AMMONIA-N REMOVAL USING SOIL MIXED CULTURE: KINETIC STUDY

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DECLARATION

I declare that this thesis entitled "Ammonia-N Removal Using Soil Mixed Culture: Kinetic Study" is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree student.

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Date: 1st February 2013

DEDICATION

Dedicated to God;

He makes everything possible and helps me in my research

He confirms once again His grace is sufficient…

And also Special Dedication to my family members,

Especially my father and mother,

That always support and give me strength in many ways,

My friends, my fellow colleagues,

And all of the faculty members…

Thank you for caring, supporting and believing in me.

Sincerely,

Suryati

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AMMONIA-N REMOVAL USING SOIL MIXED CULTURE: KINETIC STUDY

ABSTRACT

Ammonia is a colorless, water-soluble gas by-product of the microbiological decomposition of organic nitrogen by the nitrification process. This ammonia is a natural chemical substance that contains inside the earth which can cause disturbance to living organism especially animals. The most affected animal due to this toxic gas is poultry which is in the industry. In Poultry Farm Wastewater (PFW), there contains a lot of ammonia-N (NH_3) compound which is excreted by poultry. This high concentration of ammonia-N compound will produce a high level of toxic gas that will cause the poultry to be unhealthy or worst which is mortality. Since poultry is a source of food for people around the world, thus maintaining the quality of poultry is necessary. The high demand of poultry around the world nowadays leads to find a way to reduce the mortality of the poultry in industries. In order to reduce the mortality of poultry, a new way in reducing of ammonia-N in PFW in industries is needed in which by using soil mixed culture. There are some researches that have been found to use soil mixed culture as a medium of reducing this ammonia-N concentration inside this PFW. In this research, the objective is to study the kinetic parameter involving ammonia-N removal by using soil mixed culture. DR/2800 HACH Spectrometer will be utilized in order to determine concentration of ammonia-N. Since reduction of ammonia-N concentration is the one of the objective of this research, the determination of the best kinetic parameter for this soil mixed culture is required. An ammonia-N solution will be prepared by taking the pure ammonia-N solution mixed with water to get the solution. Kinetic parameter will be determined by ammonia-N removal using the soil mixed culture from University Malaysia Pahang (UMP) and poultry farm in which has been conducted in the experiment which follows the kinetic modeling. At the end of this research, results will show for the kinetic values for nitrification which are the time taken for the ammonia-N

to be reduced, k, and the amount of ammonia-N concentration can be reduced, K_N, will be determine to compare and analyze using Linear Regression Method. Lastly, Monod Model represents the growth of the microorganism inside the soil mixed culture which determines the rate of nitrification process. From the experiment that has been done, the results that want to be obtained were for the value of the coefficient k and K_N for both the UMP soil mixed culture (UMPC) and poultry farm soil mixed culture (PFC). By using the experimental data obtained in this research, the kinetic constants for nitrification were determined as k = 1.227 h^{-1} and K_N = 67.609 mg/L for UMPC and k = 1.090 h^{-1} and K_N = 68.454 mg/L for PFC. These value of K_N and k will determine the maximum reduction of ammonia-N concentration and the time taken for the process. From the result, the best solution in reducing the ammonia-N concentration was by using the PFC because the PFC can reduce the most ammonia-N in lesser time than UMPC. Other than that, the PFC can reduce more ammonia-N than other research which they obtained a lower reduction of ammonia-N concentration and slower time taken to reduce the ammonia-N concentration.

