *Nitrobacter* that are autotrophs merely reflects failure to discover an optimal medium for the activity of the heterotrophs *Actinomyces flavus* and *A. wentii* ("Nitrification,").

Nitrification process is exothermic and the reduction of CO_2 to carbohydrates uses a portion of the energy of the nitrification reactions ("The nitrifying organisms,"). Only about 7% of the energy of nitrification for carbohydrate formation is used, where nitrifies are not very efficient at this conversion. Ammonia or nitrites must be oxidized extensively in order to grow, and aeration is forced in the laboratory in order to get good growth of the bacteria. The oxidation of NH_3 to NO_3 is via obvious path that is NH_2OH and $H_2N_2O_2$. Since NH_2OH is not oxidized by *nitrosomonas* bacteria, this does not seem to be followed in the first step and is fairly toxic to them even in very low

concentrations. Utilizing a carboxylic acid combining with NH_3 to form an amide and then a hydroxamic acid, R.CO.NHOH, the formation of the necessary hyponitrous acid can be explained if it is assumed as cycle. If they are antagonists to the true hydroxamic acid taking part in the nitrification reaction, compounds having structures similar to hydroxamic acid are toxic to nitrification ("The nitrifying organisms,").

There are few factor that will affect nitrification, that are temperature, pH, dissolved oxygen, conductivity, salinity, sediment ash-free dry mass (AFDM), specific ultraviolet absorbance (SUVA), stream water NH_4^+ concentration, DOC concentration, redox potential, soluble reactive phosphorus concentration (SRP), and total extractable NH_4^+ (Strauss, Mitchell & Lamberti, 2002).

2.3.1 Temperature

Sommer et al (1991) claimed that when the temperature increases, the rate of ammonia volatilization increases with greater effect observed in the first several hours after application (Meisinger & Jokela, 2000). By decreasing the solubility of NH_3 gas, the higher temperature increase ammonia losses in the soil solution and by increasing the proportion of TAN as NH_3 gas. Higher temperature should cause ammonia losses to increase by a factor of about 3 for every 18°F (10°C) rise in temperature as predicted in physical chemistry. As an example, a slurry contain 1500 mg NH_4-N/1 at pH 7.8 would support equilibrium gaseous ammonia pressure of about 7, 23, and 69 mbar at temperatures of 50, 68 and 86°F (10, 20, and 30°C, respectively (Meisinger & Jokela, 2000). Beauchamp et al (1982), Harper et al (1983), Nathan & Malzer (1994), Sommer & Olesen (1991) also claimed that temperature effects on ammonia loss but all the temperature effects have been less dominant than theory would suggest. This is because the ammonia concentrations are seldom at equilibrium and because losses are also influenced by gaseous transport factors. Near-freezing temperatures do not stop ammonia losses (Meisinger & Jokela, 2000). Losses near freezing can occur because a lower, but still substantial rate of volatilization contuse for a longer perios of time at claimed by Sommer et al (1991) and because freezing can have the same NH_4-N/1 concentrating effect as drying stated by Midgely & Weiser (1937) and Lauer et al (1976) (Meisinger & Jokela, 2000).

2.3.2 pH

Strauss, Mitchell & Lamberti (2002) claimed that pH has received considerable attention in soils, pure culture and wastewater treatment. For example, it is reported increased nitrification through a pH gradient of 4.2–6.2 in a spruce-forest soil (Paavolainen and Smolander, 1998). The optimum pH for nitrification was approximately 7.8 over a range of 6.4–8.2 in wastewater treatment sludge (Antoniou et al. 1990). Watson et al. (1989) recommend NH4 +-oxidizing bacteria in media pH of 7.5–7.8 for culturing pure strains. Strauss et al. (2002) also observed an optimum pH of 7.5 consistent with previous studies, thus create positive relationship between pH (up to 7.5) and nitrification and is related to the increasing availability of NH_3, which is believed to be the true substrate for oxidation as said by Suzuki et al. (1974). The relative NH_3 concentration increases as pH increases, by nearly a full order of magnitude for each pH unit (Emerson et al. 1975). The advantages of increased availability of free NH_3 may be counterbalanced by the energy required to maintain the cytoplasmic pH below that of the external environment at above the optimum pH for nitrification (Wood 1988). In the other hand, enzyme activity is negatively affected when pH falls below the optimum, cause nitrification reducing (Prosser 1989). Strauss et al. (2002) claimed that in the 36 streams they surveyed, nitrification rates were low at low pH, but variable at higher pH. This shows that nitrification is enhanced at circumneutral pH values but inhibited at low pH.

2.3.3 Dissolved Oxygen (DO)

Hocaoglu, Insel, Cokgor & Orhon (2010) studies shows that a fully aerobic membrane bioreactor was run at a sludge age of 60 days fewer than three low dissolved oxygen (DO) levels below 0.5 mg/L, where effective simultaneous nitrification/denitrification is sustained for the entire observation time. They also claimed the adverse effects of a number of factors such as low DO level, alkalinity limitation will cause incomplete nitrification. Within the DO range of 0.15–0.35 mg/L, nitrogen removal remained optimal (Hocaoglu et al., 2010). The extent of simultaneous nitrification and denitrification in MBR operation with different MLSS levels is regulated in dissolved oxygen level that acts as the main diffusion control parameter. In

this study, low DO range of 0.15–0.35 mg/L will result in optimal efficiency of nitrogen removal from black water, which allowed full removal of the available oxidized nitrogen (Hocaoglu et al., 2010).

Zafarzadeh, Bina, Nikaeen, Attar & Khiadani (2011) claimed that as the oxygen functions as the electron acceptor for microorganisms over nitrate, and aerobic conditions repress enzymes involved in denitrification, DO can inhibit the denitrification reaction. Their study also shows that nitrate removal decreased in the anoxic reactor, as DO concentrations in the aerobic reactor increased (above 2.7 mg O2/l) (Zafarzadeh et al., 2011).

2.3.4 Conductivity

Akahane, Makino & Maejima (2010) claimed that the changes in electrical conductivity (EC) and pH could be attributed to the nitrification of urea and soil organic matter, which in turn increases of Cadmium (Cd) desorption from the soil. Nitrification will cause increases of pH and decreases of EC. W-Cd was affected by EC more strongly than pH for the gray lowland soil that is used in this study (Akahane et al., 2010).

Levlin claimed it is the three water treatment process (WWTP) with biological nitrogen removal that has a decrease in conductivity in the treatment process shows that ammonium nitrogen and alkalinity, which is reduced at biological nitrogen removal, contributes to conductivity with about 33 % and 14 % respectively.

2.3.5 Soil Mixed Culture

The oxidation of alternative substrates as well as on the oxidation of NH_3 by *N. europaea* can have a profound impact of soil. Hommes et al. (1998) claimed that NH_3 oxidation was influenced both by exchangeable acidity and by the adsorption of NH_4^+ to the soil with the Willamette silt loam used in this study. The factor that is identified is also likely to be important in structured soils at or below field moisture capacity that is intact soils with different moisture contents. The importance of soil moisture to the availability of NH_4^+ for nitrification is described by Stark and Firestone (Hommes et al., 1998).

Biocidal effects of chlorinated aliphatic hydrocarbons on cell growth of ammonia oxidizers in experiments that occurred over long time periods is observed by Fuller and Scow (Hommes et al., 1998). Rates of co-substrate oxidation would be sensitive to the sequestering of NH_4^+ by the soil, thus the effects of soil would be particularly relevant to in situ bioremediation or bio augmentation plans, with the result that rates of co-substrate oxidation could be reductant limited (Hommes et al., 1998).

Gundersen (1955) stated that it has always been considered a difficult task to isolate the nitrifying bacteria in pure culture. Getting rid of heterotrophic soil bacteria that seem to adhere strongly to the nitrifiers is the difficulty mention. Whether a sort of symbiotic relationship exists between the nitrifying bacteria and certain heterotrophic micro-organisms in the soil is issued by Gundersen.

Kingma Boltjes investigated a small bacterium which was identified as *Hyphornicrobium vulgare* and frequently was seen on mineral silica gels in association with *N itrobacter*. Kingma Boltjes succeeded in isolating the nitrifier by the use of a micromanipulator found that a mycobacterium named *Sorangiurn syrnbioticum* developed together with nitrifying bacteria when silica gels were inoculated with soil particles (Gundersen, 1955). When the mycobacterium later became the dominating organism, growth of the nitrifying bacteria took place initially but was followed by autolysis of the cells (Gundersen, 1955). This phenomenon is called a symbiosis by Imsenecki, but it should called metabiosis a rather common phenomenon in nature according to general terms.