PENYINGKIRAN AMMONIA-N DENGAN MENGUNAKAN TANAH CAMPURAN KULTUR: KAJIAN KINETIC

ABSTRAK

Ammonia adalah sejenis gas yang tidak berwarna serta larut dalam air yang merupakan hasil sampingan dari penguraian mikrobiologi nitrogen organik melalui proses nitrifikasi. Ammonia ini adalah bahan kimia semulajadi yang mengandungi di dalam bumi yang boleh menyebabkan gangguan kepada organisma hidup terutama haiwan. Haiwan yang paling terjejas kerana gas toksik ini adalah ayam yang berada dalam industry. Terdapat banyak ammonia-N (NH_3) kompaun mengandungi dalam Air Sisa Ladang Ayam (ASLA) yang dikeluarkan oleh ayam dalam industri. Gas toksik yang tinggi tahapnya akan terhasil apabila kepekatan sebatian ammonia-N yang tinggi berada di tahi ayam akan menyebabkan ayam menjadi tidak sihat atau kematian. Oleh sebab ayam merupakan sumber makanan bagi manusia di seluruh dunia, mengekalkan kualiti ayam adalah sangat diperlukan. Permintaan yang tinggi untuk memperolehi ayam di seluruh dunia pada masa kini membawa kepada mencari cara untuk mengurangkan kadar kematian ayam dalam industry. Dalam usaha untuk mengurangkan kadar kematian ayam, cara baru dalam mengurangkan ammonia-N dalam ASLA pada industri diperlukan di mana dengan menggunakan tanah campuran kultur. Terdapat beberapa kajian yang telah ditemui dengan menggunakan kultur tanah bercampur sebagai medium mengurangkan kepekatan ammonia-N dalam ini ASLA. Dalam kajian ini, objektif utama adalah untuk mengkaji parameter kinetik melibatkan ammonia-N penyingkiran dengan menggunakan kultur tanah campuran. DR/2800 HACH Spektrometer akan digunakan untuk menentukan kepekatan ammonia-N. Oleh kerana pengurangan kepekatan ammonia-N adalah salah satu objektif kajian ini, penentuan parameter kinetik terbaik untuk kultur tanah bercampur ini adalah diperlukan. Larutan ammonia-N akan disediakan dengan mengambil ammonia-N tulen dengan menyampurkannya dengan air untuk mendapatkan larutan yang berkaitan. Parameter kinetik akan ditentukan untuk penyingkiran ammonia-N dengan menggunakan kultur tanah yang bercampuran dari Universiti Malaysia Pahang (UMP) dan ladang ternakan yang telah dijalankan dalam eksperimen yang mengikuti model kinetik. Pada akhir kajian ini, keputusan akan menunjukkan nilai kinetik untuk nitrifikasi iaitu k, dimana ia adalah menunjukkan masa yang diperlukan untuk ammonia-N dikurangkan, dan K_N, dimana jumlah kepekatan ammonia-N boleh dikurangkan, bagi menganalisis dan membandingkan dengan menggunakan Kaedah Regresi Linear. Akhir sekali, Model Monod akan digunakan bagi mewakili pertumbuhan mikroorganisma di dalam kultur tanah bercampur yang menentukan kadar proses nitrifikasi. Dari eksperimen yang telah dilakukan, keputusan yang hendak diperolehi adalah bagi nilai pekali k dan K_N untuk kultur tanah campuran UMP (UMPC) dan ladang ternakan tanah kultur campuran (LTC). Dengan menggunakan data ujikaji yang diperolehi dalam kajian ini, pemalar kinetik untuk nitrifikasi telah ditentukan dimana k = 1.227 h^{-1} dan K_N = 67.609 mg/L untuk UMPC dan k = 1.090 h^{-1} dan K_N = 68.454 mg/L untuk LTC. Nilai yang telah diperolehi iaitu K_N dan k akan menentukan pengurangan maksimum kepekatan ammonia-N dan masa yang diambil untuk proses nitrifikasi tersebut. Dari hasil yang telah diperolehi, cara yang terbaik dalam mengurangkan kepekatan ammonia-N adalah dengan menggunakan LTC kerana LTC boleh mengurangkan kepekatan ammonia-N yang paling banyak dalam masa yang singkat berbanding UMPC. Selain daripada itu, LTC boleh mengurangkan lebih ammonia-N daripada pengkaji lain yang dimana mereka dapat pengurangan kepekatan ammonia-N yang lebih rendah dan masa yang diperlukan adalah sangat lama untuk mengurangkan kepekatan ammonia-N.

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LIST OF SYMBOLS

h	hour
mg	milligram
L	liter
%	percentage
ppm	parts per million
pH	potential hydrogen
^{o}C	degree Celsius
mL	milliliter

CHAPTER 1

INTRODUCTION

1.1 Background of Proposed Study

In this few years, there has been a lot of paper works reported that poultry industries have been increase in loss due to a large number of poultry cannot be sold because of health problem occurred in the industry. The health problem occurred in this industry is the high concentration of ammonia-N in their farm. This ammonia-N is present from the excretion of the poultry itself where it turns the ammonia-N into nitrogen gas. The concentration of ammonia-N need to be reduced as low concentration as possible in order to protect the workers as well as the poultry health in the industry. This problem has not been resolved yet due too many people do not know how to utilize and manipulate the uses of nitrification process to the fullest. This can be proven because the bacterium for nitrification process uses the ammonium, NH_4^+ as source of energy. If this natural process is used wisely and correctly, the reduction of ammonia-N will occurred and it will reduced the concentration around the poultry farm to the lowest concentration. In order to utilize the nitrification process to the fullest potential, the determination of kinetic parameter and uses the kinetic modeling of the soil is needed. By knowing and applying this kinetic parameter and modeling, the people from the poultry industry can know which type of soil to be used in order to reduce the ammonia-N concentration in their industry. By knowing the soil used, the ammonia-N concentration can be reduced to the lowest value.

1.2 Introduction

Ammonia-N is one of the natural chemical substances that contains inside the soil of the earth. The increase in the concentration of ammonia-N is due to the soil of the earth is mixed up with manure from living organism such as poultry, cow, sheep and others. Poultry in poultry farm gives a high percentage or concentration of ammonia-N rather than earth itself due to the poultry farm has a wastewater which can form a nitrification process where ammonia-N is convert to nitrate through nitrification and ammonia-N that is not good for health. In case of poultry, nitrogen is excreted as uric acid and as undigested protein in fecal waste where 50% of nitrogen is present inside fresh manure which is ready to be converting the ammonia-N by hydrolysis, mineralization and volatilization (Ritz, Fairchild, & Lacy, 2004). Based on the researcher of Hale III (2004), the researcher uses barn as a research place where the researcher used 20 chickens and four barns where one barn consists of 5 chickens in order to compare with different diet used to reduce the ammonia-N emission. But for the research part, we only take the required data that is before the chicken is fed with different diet for a week. The total amount of ammonia-N presented in the barns for a week for 20 chickens is 602.70 mg/L of concentration (Hale III, 2004) where for each chicken for a week is only about 30.14 mg/L of ammonia-N concentration. Based on the other researcher, the researcher uses the animal housing as the research place. The researcher only uses one chicken for the experiment for a whole week and get that the concentration of ammonia-N in the poultry farm wastewater is 38.56 mg/L (Moore et al., 2008). From the two researchers, the average poultry farm wastewater will give around 30 to 40 mg/L concentration of ammonia-N that is presented. Thus the concentration of ammonia-N in the poultry farm wastewater is around 30 to 40 mg/L.

1.3 Problem Statement

Currently nowadays, the disease that killed many poultry is due to overexposed of ammonia-N concentration where it is present inside its poultry farm wastewater (PFW). The ammonia-N is a very toxic substance that it can cause death to animals. This can lead to disadvantages of the poultry company or farmer as it can affect their income on daily lives**.** The overexposed of highly concentrated ammonia-N which is more than

50 ppm causes keratoconjunctivitis, with symptoms including watery eyes, closed eyelids, rubbing of eyes with the wings and blindness (Hale III, 2004). Since ammonia-N is the main problem in PFW, thus reducing it is needed. Industries all over the world do not try to create a way in reducing the ammonia-N concentration in PFW. So, this study can help in creating a new finding and will be a pioneer to other research which can be provided in the future. By using the method of soil mixed culture, the ammonia-N can be reduced in PFW. In the soil, bacteria are containing which these bacteria have distinct characteristics that can withstand the ammonia-N level of poisonous odor and can perform a process of nitrification to reduce the ammonia-N concentration. The new way to reduce the ammonia-N concentration in PFW is by using the soil mixed culture in which knowing the kinetic parameter of the soil mixed culture so it can be used by other researchers in the world. Thus soil mixed culture can help in reducing the concentration in PFW and can reduce the death of the poultry in poultry industries globally.

1.4 Research Objective

The main purpose of this research is

i. To study the kinetic of ammonia-N removal using soil mixed culture.

1.5 Scope of Research

The scopes of this study are:

i. To use the soil mixed culture where it can be obtained from area around University Malaysia Pahang (UMP) and poultry farm, as the medium to reduce ammonia-N concentration.

ii. To reduce ammonia-N to the lowest concentration by using soil mixed culture.

iii. To determine kinetic parameter of ammonia-N removal using soil mixed culture from UMP and poultry farms by kinetic equation represent nitrification process.

iv. To compare the value of k and K_N from nitrification process using soil mixed culture from UMP source and poultry farms source and compare both value of k and K_N with other research.

v. The method for analyzing the data that will be use is Linear Regression method. This method is by using Microsoft Office Excel Solver program.

1.6 Significant of Research

This study carries the significance of achieving the lowest ammonia-N content in poultry farm wastewater (PFW) that can help in providing a good and healthy poultry all over the world from the poultry industry. This study would also provide the information of concentration of ammonia-N with using the HACH Spectrometer DR/2800 where by using kinetic theory or parameter of nitrification can help in lowering the concentration of ammonia-N. This kinetic parameter is needed in order to know the amount of ammonia-N can be reduced and the time taken to reduce the ammonia-N. By this kinetic parameter, the appropriate soil can be determined in order to reduce the most amount of ammonia-N. The kinetic value is an important nitrification system design and to ensure that the nitrifying microorganisms are capable of presenting efficient metabolic activity.

CHAPTER 2

LITERATURE REVIEW

2.1 Ammonia

Ammonia is a colorless, water-soluble gas by-product of the microbiological decomposition of organic nitrogen compounds in poultry farm wastewater (PFW) (Ritz et al., 2004). Ammonia-N is a chemical substance that consists of ammonia (NH_4) and nitrogen (N). This chemical substance is a very dangerous chemical substance that can cause a lot of negative effect. Figure 2.1 shows the ammonia structure and Figure 2.2 shows chemical formula of ammonia.