Nitrosomonas is cultured together with *B. megaterium, B. rnycoides, Azotobacter chroococcum* and other bacteria by Pandalai, and found in all cases enhanced nitrification in his mixed cultures. Several heterotrophic soil bacteria are isolated by Stapp from enrichment cultures of nitrifying bacteria. All of them were pigment producers of the Bacterium- and Pseudomonas-type with one exception. These heterotrophs that are three species from ammonia-oxidizing bacteria (*Nitrosomonas*) and four from nitrite-oxidizing (*Nitrobacter*) were extremely tolerant towards high concentrations of ammonia and nitrite, even in a 30 per cent~/mmonium sulphate medium growth was visible, and one (a Pseudomonas) tolerated 2 per cent sodium

nitrite in the medium (Gundersen, 1955). The viability of the heterotrophic bacteria in inorganic media was higher in the presence of nitrifiers than when in pure culture. When cultured together with *Nitrosomonas*, growth of the heterotrophs associated with the *Nitrosomonas* group was not accelerated, whereas growth of the heterotrophs from the *Nitrobacter* group was so when cultured together with *Nitrobacter*. In mixed cultures of these bacteria, it is found accelerated nitrification by Stapp. He does not believe in a specific action on the nitrification process, as different investigators always find different heterotrophic bacteria adhering to the nitrifiers. Gundersen (1955) found that heterotrophic soil bacteria isolated from enrichment cultures of nitrifiers were able to neutralize the toxic effect of peptone, phenylalanine and tyrosine on *Nitrosomonas* when in mixed culture.

Jensen stated that nitrification can take place rapidly in soil and even in farmyard manure is explained by this observation in presence of considerable quantities of organic matter. These autotrophic bacteria are very sensitive to a variety of organic compounds in pure cultures. Some observations made on mixed cultures of *Nitrosomonas* and heterotrophic bacteria from enrichment cultures of the nitrifier is the present investigation comprised (Gundersen, 1955). Gleeson, Herrmann, Stephen J. & Murphy (2008) suggest that in semiarid soils water potential plays a key role in determining the structure of ammonia oxidizing bacteria (AOB), and that additionally AOB community structure is correlated to potential nitrification rate in these soils. A microbial functional group such as autotrophic ammonia oxidizing bacteria (AOB) mediate nitrification, and are influenced by a variety of environmental factors, including water content, that dictate community parameters. It is found that between AOB community structure and potential nitrification rate (Gleeson et al., 2008).

2.4 Previous work on Ammonia-N removal

2.4.1 Interactive effect of ammonia and nitrate on the nitrogen uptake by *nannochloropsis* sp.

Hii, Soo, Chuah, Mohd-Azmi & Abol-Munafi (2011) claimed that microalgae are able to assimilate various types of nitrogen. Concerning microalgae are used as a

biological treatment in waste water, the interaction of nitrogen on their removal by microalgae should be of grea. This investigation aims by using *Nannochloropsis* sp. to reveal the preference of nitrogenous compounds (Hii et al., 2011). 900 μM nitrates, 900 μM ammonium, and 900 μM nitrates plus 900 μM ammonium are the medium for microalgae cultivation.

When both compounds that are ammonium and nitrate were available to the microalgae, *Nannochloropsis* sp. preferred ammonium rather than nitrate. Hii et al. & Abol-Munafi (2011) said that nitrate was utilized in the absence of ammonium. There was significantly higher than nitrate ($p = 0.004$) in the uptake rate of ammonium. With different nitrogen sources ($p < 0.000$), there was significant difference in the growth rate of the microalgal. *Nannochloropsis* sp. growths are faster in treatment containing ammonia. However, cell density produced by the cultures in different nitrogenous nutrients shows that there were no significant differences. *Nannochloropsis* sp. can be used as a biological treatment for waste water. Ammonium will be first remove by the microalgae and ammonia toxicity is remediated to most fish. Nitrate can be only removed by the microalgae once after ammonium has been removed or brought down to a safer level, nitrate can be removed by the microalgae.

Nitrate would be utilized in the lack of ammonia. *Nannochloropsis* sp. was able to produce same cell densities growing on both ammonia and nitrate because uptake rate of ammonia was higher than nitrate. Based on Hii et al. studies, the results showed that *Nannochloropsis* sp. can be a potential biological treatment for aquaculture water. The problems of ammonia toxicity in the culture system was solved by the preferential uptake of ammonia by *Nannochloropsis* sp.. Before discharge the microalgae will utilize nitrate from the aquaculture water and bring it down to a safe level (Hii et al., 2011)

2.4.2 Nitrogen Removal from Wastewater Using Simultaneous Nitrate Reduction and Anaerobic Ammonium Oxidation in Single Reactor

Sumino, Isaka, Ikuta, Saiki & Yokota (2006) claimed that in this system, nitrogen removal ratio was affected by C/N ratio and TOC loading, not by the amount

of granular sludge which is taken from methane fermentation reactor was placed in an up flow reactor and supplied with synthetic wastewater containing nitrate at a C/N ratio of 1 to grow heterotrophic denitrifying bacteria. ANAMMOX sludge attached to nonwoven-carrier was added into the same reactor when nitrogen removal ratio reached 30%, and then ammonia was added to the synthetic wastewater (Sumino et al., 2006). Nitrogen removal ratio was markedly increased to 80–94%. N2 gas was formed by anammox reaction is shown by a stable isotopic analysis using 15N-labeled nitrate showed (Sumino et al., 2006).

Sumino et al. (2006) also claimed in this study, they are examined the possibility of combining nitrate reduction and ANAMMOX through the process involving a sequence of nitrate reduction to nitrite taking place in granular sludge and anaerobic ammonium oxidation with nitrite as the electron acceptor occurring in ANAMMOX sludge attached to nonwoven carrier in a single reactor. Sumino et al. (2006) said that, both ANAMMOX and heterotrophic denitrification proceeded simultaneously.

From the studies, nitrogen removal through anaerobic ammonium oxidation and simultaneous nitrate reduction in a single reactor is considered to be feasible for the treatment of wastewater containing low nitrogen content such as industrial wastewater and domestic wastewater (Sumino et al., 2006).

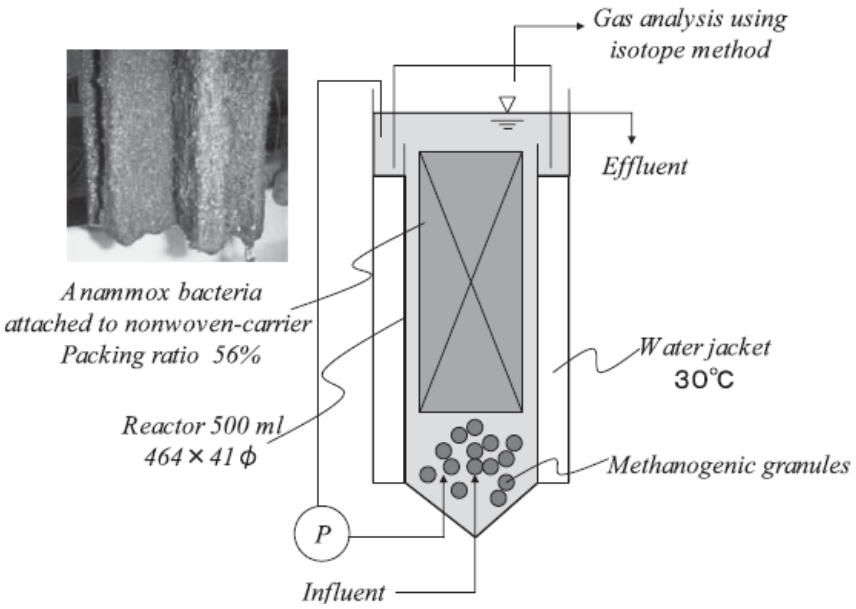

Figure 2.4.2: Bench-scale reactor.

2.4.3 Using *Bacillus amyloliquefaciens* for remediation of aquaculture water

Xie, Zhu, Li, Zhang & Zhou (2013) said that remediation of aquaculture water using microorganisms like *Bacillus* species is a burgeoning trend for the sustainable development of aquaculture industries. Based on this work, a *Bacillus amyloliquefaciens* strain known as namely *B.amyloliquefaciens* HN, isolated from activated sludge of a polluted river, and was evaluated for its potential in water remediation using simulated aquaculture water. *B. amyloliquefaciens* HN exhibited high tolerance towards 80 mg l-1 of nitrite-N and ammonia-N which shows that it could effectively remove 20 mg l-1 of nitrite-N. Unfortunately, when the ammonia-N concentration was below 20 mg l-1, it was inefficient in eliminating ammonia-N. After that, further studies had been done and showed at 30°C and 35°C than 25°C the ammonia-N removal by *B. amyloliquefaciens* HN was more efficient, and that maximum nitrite-N removal rate was achieved at pH 8 (Xie et al., 2013). Microorganisms have become burgeoning trends in these few recent years to improve water quality.

Since *Bacillus* they are stable for long period due to spore formation, easily prepared by fermentation and possess antagonistic effects on pathogens species, they are widely used for water remediation. For their potential as biological agents for water quality enhancement, strains belonging to several Bacillus species, such as *Bacillus subtilis, Bacillus cereus, Bacillus licheniformis, Bacillus pumilus* were isolated and evaluated (Xie et al., 2013). Towards developing commercial microbial agents, screening strains with good remediation characteristics still remains a fundamental step.