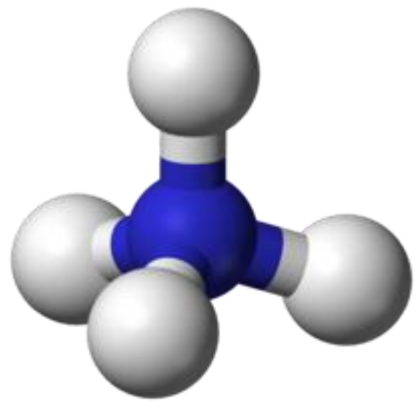

Figure 2.1 Ammonia structure

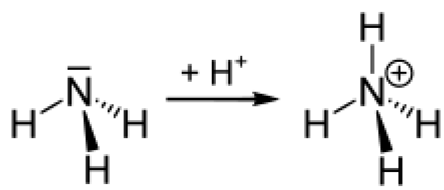

Figure 2.2 Chemical formula of ammonia

Ammonia-N is one of the components or chemical that is present inside the PFW for many years which cause health issues to the poultry industry. Ammonia-N can be dangerous to the poultry and the workers inside the industry. This have been proven by Hale III (2004), that ammonia-N vapors causes damage to the respiratory tract after only six week and when the poultry exposed to 20 ppm ammonia-N for 72 hours, the chicken or turkey will get infected with the Newcastle disease. This disease is *Paramyxovirus 1* a highly contagious viral disease affecting poultry of all ages. Affected species include chickens, turkeys, pigeons and ducks. PFW is a wastewater treatment where poultry manure from the excretion of poultry contains ammonia-N. This ammonia-N does not have an ionic charge, making it readily released into the atmosphere in gaseous form can affect the health of poultry and the worker in poultry industries. However, gross and microscopic damage to the respiratory tract could be detected only after six week of continuous exposure to 20 ppm of ammonia-N (Hale III, 2004). The level of ammonia-N concentration which is from 75 to 100 ppm is associated with changes in the respiratory epithelium, including loss of cilia and increased number of mucus-secreting cells. Excess ammonia-N concentration of 100 ppm in the atmosphere in layer house can also affect egg production of poultry (Kim & Patterson, 2004). From a global perspective, ammonia-N emissions also play a key role in acid rain formation. Besides that, Beker et al. (2004) has found out that NH_3 in poultry houses lowers performance and may increase disease susceptibility. This also has been proven by Olanrewaju et al. (2009) and Kristensen & Wathes (2000) that ammonia-N has shown to be detrimental to poultry health and performance and is cited as an environmental concern as well (National Research Council, 2003). It has been suggested that ammonia-N concentration should not exceed 25 ppm in poultry houses. However, the prolonged exposure of ammonia-N concentrations, as low as 20 ppm, can be detrimental to poultry health and its performance throughout the production period. An exposure of 300 ppm of ammonia-N concentration is considered an immediate danger to life and human health (Ritz et al., 2004). The National Institute of Occupational Safety and Health (NIOSH) have established a time-weighted human threshold limit value which can only withstand 8 to 10 hours exposure of ammonia-N concentration at level of 25 ppm. The Occupational Safety and Health Administration (OSHA) stated that only 8 hours of exposure to the ammonia-N can be withstand at a the concentration of 50 ppm. The American

Conference of Governmental Industrial Hygienists (ACGIH) determined that human can withstand only for a short-term exposure limit of 5 minutes at the ammonia-N concentration of 35 ppm.

2.2 Ammonia Wastewater

Wastewater is any water that has been adversely affected in quality by human activity and also known as anthropogenic. The wastewater consists of waste that can contaminate the pure water in sense of their concentration. The waste can be discharge by many methods that are domestic residences, commercial properties, industry, and agriculture. The areas in which majority of wastewater are gather are known as the municipal wastewater. This municipal wastewater contains a broad spectrum of contaminants resulting from the mixing of wastewater from homes, businesses, industrial areas and often storm drains, especially in older sewer systems. Wastewater usually contains one important chemical that is ammonia-N. This ammonia-N usually present inside the wastewater due to the waste contains ammonia-N. In this part, there will be an explanation about the wastewater in poultry farm, in agriculture; wastewater in municipal and also comparing the concentration of ammonia-N in the wastewater between these three places will be elaborated.

2.2.1 Ammonia-N Content in Poultry Farm Wastewater (PFW)

Poultry farm is a place where industries breed and grow their poultry. These breeding and growing of poultry causes increasing number of poultry in poultry farm. From these poultry, they will produce a large amount of waste or excrement that will lead to over waste produced. The waste contains a lot of ammonia-N and thus causes the poultry farm to be contaminated or more toxic due to the ammonia-N released. The excessive waste produce from the poultry causes the waste to spoil the water near the farm and thus forming wastewater. This wastewater will release high concentration of ammonia-N that will lead to the poultry health issues and finally the poultry will die and people can get a disease from this. Figure 2.3 shows the poultry farm and the poultry inside the farm place.

Figure 2.3 The poultry farm and the poultry inside the farm place

There is this one research journal from a company of bio treatment where they have done a research about poultry waste water in the month of June. They take the sample from a poultry farm water supply and found out that at this month, the ammonia-N concentration was 170.80 mg/L. This shows that poultry waste contains a lot of ammonia-N and can contaminate any water supply.

2.2.2 Ammonia-N Content in Agriculture Wastewater

Agriculture is a farming or cultivation of animals, plants, fungi, and other life forms for food, fiber, and other products used to sustain life. Agriculture is the key development in the rise of sedentary human civilization, whereby farming of domesticated species created food surpluses that nurtured the development of civilization. Agriculture, generally speaking, refers to human activities of farming and plantation but due to the pest present such as ant and termite, they disrupt and destroy crops and plantation. Because of this termite and ant, many farmers use pesticides in order to kill them. The pesticides are widely used nowadays by farmers to control plant pests and enhance production, but these chemical pesticides can cause water quality problems. Pesticides can be transmitted to the surface of water via direct application, runoff during rain storms and aerial drift. Figure 2.4 shows the agriculture wastewater and aerial drift pesticides implementation.

Figure 2.4 The agriculture wastewater and aerial drift pesticides implementation

The pesticides are the main causes for the ammonia-N to spread to the water of the agriculture. The pesticides travel to the air and flow directly to the water causes the water to contaminate and become wastewater. Based on a Liess and Schulz (1998), they have taken the samples of wastewater at the agriculture area and have found out that the minimum ammonia-N concentration that contains inside the water due to pesticides was 0.05 mg/L and the maximum ammonia-N concentration was 1.0 mg/L. This shows that the pesticide causes the water to be contaminated with ammonia-N causes the average concentration of ammonia at around sites is ranges from 1 to 100 mg/L in soil. Ammonia-N can also be detected dissolved in pond and other bodies of water at a waste site. Ammonia-N levels are highest in the summer and spring seasons due to these pesticides.

2.2.3 Ammonia-N Content in Municipal Wastewater

Municipal has one major problem in wastewater treatment that is the separation of solids from water. The solids come from houses and other adjacent places to this wastewater treatment place because the wastewater plant to be treated by using two methods that are biological and chemical treatment. In general, the higher the degree of treatment of this wastewater treatment plant, the larger the amount of sludge that is being produced. Wastewater if not separate completely and correctly can cause the water to be higher in ammonia-N than normal. This is due to if the separation is not correctly, the solid waste can break up even more and thus more ammonia-N will be released into the water easily. This will also cause the solid waste to become harder to be extracted from the water due to it has been broken and thus can lead to complete dissolve of the solid to the water making it untreated wastewater. Municipal wastewater is usually treated in a combined sewer, sanitary sewer, effluent sewer and tank. Figure 2.5 shows the municipal wastewater.

Figure 2.5 The municipal wastewater

The municipal solid wastes include garbage, organic wastes, animal wastes and combustibles. Ammonia-N concentrations in the municipal wastewater are typically higher than in natural systems, ranging from 12 to 50 mg/L (Metcalf and Eddy, 1991). Based on Kim et al. (2005), nitrification process is the most economical process in removal of nitrogen in municipal wastewaters.

2.2.4 The Comparison between Concentrations of Ammonia-N in These Three Places

Based on the above three situations, it shows that the concentration of ammonia-N is higher at poultry farm rather than at the agriculture place and the municipal waste. This is due the ammonia-N concentration inside the poultry farm has the poultry waste and poultry waste produce higher concentration of ammonia-N than the pesticides and combined sewer, sanitary sewer, effluent sewer and tank from municipal waste. The concentrations of ammonia-N in poultry farm wastewater (PFW) are 170.80 mg/L compare to the agriculture place that is ranges from 1 to 100 mg/L and the municipal ranges between 1 to 50 mg/L. This proves that the ammonia-N in the agriculture place has been affect by the pesticides and this pesticide only contains a little amount of ammonia-N concentration. By comparing these three places, the ammonia-N concentration is the highest at poultry farm and this is a better place to make an experiment rather than the agriculture place and also municipal waste.

2.3 Nitrification

Nitrification is the conversion of ammonia-N to nitrate by action of microbial *Nitrosomonas and Nitrobacter* species. Ammonium is aerobically nitrified by autotrophic bacteria and then, the nitrate and nitrite is anaerobically converted to nitrogen gas by heterotrophic bacteria (Wen & Wei, 2011). Microbial activity is responsible for the two steps of nitrification which conversion of ammonia-N to nitrite and conversion of nitrite to nitrate. These two-step process begin with the first step which is ammonia-N is converted to nitrite by *Nitrosomonas* species. The second step of nitrification occurs through *Nitrobacter* species, which convert nitrite to nitrate. This step rapidly follows ammonia-N conversion to nitrite, and consequently nitrite concentrations are normally low in soils (Ritz et al., 2004). Nitrification is understood to be an aerobic process there is strong evidence that it can also occur under anaerobic conditions. The effect of nitrification process is acid production. This acid will lower the pH of environment which then reduces the rate of growth of nitrifying bacteria. These two groups of autotrophic bacteria are called nitrifying bacteria because the bacteria of the *Nitrosomonas* sp. oxidize NH_3 to nitrites (NO_2^-) and *Nitrobacter* sp. bacteria oxidize the nitrites (NO_3^-). Nitrifying bacteria are chemoautotrophic which depending on the genera like *Nitrosomonas, Nitrosococcus, Nitrobacter,* and *Nitrococcus* species. These bacteria grow by consuming inorganic nitrogen compounds. They are widespread in soil and water, and are found in highest numbers where considerable amounts of ammonia are present such as areas with extensive protein decomposition and sewage treatment plants. Besides this, they also thrive in lakes and streams with high inputs of sewage and wastewater because of the high ammonia-N content. Eq. 2.1 shows the nitrification of ammonia-N:

$$(ammoniacal - N) \overset{K_1}{\rightarrow} (NO_2^- - N) \overset{K_2}{\rightarrow} (NO_3^- - N) \qquad (2.1)$$

The nitrifying activity was estimated by determining the kinetic parameters k_1 and k_2 based on the fitting of the kinetic models represented by the equations of consumption of the substrate $N\text{-}NH_4^+$ (Eq. 2.2) as a function of the production of the oxidized forms (NO_2^- – N and NO_3^- – N) in the nitrification stages (Eq. 2.3) and nitritation (Eq. 2.4).

$$(ammonialcal - N) = (ammoniacal - N)_0 . e^{-kl.t} \qquad (2.2)$$

$$(NO_2^- - N) = (ammoniacal - N)_0 . k_1 (\frac{e^{-k1.t}}{k2-k1} + \frac{e^{-k2.t}}{k1-k2}) \qquad (2.3)$$

$$(NO_2^- - N) = (ammoniacal - N)_0 . k_1 (1 + \frac{k2}{k1 - k2} e^{-k1.t} + \frac{k1}{k2 - k1} e^{-k2.t}) \qquad (2.4)$$