Xie et al., (2013) claimed that they isolated a *Bacillus amyloliquefaciens* strain, named as *B. amyloliquefaciens* HN from the activated sludge of a polluted river and it has being proved that this strain was shown to effectively remove nitrogenous compounds and grow in broad temperature, pH, and salts concentration in preliminary studies. No previous studied has characterized the nitrogen removal ability of *B. amyloliquefaciens*, thus aiming to evaluate *B. amyloliquefaciens* HN for its remediation properties using simulated aquaculture water.

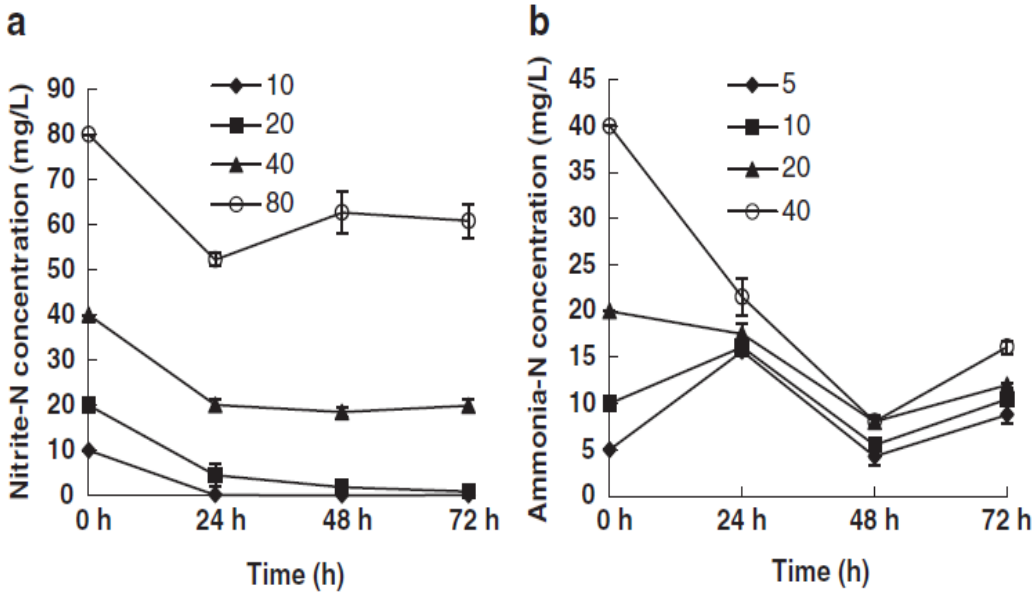

Figure 2.4.3a Nitrite and ammonia-N removal tests *for B. amyloliquefaciens* HN. A) Nitrite removal tests; b) Ammonia-N removal tests.

2.4.4 Removal of a High Load of Ammonia by a Marine Bacterium, *Vibrio alginolyticus* in Biofilter

Kim & Shoda (2002) claimed that a newly isolated heterotrophic marine bacterium, *Vibrio alginolyticus*, was used to remove a high load of ammonia gas under non-sterile condition. High load of ammonia, in the range of ammonia gas concentration of 170 ppm to 880 ppm, was introduced continuously after the cells were inoculated onto an inorganic packing material in a fixed-bed reactor (biofilter). Result shows about three times greater than those obtained in nitrifying sludge inoculated onto the same packing material when maximum removal capacity and the complete removal capacity were 19 g-N kg-1 dry packing material day-1 and 16 g-N kg-1 dry packing material day-1, respectively. During this operation, the non-sterile condition had no significantly adverse effect on the removability of ammonia by *V. alginolyticus* (Kim & Shoda, 2002).

A high load of ammonia from the start of cultivation could be applied by using a heterotrophic marine bacterium (Kim & Shoda, 2002). Direct introduction of 300 ppm

of ammonia for nitrifying bacteria, as a starting of experiments had a severe inhibitory effect on growth and at 880 ppm, they could not carry out nitrification. These shows for the removal of relatively high loads of ammonia, *V. alginolyticus* will be applicable (Kim & Shoda, 2002). About 36% of removed nitrogen was converted to alanine by *V. alginolyticus*. Acidic products extracellular like pyruvic acid and uronic acid that can neutralize ammonium ions were reported produced by *V. alginolyticus*. The similar metabolism by *V. alginolyticus* may have occurred in spite of the existence of contaminated microorganisms in the biofilter experiment (Kim & Shoda, 2002).

Alanine or ammonium ions supposed utilized by the contaminated microorganisms because only small amount of alanine or ammonium ions were detected in the elute from the biofilter. A stable removal of high load of ammonia gas in the biofilter may lead by this coexistence of the marine bacterium and the contaminants may lead (Kim & Shoda, 2002). To remove a high load of ammonia gas in a biofilter, a heterotrophic marine bacterium was firstly used where nitrifying bacteria cannot be applicable. Ammonia gas was removed efficiently without acclimation period of the bacterium to ammonia gas from the start of experiment (Kim & Shoda, 2002).

Kim & Shoda (2002) said that high removability was maintained even under the non-aseptic condition where the accumulated cells on the inorganic packing material after a long operation were detached by NaOH treatment. The marine bacterium can give an alternative method to treat high concentration of ammonia gas, as the maintenance of the heterotrophic marine bacterium is easier than that of autotrophic bacteria (Kim & Shoda, 2002).

2.5 Analysis method for Ammonia-N content

2.5.1 Ammonia-N in Water and Biosolids by Automated Colorimetry with Preliminary Distillation

Ammonia-N in drinking, ground, and surface water; domestic and industrial waste; and bio solids can be determined by using these procedures. Ammonia is distillate from the sample before analyze by automated colorimetric analyzer ("Ammonia-n in water," 2001). This method is based on U.S. Environmental Protection

Agency (EPA) Methods 350.1: Nitrogen, Ammonia (Colorimetric, Automated Phenate) and 350.2: Nitrogen, Ammonia (Colorimetric; Titrimetric; Potentiometric Distillation Procedure). This method is associated with Method 1691: Municipal Biosolids Sampling Guidance ("Ammonia-n in water," 2001).

Much potential interference is eliminated through separation by distillation of ammonia. The ammonia released is captured in a dilute sulfuric acid solution. Colorimetric measurement of indophenol blue is used in determining the ammonia which is formed when ammonia reacts with alkaline phenol and hypochlorite. Quality is assured through calibration and testing of the analytical instruments and testing of the sample preparation ("Ammonia-n in water," 2001).

2.5.2 Ammonia-N in wastewater by HACH Spectrophotometer

This method is used to identify the concentration of ammonia-N in the waste water where it is called Salicylate Method (*Nitrogen, ammonia,* 2007). The items needed are Ammonia Cyanurate Reagent pillows, Ammonia Salicylate Reagent pillows, and Sample Cells, 1-inch square, 10-mL. Ammonia compounds combine with chlorine to form monochloramine. Monochloramine reacts with salicylate to form 5-aminosalicylate (*Nitrogen, ammonia*, 2007). The 5-aminosalicylate is oxidized in the presence of a sodium nitroprusside catalyst to form a blue-colored compound. The blue color is masked by the yellow color from the excess reagent present to give a final green-colored solution. Test results are measured at 655 nm (*Nitrogen, ammonia*, 2007).

2.6 Optimization

2.6.1 Research Surface Methodology (RSM)

Response surface methodology (RSM) which is introduced by G. E. P. Box and K. B. Wilson in 1951 has the function to use a sequence of designed experiments to obtain optimal parameters. Aslan (2007) said that RSM is a collection of statistical and mathematical methods that are useful for the modeling and analyzing engineering problems. In this technique, optimization of the response surface that is influenced by various process parameters is the main objective. The relationship between the

controllable input parameters and the obtained response surfaces also quantifies by RSM (Aslan, 2007).

Aslan (2007) also shows the design procedure for the RSM, which is as follows:

1. A series of experiments for adequate and reliable measurement of the response of interest is designed.
2. A mathematical model of the second-order response surface with the best fittings is developed.
3. The optimal set of experimental parameters that produce a maximum or minimum value of response is found.
4. The direct and interactive effects of process parameters through two and three-dimensional (3D) plots is represented.

Khuri & Mukhopadhya (2010) said that Response surface methodology (RSM) consists of a group of mathematical and statistical techniques used in the development of an adequate functional relationship between a response of interest, y, and a number of associated control (or input) variables denoted by $x_1, x_2, \ldots, x_k$.

2.6.2 The Central Composite Design

The RSM will optimize the data by using The Central Composite Design (CCD). CCD consists of the following three portions:

1. Factorial portion, which is a complete 2k factorial design whose factors' levels are coded as $-1, 1$.
2. An axial portion that consist 2k points arranged so that two points are chosen on the axis of each control variable at a distance of α from the design center
3. N0 center points.

CCD is obtained by augmenting a first-order design, namely, the 2k factorial with additional experimental runs, namely, the 2k axial points and the n0 center-point replications (Khuri & Mukhopadhya, 2010). Therefore, a manner consistent is developed by this design with the sequential nature of a response surface investigation in starting with a first-order design, to fit a first-degree model, and then followed by the addition of design points to fit the larger second-degree model (Khuri & Mukhopadhya,

2010). The first-order design serves in a preliminary phase to get initial information about the response system and to assess the importance of the factors in a given experiment. The additional experimental runs are chosen for the purpose of getting more information that can lead to the determination of optimum operating conditions on the control variables using the second-degree model.

Determination of Optimum Conditions One of the main objectives of RSM is the determination of the optimum settings of the control variables that result in a maximum (or a minimum) response over a certain region of interest, R (Khuri & Mukhopadhya, 2010). This requires having a 'good' fitting model that provides an adequate representation of the mean response because such a model is to be utilized to determine the value of the optimum (Khuri & Mukhopadhya, 2010). Optimization techniques used in RSM depend on the nature of the fitted model. For first-degree models, the method of steepest ascent (or descent) is a viable technique for sequentially moving toward the optimum response.

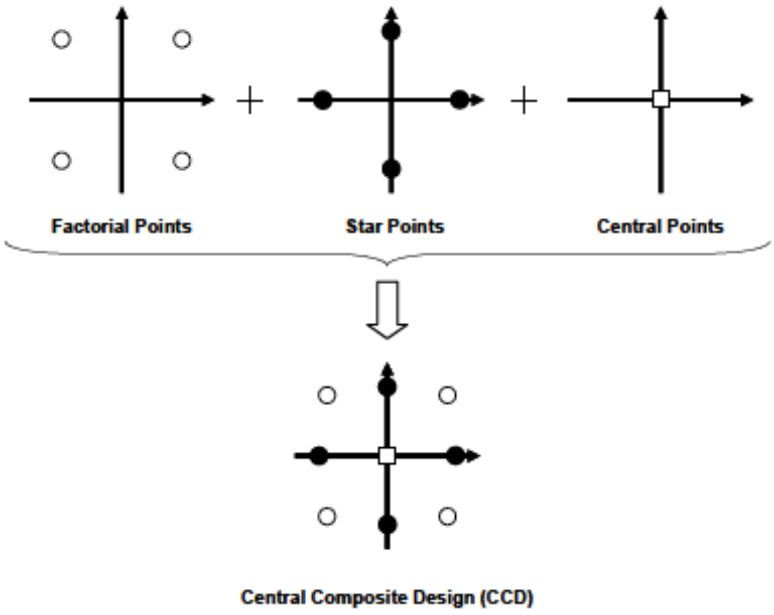

Figure 2.6.2 Central Composite Design (CCD) for two factors

2.7 Summary

Ammonia-N is the nitrate of ammonia that is commonly used as fertilizer. It has the structure of white crystalline solid at room temperature and standard pressure.

Ammonia-N have several effect on the environment such as it cause eutrophication, toxic substances for aquatic life and can cause acidification of soil. Ammonia-N may also cause rainfalls that contain high concentration of nitrogen that can cause several complications. Ammonia-N concentration can be reduce by process nitrification, that is reduction of ammonia to nitrite by *Nitrosomonas* bacteria and by using *Nitrobacter* bacteria convert nitrite into nitrate. Nitrification can be affected by a few parameters such as temperature of soil, pH, and soil to water ratio. Higher temperature leads to higher ammonia losses while additionally AOB community structure is correlated to potential nitrification rate in this soil, which means the higher the soil to water ratio, the better the removal of ammonia process. There are a few studies on Ammonia-N removal done by researcher that are through the usage of microbial. To obtain the best parameter, optimization by using research surface methodology (RSM) was used.

CHAPTER 3

MATERIALS AND METHODOLOGY

3.1 Introduction

Ammonia-N removal was identified by using HACH Spectrophotometer where concentration of ammonia-N was obtained. The ammonia-N undergoes the process of nitrification to remove the ammonia-N content. Two parameters were selected to optimize the ammonia-N removal were temperature of soil and soil to water ratio. Ammonia-N solution was added with soil mixed culture to enable nitrification process happened. The result obtained was analyzed by using RSM with factorial central composite design (CCD) using software package Design Expert.

The research was done as the flow chart shown in Figure 3.1. Firstly, samples were collected, that was the soil. Secondly inoculums were prepared, where the inoculums were the soil mixed culture. Ratios of 1:4 to 1:8 were prepared. Thirdly was the preliminary experiment which was important to obtain suitable range of values of ammonia-N concentration versus time before real experiment being conducted. After preliminary done, the experimental runs were conducted and the data were collected and analyzed through optimization by using response surface methodology (RSM). Software package Design Expert was used to obtain the best parameters.

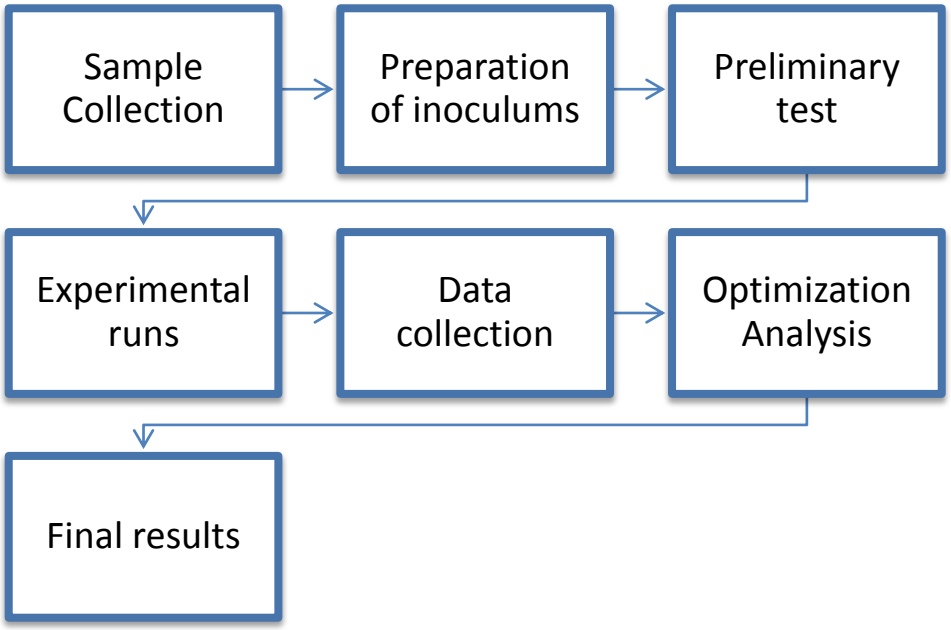

Figure 3.1: Flow Chart Research of Methodology

3.2 Preliminary

Preliminary was conducted to determine a suitable range of values of ammonia-N concentration versus time before real experiment being conducted. For preliminary experiment, soil to water ratio of 1:6 and temperature of mixture of 25°C were used as the parameters. The ammonia-N's concentrations were measured for each hour for about 5 hours. From the data that was obtained, optimum time for measurement of ammonia-N's concentration was identified to be carried out for the real run.

From the data obtained, it was shown that the concentration of ammonia-N reduced by time until the time reached 3 hours, where the maximum ammonia-N removal achieved. After 3 hours, the concentration of ammonia-N slowly increases by time. Therefore, by using preliminary's result, the real experimental result condition was obtained, where the experimental time used in each experimental run was 3 hours.

Table 3.2: Preliminary result

Time (h)	Concentration of Ammonia-N (mg/L)

0	124
1	80
2	76
3	48
4	64
5	64

3.3 Soil mixed culture

Soil mixed culture was a mixture of soil and distilled water that was important for nitrification process. This study was a continuation of previous study, where method used to obtain the parameters was kinetic study. Therefore, to avoid variations in the result, one type of soil was collected, which is UMP's soil. Firstly, the soil was filtered to remove stones and unwanted materials from the soils. Soil was added with distilled water at ratio of 1:4 to 1:8 in the beakers. The mixtures were stirred for a few minutes until the mixture was mixed well as shown in Figure 3.3a. The soil mixed culture was then filtered using filter paper to remove soil and other particles as shown in Figure 3.3b and filtrate obtained was stored in a refrigerator at a temperature of -4°C to stop the activity of microorganisms.

Figure 3.3a: Soil Mixed Culture Preparation

Figure 3.3b: Soil Mixed Culture after filtration

3.4 Ammonia-N solution

Ammonia-N was an important material where the reduction of ammonia-N was observed in this study. Ammonia-N solution was prepared by mixing ammonium nitrate salt and distilled water. Ammonium nitrate, which is a salt of ammonia-N and nitric acid, is a colorless and have high melting point, but can easily soluble in water, alcohol and liquid ammonia-N. 2 liters of ammonia-N solution with concentration of 100 mg/L was prepared by adding 940.14 mg ammonium nitrate salt with distilled water and the solution was stirred. 2 liters of Ammonia-N solution prepared was used for 13 experimental run. The solutions were stored in the -4°C refrigerator to deactivate any interaction. For each run, only about 150 ml was mixed with 100 ml soil mixed culture.