2.4 Microbe of Nitrification

Soil mixed culture is a type of soil that is mixed with other substances to form mixed culture. This soil mixed culture is good for reduction of any high concentration of chemical substances. This is because the soil mixed culture contains different kinds of bacterium that is used for the treatment. The treatments are bioremediation, nitrification, denitrification and others. Based on Khan et al. (2008), mixed cultures have been shown to be more suitable for bioremediation compared with pure cultures. They also restated that rationale of using this mixed culture is that their biodiversity can enhance environmental survival and increase the number of catabolic pathways available for contaminant biodegradation. For nitrification, the soil mixed culture is used in order to reduce ammonia-N that is present inside any soil or poultry waste. Based on Ritz et al. (2004), there are a bacteria that contains inside the soil mixed culture that can help the reduction of ammonia-N inside the poultry waste that is *Nitrosomonas* and *Nitrobacter* bacteria. These two bacteria are responsible in reducing the ammonia-N concentration in poultry wastewater (PFW) plant. Thus, this shows that mixed culture is good for any treatment including nitrification and others. Figure 2.6 shows the example of soil mixed culture.

Figure 2.6 Example of soil mixed culture

There are two types of soil will be used that is University Malaysia Pahang soil mixed culture (UMPC) and poultry farm soil mixed culture (PFC). The microbes that will be inside this soil will be elaborate and explained under the nitrifying bacteria species.

2.4.1 Nitrifying Bacteria Species

Nitrifying bacteria is either chemoautotrophic or chemolithotrophs depending on the genera bacteria by consuming the inorganic nitrogen compounds for growth. The genera are *Nitrosomonas, Nitrosococcus, Nitrobacter,* and *Nitrococcus*. Many of the bacteria species has a complex internal membrane system that is the location for key enzymes in nitrification. The enzymes are ammonia monooxygenase and nitrite oxidoreductase. These two enzymes function are to firstly oxidize the ammonia into hydroxylamine (nitrite) via ammonia monooxygenase and secondly are oxidize nitrite to nitrate via nitrite oxidoreductase. These bacteria like to thrive or live at the areas where there are a lot of ammonia-N present and with extensive protein decomposition, and sewage treatment plants. Among them, the nitrification process has been thought to be carried out mainly by ammonia-N and nitrite-oxidizing bacteria that are obligate aerobic and chemoautotrophic (Kim et al., 2005). Since the nitrifying bacteria have four types, this research has only two types of genera inside the poultry waste that are *Nitrosomonas* sp. and *Nitrobacter* sp. These two bacteria have different role in the process that is based on Boas et al., (2011) the *Nitrosomonas* undergoes nitritation process whereas *Nitrobacter* undergoes nitration.

Nitrosomonas species is a genus comprising shaped chemoautotrophic bacteria. This is a rare bacterium that oxidizes ammonia into nitrite as a metabolic process. This process is called nitritation. The bacterium is useful in many areas of treatment such as industrial treatment, sewage treatment, and bioremediation. In a new treatment, it is also going to be useful for poultry waste treatment where it will reduce the ammonia-N in this waste. The reasons they use this bacteria species is due to they are important in nitrogen cycle. They are important due to they can increase the availability of nitrogen to plants and soil while reduce the carbon dioxide fixation. This species is usually found in soil mixed culture, sewage, freshwater, building surfaces and in poultry waste where nitrogen concentration is high. Figure 2.7 shows the *Nitrosomonas* species bacterium.

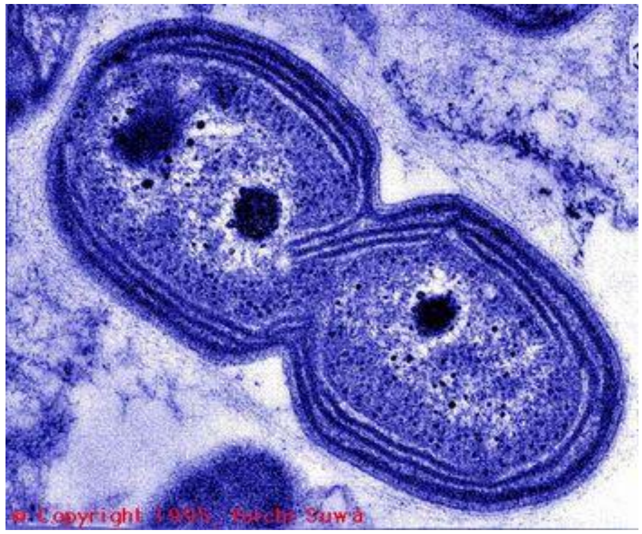

Figure 2.7 *Nitrosomonas* species bacterium

The *Nitrosomonas* bacterium prefers an optimum pH of 6.0 - 9.0 and temperature range of 20 to 30°C. They have a flagellum located in the Polar Regions that makes most of this species are motile. These bacteria multiply by consuming high concentration of ammonia-N and thus they need to be in a place where a lot of ammonia-N is present. Most N_2O is formed from dissimilatory reduction of nitrate in oxygen deficient environment; although it can also be produced from chemolitotrophic and heterotrophic nitrification and assimilatory reduction of nitrate in aerobic conditions (Wlodarczyk, Glinski, & Kotowska, 2004). They also describe that in most soils, nitrification of applied ammonium is rapid (2-3 weeks), but nitrification rates are greatly reduced by cool soil mixed culture temperature 10°C, low pH (5.5), and waterlogged conditions. Under this condition, the *Nitrosomonas* bacteria species cannot perform well as their optimum condition for the ammonia monooxygenase process is pH between 6.0 and 9.0 and the temperature of 20 to 30°C. Based on Boas et al. (2011), nitrification is the most sensitive process among the processes of biological removal of nutrients from residual waters. The autotrophic mass is approximately 10-fold more sensitive than the heterotrophic biomass. This is due to this bacteria is sensitive to the surrounding environment.

Nitrobacter species is almost the same species as *Nitrosomonas* but it is a gram negative bacteria. It has almost the same role as *Nitrosomonas* where its role is also for nitrogen cycle but the difference is that this bacterium oxidizes nitrite to nitrate. This process is called nitration. For *Nitrobacter,* they are different from *Nitrosomonas* because *Nitrosomonas* use energy from ammonia-N oxidizes to nitrate while *Nitrobacter* use energy from the oxidation of nitrite ions, NO_2, into nitrate ions, NO_3^- to fulfill their carbon requirements. This process undergoes anaerobic process where it converts NO_2^- to NO_3^- (Verchot, Groffman, & Frank, 2002). Figure 2.8 shows the *Nitrobacter* species bacterium.

Figure 2.8 *Nitrobacter* species bacterium

Nitrobacter have an optimum pH between 7.3 and 7.5, and will die in temperatures exceeding 49°C or below 0°C. According to Grundmann et al. (2000), *Nitrobacter* seem to grow optimally at 38°C and at a pH of 7.9, but Holt et al. (1993) states that *Nitrobacter* grow optimally at 28°C and grows within a pH range of 5.8 - 8.5 and has pH optima between 7.6 and 7.8. Based on Huber and Nelson et al. (1992), nitrite is further oxidized to nitrate (NO_3) by *Nitrobacter* bacteria in a process termed nitrification. They also stated that nitrate is normally the form of N taken up by plants; however, most plants can also assimilate ammonium.

2.5 Kinetic Study

There are two parts in this section, the first part is to compare kinetic studies between researches and the second part is to choose suitable kinetic study for this research.

2.5.1 Comparing Kinetic Study

Kinetic study is one of the methods to get the value for the nitrification process. In this part, there will be comparison between two different journals about the kinetic study that they have done on their research. The research study is based on one journal from Ritz et al. (2004) that is the determination of kinetic constants for nitrifying bacteria in mixed culture, with the aid of an electronic computer and the other journal is from Dincer and Kargi (2000) that is kinetics of sequential nitrification and reduction of nitrification processes. This journal has been stated on the kinetic study of this research.

Based on the researcher of Ritz et al. (2004), the researchers use the equation for growth of *Nitrosomonas* in the oxidation process of ammonium. This can be described by the Monod equation:

$$\frac{dXn}{dt} = \left(\frac{dXn}{dt}\right)g + \left(\frac{dXn}{dt}\right)d \tag{2.5}$$

$$\left(\frac{dXn}{dt}\right)g = \mu . X_n = \mu_m . X_n . \frac{Na}{(Na + K_n)} \tag{2.6}$$

$$\left(\frac{dXn}{dt}\right)d = -b_n . X_n \tag{2.7}$$

Where is X_n = *Nitrosomonas* concentration (mg VSS/L), $(\frac{dXn}{dt})$ = rate of change of the *Nitrosomonas* concentration (mg VSS/L.d) = net growth rate of *Nitrosomonas*, $(\frac{dXn}{dt})g$ = *Nitrosomonas* growth rate (mg VSS/L.d), $(\frac{dXn}{dt})d$ = *Nitrosomonas* decay rate (mg VSS/L.d), μ = specific growth rate for *Nitrosomonas* (d^{-1}), μ_m = maximum specific growth rate for *Nitrosomonas* (d^{-1}), b_n = decay rate for *Nitrosomonas* (d^{-1}), K_n = Monod half saturation constant (mg N/L).

In the Monod equation, the parameter μ represents the growth rate of the micro-organisms per time unit. The Eq. 2.6 shows that the μ value depends on the substrate concentration (N_a). At high N_a concentration (saturation), the maximum growth rate μ_m is attained. The constant K_n is equal to the substrate concentration for which $\mu_m = \frac{1}{2\mu}$, and for that reason is called the "half" saturation constant. The basic equation of Ritz et al. (2004) can be used to calculate the residual ammonium concentration in a completely mixed advance sludge process. Under these conditions, there is no variation of the mass of *Nitrosomonas* in the system: the net growth rate (defined as the growth rate minus the decay rate) is equal to the discharge rate due to abstraction of excess poultry waste. Hence:

$$\left(\frac{dXn}{dt}\right) = 0 = \left(\frac{dXn}{dt}\right)g + \left(\frac{dXn}{dt}\right)d + \left(\frac{dXn}{dt}\right)e \tag{2.8}$$