Figure 3.4: Ammonia-N solution ready before stored in refrigerator

3.5 Ammonia-N test

Firstly, 150 ml of ammonia-N solution was added with 100 ml of soil mixed culture of 6:1 of water to soil ratio. To obtain a constant temperature for 3 hours, the mixture was placed in the incubator. After 3 hours, 5 ml of the mixture was taken out and was added with 1995 ml of distilled water to dilute it. The solution was mixed well before 10 ml of the solution was taken out and place in the sample bottle. 10 ml of distilled water was used as a blank for the each experimental run. Both sample and blank bottles were added with Ammonia Salicylate Reagent and shake well for 3 minutes. Then both bottles were added with Ammonia Cyanurate Reagent and shake well for 15 minutes. A green color was developed if ammonia nitrogen was present as shown on Figure 3.5. The darker the green color, the higher the concentration of ammonia-N. Most reliable results were obtained when samples were analyzed as soon as possible after the samples were collected.

The measurement of ammonia-N's concentration was carried out by using HACH Spectrophotometer. When the timer was expired, the blank was inserted into the cell holder with the fill line facing right. Zero buttons was pressed to calibrate it, which was known as "zeroing" procedure. 0.00 mg/L NH3–N was displayed. The sample was wiped before inserted into the cell holder to avoid any impurities that will cause inaccurate reading taken. The fill line was made sure facing right. Read button was pressed and results are in mg/L NH3–N was displayed.

The experiment was repeated with another 12 experimental runs as in Table 3.5. The data obtained was tabulated and undergo optimization by using response surface methodology (RSM). Software package Design Expert was used to obtain the data.

Table 3.5: Experimental run

Run	Factor 1 Temperature (°C)	Factor 2 Water: Soil Ratio
1	25.0	6:1
2	32.5	5:1
3	30.0	6:1
4	35.0	6:1
5	30.0	4:1
6	27.5	7:1
7	32.5	7:1
8	30.0	6:1
9	27.5	5:1
10	30.0	6:1
11	30.0	8:1
12	30.0	6:1
13	30.0	6:1

Figure 3.5 Blank and sample after addition of Ammonium Salicylate and Ammonium Cynurate

3.6 Analysis using Design Expert

The experiment was repeated with another 12 experimental runs. The data obtained was tabulated and undergo optimization by using response surface methodology (RSM). Software package Design Expert was used to obtain the data. The main idea of RSM was to obtain optimal parameters by using a sequence of designed experiments. RSM also design employing the multivariate approach enables substantial improvement in the method development using fewer experiments, without wastage of large volumes of organic solvents, which leads to high analysis cost. Therefore, by using results from experimental runs, the data was generated in software package Design Expert and represented in the form of graph and figure.

3.7 Summary

For nitrification process, ammonia-N solution and soil mixed culture were mixed before being tested by using spectrophotometer. Each of the procedure must be followed to enable accurate reading was obtained. The data tabulated was used to

observe the best parameters in ammonia-N removal. As the summary, the flow of experiment was as follow.

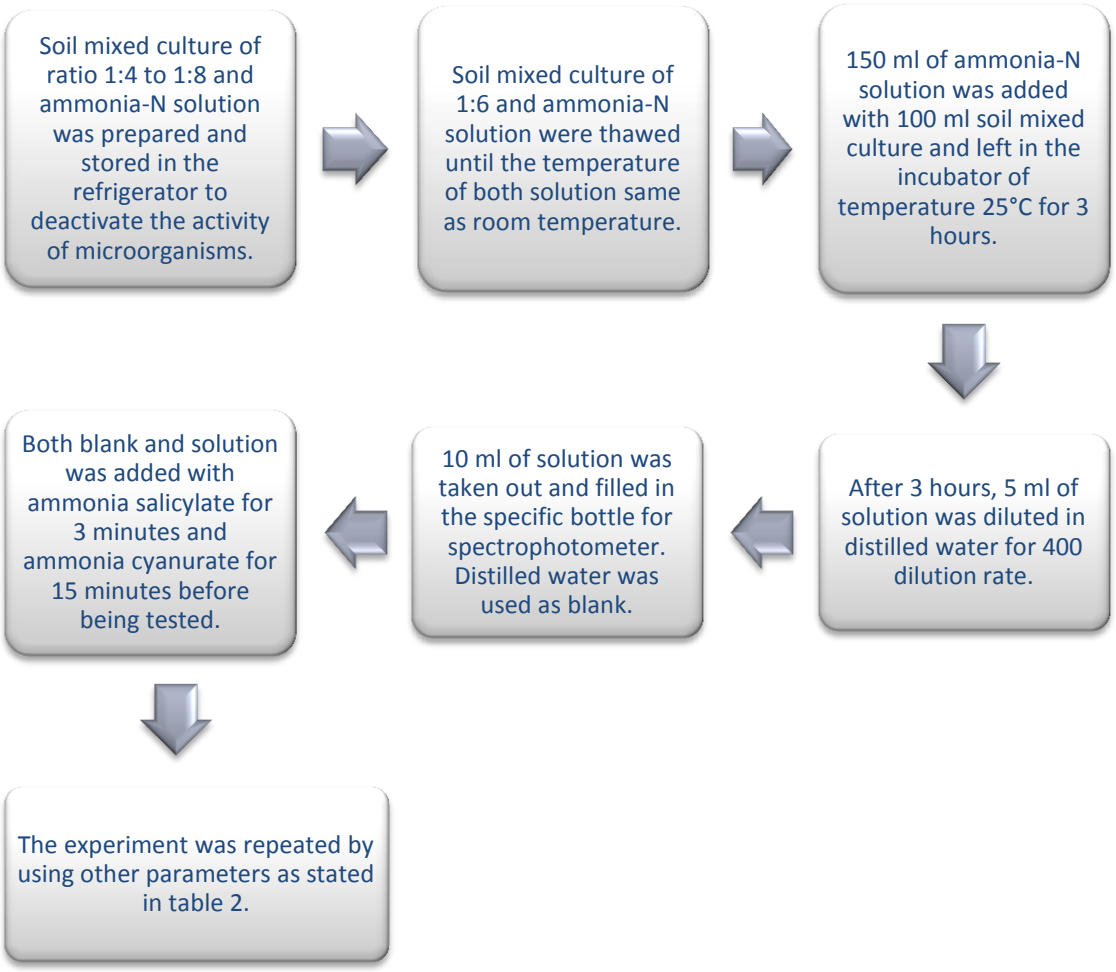

Figure 3.7: Flowchart of experiment

CHAPTER 4

DISCUSSION

4.0 Introduction

In this study, a few modelling was used to illustrate the optimization method such as Design of experiment (DOE), Response surface methodology (RSM), and Central Composite Design (CCD).According to Nath and Das (2011), DOE referred to structured method by which certain factors were selected and deliberately varied in a controlled manner to obtain their effects on the output of a process, often followed by the analysis of the experimental results. By using this method, the important parameters and the relationship between them was predicted. DOE was divided into two catogeries that were single-factor design and multiple-factor design (Nath & Das, 2011).

4.1 Preliminary

Preliminary was done to identify the suitable range of time for ammonia-removal. The conditions that were used were 25°C and 1:6 ratio of soil mixed culture. The duration of preliminary test was 5 hours, where the time used was because the study was based on the previous study. After 5 hours, the result was taken and tabulated in table 3.2. The data was used to generate the graph as shown in figure 4.1. Based on the result, the concentrations of Ammonia-N were reduced as time goes by until the time reached 3 hours from 124 mg/L to 48 mg/L. After 3 hours, the result was increased and maintained at 64 mg/L until 5 hours.

Nitrification process was occurred efficiently at 0 time to until 3 hours. At this stage, ammonia-N was converted, therefore, the concentration of ammonia-N was

reduced. After 3 hours, the nitrification process where unable to convert ammonia-N. On the other hand, ammonia-N concentration was increased. This is because in the soil mixed culture, there must be an organism that will increase the production of ammonia-N, thus increase its concentration.

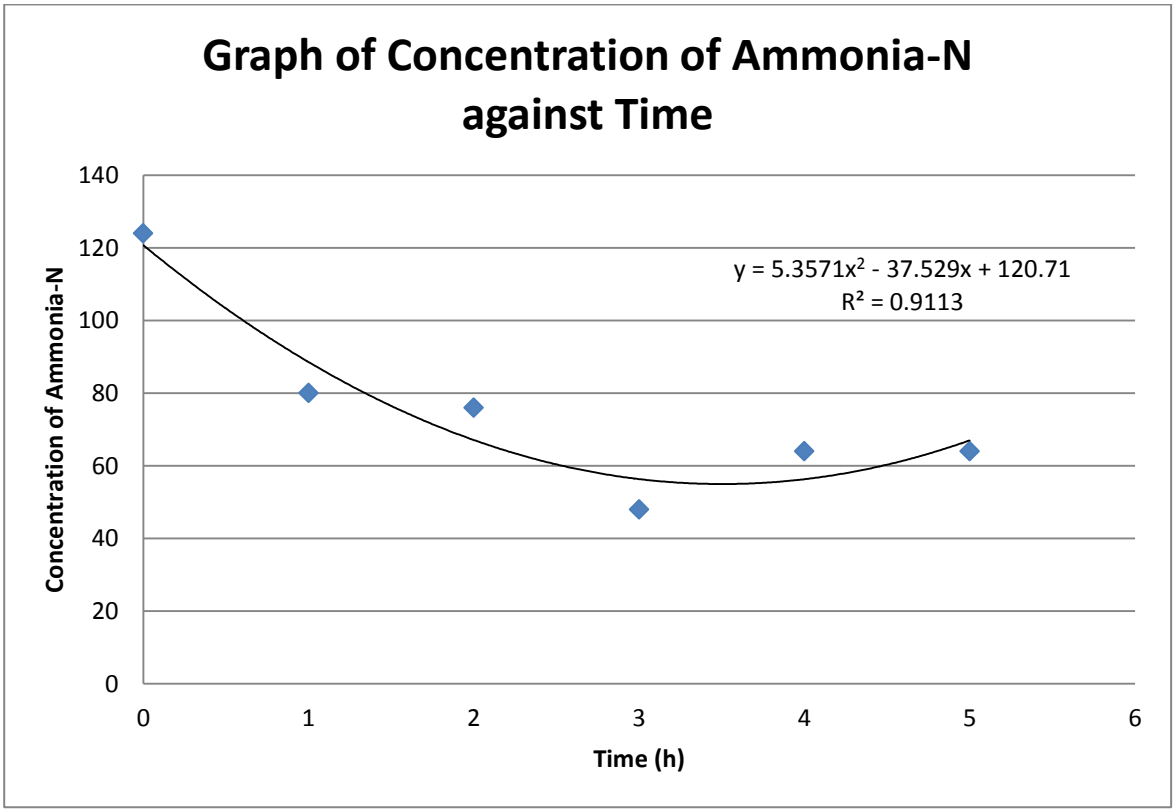

Figure 4.1: Graph of concentration of ammonia-N against time

In order to achieve the objective, preliminary study was done to obtain the best conditions for the real experiment to be conducted later. In this case, the preliminary was conducted to obtain the optimum experiment's duration. The preliminary experiment was conducted for 5 hours, where the concentration of Ammonia-N was measured each hour. The initial ammonia-N concentration was 124 mg/L where it should actually 100 mg/L due to weight balance's error.

The experiment was conducted for 5 hours due to result of the previous study of kinetic study. The soil to water ratio of 1:6 and temperature of mixture of 25°C were

used as the parameters. The result was tabulated in Table 3.2 while tha graph of the experiment was tabulated in Figure 4.1.

Based on the graph, the ammonia-N concentartion suddenly dropped from 124 mg/L to 80 mg/L at the first hour before it slowly reduced to 76 mg/L at the second hour. It was shown that the lowest concentration gained in the experiment was during 3 hours after the experiment was started. After that, the concentration rose and does not changed. This situation was called fluctuation, where the the concentration of ammonia-N was determined by the kinetic study of nitrification by nitrifying bacteria.

By this situation, the present of other microorganisms in the soil mixed culture also effecting the result of nitrification. The differences of nitrifying rate may cause due to differences in active nitrifying population in the soil mixed culture. Based on the result, the duration time of 3 hours for each experimental run was determined.

Kim & Makoto (2002) stated that they can achieve the maximum removal capacity and the complete removal capacity were 19 g-N/kg dry packing material per day and 16 g-N/kg dry packing material per day for experiment duration of 15 to 20 days. They achieved 85% ammonia gas removal after 107 operation days. They were using *Vibrio alginolyticus* for removal of high load ammonia gas under non-sterile conditions, where the microbes were a newly isolated heterotrophic marine bacterium. In the pamphlet "A55L ammonia-reducing bacteria", they had experienced a 40% reduction in ammonia in 48 hours by using bioaugmentation method.

4.2 Experimental Design and Optimization

Research Surface Methodology (RSM) was used in determining the optimum parameters of the study. Ammonia-N removal by using soil mixed culture was optimized via RSM packages in Design Expert®. Central Composite Design (CCD) was used in the design of runs with bound of two independent variables. Each independent variable's range was obtained from previous study, where the range of first variable, temperature was from 25°C to 35°C, and the second variable, water soil ratio

was from 1:4 to 1:8. Experimental conditions of CCD runs of Design Expert® and responses were presented in Table 4.2.

Table 4.2 Experimental conditions of CCD runs of Design Expert® and responses

Initial Concentration: 100 mg/L

Experimental Run	Variable 1 A: Temperature (°C)	Variable 2 B: Water:Soil	Response 1 Ammonia-N Concentration (mg/L)
1	25.00	6.00	76
6	27.50	7.00	34
9	27.50	5.00	104
3	30.00	6.00	80
13	30.00	6.00	84
8	30.00	6.00	86
10	30.00	6.00	82
12	30.00	6.00	88
11	30.00	8.00	20
5	30.00	4.00	52
2	32.50	5.00	36
7	32.50	7.00	28
4	35.00	6.00	20

The first three columns that were experimental run numbers, and experimental variables that were temperature and water to soil ratio were arranged by the CCD. The response of ammonia-N removal was analyzed to evaluate the performance of the process. Seven experiments were augmented with five replications at the design center to access the pure error and the experiments were carried in randomized order as required in many design procedures.

The appropriate model is the quadratic equation:

Response 1(Ammonia-N Removal) = -313.24138 + 45.06897A - 51.2471B - 1.47448A^2 - 12.21552B^2 + 6.2000AB

……...Equation (1)

Where;

A: Temperature

B: Water:Soil ratio

4.2.1 Central Composite Design (CCD)

Rasdi et. al (2009) stated that bulding models, evaluating relative significance of several independent variables and optimum conditions for desirable responses determination can be achived by using RSM which is a statistical and mathematical technique for building models. The condition that could give optimum ammonia-N removal were predicted using the numerical optimization in Design-Expert.

ANOVA was frequently used as regression where its predictive variables are qualitative, which in this study, qualitative variables used were temperature and soil mixed culture ratio. The result of the statistical analyses were interpreted by using statistic R^2, where the percentage of variation in response variable that were represented by R^2 were explained by its relationship with the two predictor variables; temperature and soil mixed culture.

In addition, how much variation in the output variable was determined by using R^2. Based on the Table 4.2.1a, it shows that a multiple regression model with an R^2 of 0.9175, where it suggests that 91.75% of the variation in regression model was explained with the Equation (1). The high R^2 shows that the experimental and predicted values was nearly the same and it indicates a better correlation between the values.

Table 4.2.1a Table of R^2

Std. Dev	11.20	R-Squared	0.9175
Mean	60.77	Adj R-Squared	0.8586

C.V.18.43		Pred R-Squared	0.3295
PRESS	7136.77	Adeq Precision	12.348

Degree of Freedom (DF) indicates the number of values in the final calculation of a statistic that were free to vary. Dallal (2007) stated that DF was a difference in dimensionalities of parameter spaces. DF was used in for numerator degrees of freedom for F tests and many chi-square tests. The ANOVA error was the interaction between subjects and the effect and requires degrees of freedom from both.

The degrees for freedom were much higher for the mixed effects model. Sum of Squares (SS) was given by taking each value of a factor minus the average and then square each difference. Mean Squares (MS) shows the estimation of the population variance based on variability among a two sets of parameters. There were no differences between the values of A, B, A^2, B^2, and AB of SS and SM.

Table 4.2.1b The analysis of varience (ANOVA) for response surface quadratic model

Source	**Sum of Squares**	**DF**	**Mean Square**	**F Value**	**Prob > F**
Model	9766.61	5	1953.32	15.58	0.0011 (significant)
A	2883.00	1	2883.00	22.99	0.0020
B	1680.33	1	1680.33	13.40	0.0081
A^2	1945.95	1	1945.95	15.52	0.0056
B^2	3419.14	1	3419.14	27.27	0.0012
AB	961.00	1	961.00	7.66	0.0278
Residual	877.70	7	125.39		
Lack of Fit	837.70	3	279.23	27.92	0.0038 (significant)
Pure Error	40.00	4	10.00		
Cor Total	10644.31	12			

From Table 4.2.1b, it can be concluded that the main effect of parameter A (temperature) and B (soil to water ratio) along with their dual interaction (AB) are significant. The Model F-value of 15.58 implies the model is significant. There was only a 0.11% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant and this showed that these quadratic functions can be applied for the purpose of successful prediction for the future response of ammonia-N removal by using soil mixed culture. In this case A, B, A^2, B^2 and AB are significant model terms. Values greater than 0.1000 indicate the model terms were not significant.

Model reduction will improve the model if there are many insignificant model terms. The "Lack of Fit F-value" of 27.92 implies the Lack of Fit was significant. There is only a 0.38% chance that a "Lack of Fit F-value" this large could occur due to noise. Significant lack of fit happened because the bias component of error is much larger than the pure error, where both components estimate the nominal level of error shows the model is adequate. Significant lack of fit can be get rid by adding either of the linear-by-quadratic interaction terms to the full quadratic model. Goos and Gilmour (2013) stated that for some cases, although significant lack of fit might cause a little impact on the data interpretation, but it can be effectively ignored.

However Wayne (2010) stated that higher order model is needed to indicate a significant lack-of-fit. He stated that the significant lack-of-fit can be present if the R-square estimates are relatively high where about 0.8 or higher because the pure error between replicates is very small. Since the R^2 obtained in the experiment was 0.97, the model fit the data even though the lack of fit was significant.

The residual error to the pure error was compared by using the lack of fit test from replicated design points. Something that was remains in the residuals was indicated by the residual error significantly larger than the pure error. Therefore, this residual may be removed by a more appropriate model. Because the lack of fit was significant, the model should not be used as a predictor of the response.

4.3 Effect of Temperature and Ratio of Water:Soil on Ammonia-N Concentration

The second-order polynomial equation 1 was used by applying multiple regression analysis on the experiment data, where this equation explains the ammonia-N concentration.

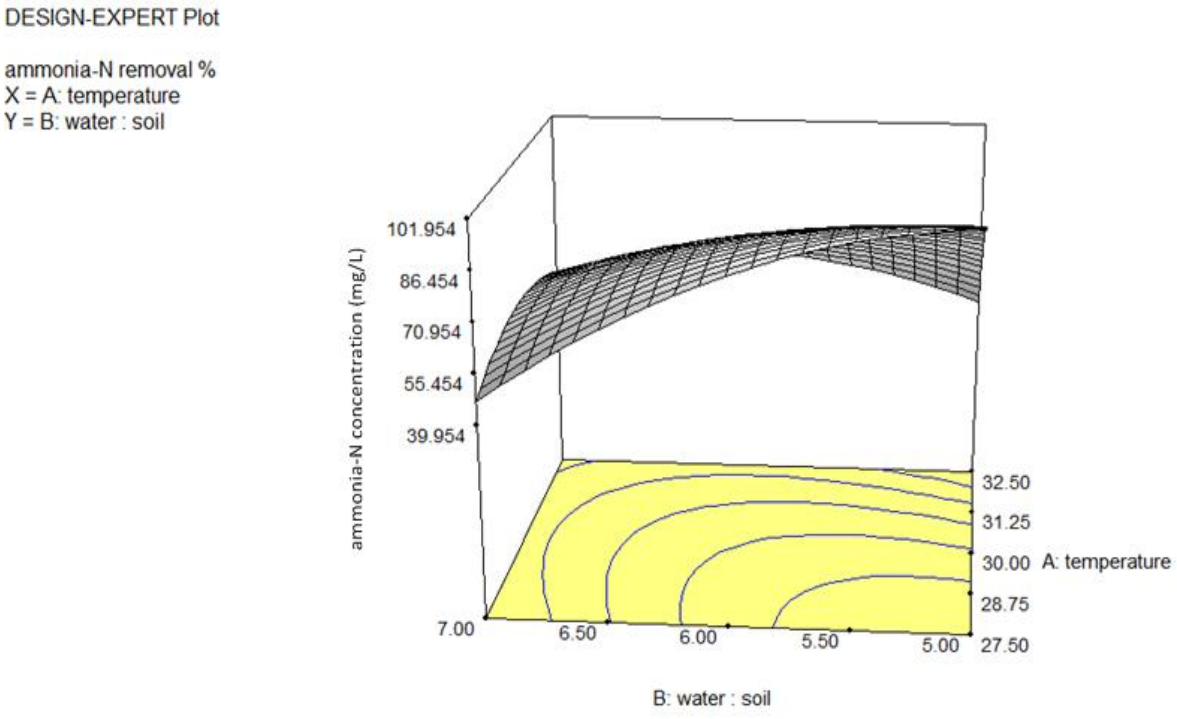

Figure 4.3a The 3D-plot and 2-D projection (Contour plot) showing the interaction between temperature and water and soil ratio on the concentration of ammonia-N

Based on the regression model equation (1), three-dimensional (3D) was plotted, and was illustrated in Figure 4.3a, where the variables was within the experimental range for response to the concentration of ammonia-N. The 2-D projection shows that the optimum point was not at the centre of the graph and the 3D plot was sloping downward from the small value of the variables toward the higher value of the variables. The contour with respect to temperature and water:soil ratio showed an elongated shaped running diagonally on the plot. Based on Rasdi et. al (2009), this indicate that the variables were interdependent, or between temperature and water:soil

ratio exist significant interaction. Sudarno, Winter & Gallert (2011) stated that the oxidation rate of ammonia and nitrate was affected by the temperature, a main factor of the kinetic behavior of biological reactions.

Their experiment result in the oxidation rate of ammonia 2.2 mg N/Lh and oxidation rate of nitrate 4.9 mg N/Lh at the temperature of 22.5°C. The ammonium oxidation rate was zero at 6 °C, increased almost linearly until 32.5 °C, stagnated until 40 °C and decreased to less than 10% of its maximum activity at 50 °C (Sudarno et al., 2011). This shows that the ammonium content of their study was affected by temperature at different stages. The higher the temperature, the higher the rate until it reached the maximum temperature for efficient oxidation. Compared to this study, where experimental run 1, 13 and 4 were took as the comparison, it shown that at temperature 25°C and 30°C, the ammonia-N concentration dropped a little compared to temperature 30°C and 35°C where the different between these two concentration was quite large. This study does not experience the stagnated stage and above as the range for temperature was only until 35°C.

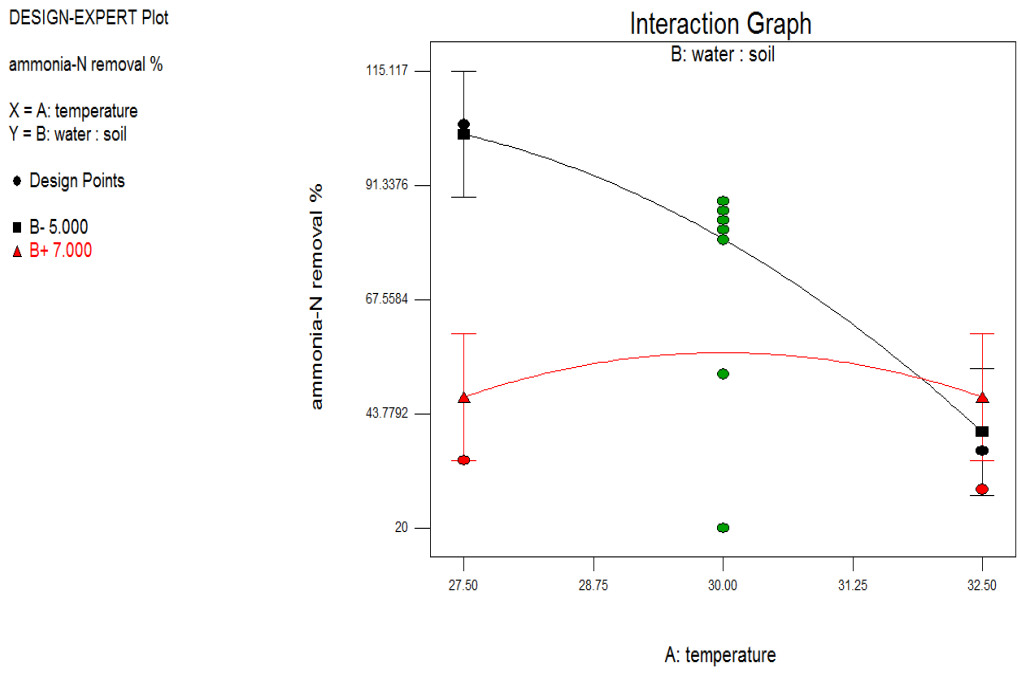

Figure 4.3b Interaction factor between temperature and soil and water ratio on the concentration of ammonia-N removal

Figure 4.3b describes how the interaction between temperature and soil to water ratio affects the percentage of ammonia-N reduction in the 3 hours experiment. The graph shows that the percentage of ammonia-N removal within the range between 43.7792 to 115.117 mg/L upon increasing of water to soil ratio between 5 to 7 and temperature between 27.50 to 32.50. Based on the graph, it shows how the interaction of temperature due to soil and water ratio, where ratio of 1:7 soil and water remove more ammonia-N content in the sample compared to ratio of soil and water 1:5.

The lines are near from parallel, which indicates that the ratio of soil water of 1:5 and 1:7 at temperature 32.5°C result in quite large difference in ammonia-N removal. The line of ratio of soil water 1:5 reduced gradually compared to ratio of soil water 1:7 that is nearly flat. This indicates that the ratio of 1:7 does not effected fully by changes in temperature compared to ratio of soil water 1:5.

Andersson et al. (2001) stated that based on their study, the ammonia removal gradually decreased from 77% to 38% when the temperature decreased. In addition, the increasing temperature from 10.5°C to 17°C caused an immediate increase of ammonia removal that was about 66% to 82.4%. Although the percentage of ammonia was changed based on the temperature, the fixed nitrifying biomass did not decreased even after 3 weeks in cold water (Andersson et. al, 2001). Based on this statement, it was shown that the temperature had not direct impact on the quantity of nitrifying biomass, but the bacterial activity was distracted by the changes of temperature. By increase the temperature of experiment, the ammonia-N concentrations were reduced.

Camberato (2001) state that the rate of nitrification will increases in the warmer temperature. As an example, when ammonium sulphate mixed with soil at variation temperature for 24 days, it shows that at 40°F 29% of the ammonium had been nitrified while at 80°F, 100% was converted to nitrate (Camberato, 2001). Meisinger & Jokela (2000) predict that ammonia removal will increase by factor of about 3 for every increases of temperature for about 10°C.

4.4 Validation of the Identified Optimal Conditions

Validity of the statistical experimental strategies were conducted by three additional confirmation experiments in order to understand the ammonia-N removal by using nitrification of bacteria in soil water and ammonia solution. Furthermore, validation runs were conducted for the confirmation of adequacy of the model equations, therefore validation runs under the optimized condition were carried out. Table 4.4c shows the chosen conditions for temperature and water soil ratio and the experimental results.

In these runs, the controls of the experiments were the values predicted by the optimization and all the validation runs were conducted in triplicate.

Table 4.4a Optimum condition from software based on constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Important
Temperature(°C)	Is in range	27.5	32.5	1	1	3
Water:Soil	Is in range	5	7	1	1	3
Ammonia-N concentration(mg/L)	Minimize	20	104	1	1	3

Table 4.4b Optimum condition from software based on solution

Number	Temperature (°C)	Water:Soil	Ammonia-N concentration (mg/L)	Desirability	
1	32.50	5.00	39.9541	0.762	Selected
2	32.50	7.00	47.2874	0.675	
3	32.48	7.00	47.4304	0.673	
4	32.50	6.98	47.6461	0.671	
5	32.50	6.97	47.9768	0.667	
6	32.50	5.43	49.7511	0.646	

From the two parameters, that were temperature and water to soil ratio, where from 27.5 to 32.5 and from 5 to 7 respectively, the concentration of ammonia-N was within the range of 20 to 104 mg/L. Based on the Table 4.4b, it was found that the best condition for ammonia-N removal is 32.5°C for the temperature and 5.00 for the ratio of water to soil.

Table 4.4c Data from validation run

Run	Temperature (°C)	Water:Soil	Ammonia-N content (mg/L)	Expected result (mg/L)	Error	Ammonia-N removal (%)
1	32.5	5	44	39.9541	9.195%	57.69
2	27.5	7	52	47.2874	9.062%	50.00
3	32.5	7	44	47.2876	7.471%	57.69

In table 4.4c, data was tabulated from the validation run, where the experimental error for the best condition was the highest among the three validation run. The desired result from Table 4.4c for Run 1 was 39.9541 mg/L, but the result obtained 44 mg/L, generates 9.195% error. For Run 2, 52 mg/L was collected for ammonia-N content, where its error reached 9.061% compared to the predicted value by software that was 47.2874 mg/L. In Run 3, 44 mg/L was measured for ammonia-N content where it generated about 7.471% error. However, the result obtained was still valid since the result does not exceed 10%. Since the result obtained showed that ammonia-N concentrations are closed to theoretical value using RSM, this can proved that RSM analysis was a useful technique for optimizing the nitrification of ammonia-N.

The optimum result of 44 mg/L ammonia-N concentration was obtained represented 9.195% error compared to the theoretical concentration that is 39.9541 mg/L. The value was higher than expected in theory because the characteristics of nitrification bacteria that were affected by temperature changes. Subsequently, the optimum ammonia-N removal was estimated from Equation (1) at temperature 32.5°C

and ratio of soil water 1:5. He, Tao, Wang & Shayya (2012) had done a research to indicate the partial nitrification and ANAMMOX in free-water surface wetlands, where pH and temperature was used as parameters.

Based on study, the optimal temperature for ANAMMOX and favor ammonium oxidizing bacteria (AOB) over nitrite oxidizing bacteria (NOB) in the bioreactor was higher than 30°C. Through the experimental, He et al. (2012) conclude that the nitrification in the free-water surface wetland were not affected by the changes of temperature unless the temperature change of more than 6 °C in the range of 13.8–24.9 °C.

Compare to the result obtained in this study, where the water to soil ratio of 1:6 was taken as comparison. At 25°C, the ammonia-N concentration was 76 mg/L at 30°C, the ammonia-N concentration was averagely 84 mg/L while at 35°C, and the ammonia-N concentration was dropped to 20 mg /L. This reading shows the large different of ammonia-N concentration.

4.5 Comparison with Previous Study

Based on Table 4.4c, it was shown that the highest removal of ammonia-N was about 57.69%. Many researchers had already done the ammonia-N removal by many ways. The research that was done effected by several parameters such as temperature, soil mixed culture, pH, salinity and free ammonia concentration. Following table shows comparison of the study with other researchers in ammonia-N removal.

Table 4.5 Comparison of Ammonia-N Removal with Other Researchers

Researcher (Year)	Title	Removal Efficiency (%)	Reaction Time (hour)
Rahim, N. (2014)	Ammonia-N Removal by Using Soil Water-Optimization	57.69	3
Constantine, T. (2008).	An Overview On Ammonia and Nitrogen Removal in	85.0	36

	Wastewater Treatment		
Rogalla, F., Ravarini, P., De Larminat, G., & Couttelle, J. (1990).	Large-Scale Biological Nitrate and Ammonia Removal	82.0	24
Kutty, S. R. M., Isa, M. H., & Leong, L. C. (2011).	Removal of Ammonia-Nitrogen (NH3-N) and Nitrate (NO_3^-) by Modified Conventional Activated-Sludge System to Meet New D.O.E Regulations	60.0	240
Pramanik, B. K., Fatihah, S., Shahrom, Z., & Ahmed, E. (2012).	Biological Aerated Filters (Bafs) For Carbon And Nitrogen Removal: A Review	98	2
Kim, N. J., & Makoto, S. (2002).	Removal of a high load of ammonia by a marine bacterium, *vibrio alginolyticus* in biofilter.	85	2568 (107 days)

From the researches that was done earlier, most of them use biological treatment to remove ammonia and nitrogen because the cost of the process was less expensive compared to the traditional one. In Constantine's studies, about 85% of nitrate removal was achieved by using 500% recirculation rate (Constantine, 2008). Although compared to this study, the ammonia-N removal was lower, the duration experiment of Constantine shows about 12 times longer that was 36 hours compared to only 3 hours of this study. The longer the time used, more money was needed for electrical and procedures. Furthermore, this study only used soil mixed culture for nitrification, where Constantine used a series of instruments for the ammonia and nitrogen removal. This will cost more compared to the study.

Research by Rogalla et al. (1990) shows that about 82% of ammonia was removed by using denitrification plant, where it incorporating both anoxic and aerated

biological filters. This situation was quite same as the previous journal, where the duration of 24 hours seems longer compared to this study and the operating cost was higher.

Kutty et al. (2011) stated that they did remove 60% of ammonia in duration of 240 hours. This bioreactor was enhanced in aeration system compared to this study, where no aeration system was installed in this study. Although there was no aeration system applied, the removal of this study was nearly as high as Kutty et al. which were 57% compared to 60% with less duration time of 3 hours compared to 240 hours. In addition, with no aeration system and expensive reactor used, this experiment achieved less instrumentation costing.

Pramanik et al. (2012) achieved 98% of ammonia-N removal with carbon to nitrogen ratio of 1 within 2 hours, where and efficient utilization of organic compounds in the aerobic process were installed. Pramanik et al. also stated that the increasing nitrification rates were due to increase of ammonium load. Pramanik achieved a very excellent result compared to other researchers due to usage of Biofilter Aerated Filters (BAFs) since the duration of the process was only two hours. However this reactor might be impaired by the decreases of surface area available for biofilm attachment, and specifically the masses of essential microorganisms present in the reactor.

Kim et al. (2002) stated that the average percentage of ammonia gas removed exceeded 85% at 107 days of operation. They were using *V. alginolyticus* which was a marine microbes for non-sterile condition which had no significantly adverse effect on the removability of ammonia by this microbes. The isolated strain was preserved in a heart infusion broth containing 1% NaCl at -80°C. A sucrose medium was supplied as a substrate for the microbes. This strain was cultured in a very strict procedure. Compared to this study, the microbes were obtained from soil mixed culture that was simply prepared by addition of water and soil at certain ratio. The nitrifying microbes, *Nitrosomonas* sp. which were often discovered in soil were used as the microbes for ammonia-N removal. The procedure was easier compared to Kim et al. study, thus used less cost for the cultivation process.

CHAPTER 5

CONCLUSION

5.1 Conclusion

Based on the result that was obtained, the best parameters for the highest ammonia-N removal were obtained by using optimization, where about 57% of ammonia-N was reduced by using the combination of parameters at of 32.5°C and soil mixed culture of ratio 1:5. The reduction of ammonia-N was done by using microorganisms that were found in the soil mixed culture that were *Nitrosomonas* sp., *Nitrobacter sp, Nitrosolobus* sp., *Nitrospira sp, and Nitrovibrio* sp. by using principle of nitrification. The time duration to remove ammonia-N was proved to be less compared to other researches that was 3 hours, yet still achieved high ammonia-N removal. From this study, it was also achieved that ammonia-N concentration can be reduced by using a simple procedure and easy to be applied. This was because the material needed during the experiment was only soil mixed culture that can be mixed without using any expensive instrument.

5.2 Future work

In this study, the research can be further study on the effect of pH on the efficient of nitrification, where pH also has a great effect on the nitrification process. The result from the study can also be applied to other industry than farming industry, such as wastewater treatment.

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