The rate of change of the *Nitrosomonas* concentration due to the discharge of excess poultry waste $\left(\frac{dXn}{dt}\right)e$ can be expressed as:

$$\left(\frac{dXn}{dt}\right)e = \frac{-X_n}{Rs} \tag{2.9}$$

By using Eq. 2.6 and Eq. 2.9 inserted into Eq. 2.8, it will become:

$$\left(\frac{dXn}{dt}\right) = 0 = \mu_m . X_n . \frac{Na}{(Na + K_n)} - b_n . X_n - \frac{X_n}{Rs} \tag{2.10}$$

$$= \mu_m . \frac{Na}{(Na + K_n)} - b_n - \frac{1}{Rs} \tag{2.11}$$

By rearranging:

$$N_a = K_n . \frac{(b_n + \frac{1}{Rs})}{(\mu_m - (b_n + \frac{1}{Rs}))} \tag{2.12}$$

From the Eq. 2.12, it gives the ammonium concentration in the mixed liquor of a completely mixed sludge process and hence also in the effluent of this process. This residual concentration, which is indicative of the efficiency of the nitrification process, depends on the numerical values of the three kinetic parameters are μ_m, K_n and b_n and one operational variable and also the poultry waste age (R_S). It is interesting to note that the residual ammonium concentration does not depend on the initial concentration. The residual ammonium concentration can never be superior to the ammonium concentration that is available for nitrification. Therefore this condition defines the minimum sludge age for nitrification as:

$$N_a = N_p = K_n . \frac{(b_n + \frac{1}{Rsn})}{(\mu_m - b_n - \frac{1}{Rsn})} \tag{2.13}$$

$$R_{sn} = \frac{(1 + \frac{K_0}{Np})}{(\mu_m - b_n \; .(1 + \frac{K_0}{Np})} \tag{2.14}$$

Where N_p = nitrification potential = ammonium concentration available for nitrification (mg N/L).

In the case of domestic sewage, the ammonia-N concentration available for nitrification will always be much greater than the half saturation value *Kn*. In that case the ratio $\frac{K_n}{R_P}$ « l and Eq. 2.14 can be simplified to:

$$R_{sn} = \frac{1}{(\mu_m - b_n)} \quad (2.15)$$

Eq. 2.15 also expresses that nitrification will not develop if the sludge age is shorter than a minimum value of $R_{sn} = \frac{1}{(\mu_{m} - b_n)}$ which because the rate of *Nitrosomonas* discharge in the excess sludge will exceed the net growth rate. When the sludge age R_s is higher than the minimum value, nitrification will develop and its efficiency will depend on the sludge age and the kinetic constants K_n, μ_m and b_n.

For Dincer and Kargi (2000), the concentration of ammonia-N unit used for nitrification detect of analysis soil mixed culture with 120 mg/L was operated at different time in HACH equipment, Θ_H. According to Dincer and Kargi (2000), the synthesis of ammonia-N is based on the nitrification process forming nitrite by using *Nitrosomonas* species present inside the poultry farm wastewater and lastly converted to nitrate by using *Nitrobacter* species. The nitrification process of the ammonia to nitrate reaction scheme is shown as from the balance:

$$2NH_3(g) + 4H_2O(l) \xrightarrow{\textit{Nitrosomonas}\text{ species}} 2NO_2^- + 7H_2(g)$$

$$NO_2^- + H_2O(l) \xrightarrow{\textit{Nitrobacter}\text{ species}} NO_3^- + H_2(g)$$

From the nitrification balance, the Eq. 2.16 is constructed:

$$U = \frac{Q(N_0 - N)}{VX} = \frac{kN}{K_N + N} \tag{2.16}$$

Where, U is specific rate of nitrification (mg N/mg Xxd); Q is flow rate of feed ammonia (L/d); V is volume of the aeration tank (L); No and N are the feed and effluent NH_4-N concentrations (mg/L); X is the concentration of soil mixed culture from University Malaysia Pahang (UMP) and poultry farm (mg/L); k (d^{-1}) and K_N (mg/L) are the maximum nitrification rate and saturation constants, respectively. Further equation will be placed at the linear regression method for Dincer and Kargi (2000) due to it will be used for the calculation of kinetic parameter of k and K_N.

In choosing the suitable kinetic study, this research chooses Dincer and Kargi (2000). The reason for picking that this research is due to it has the required data that is the U and other value to form a graph and to use it in the linear regression method. This equation is being used due to many research refer to this equation. Other than that, it is because Dincer and Kargi (2000) equation has the kinetic parameter that this research can used and also can be applied to the reduction of ammonia-N concentration to the lowest concentration.

2.5.2 Linear Regression Method

This Linear Regression Method is a method to solve the kinetic study by using Microsoft Excel Solver program. This method is the best-fit line method where it is to minimize the discrepancy between the data point and the curves. The mathematical expression for the straight line is:

$$y = a_0 + a_1 x + e \tag{2.17}$$

Where a_0 is $\frac{1}{k}$ from the y-intercept and a_1 is $\frac{K_N}{k}$ from the slope of the graph. This value of k and K_N is the kinetic parameter of nitrification process. The e is error or residual of the true and the observation (approximation) value from the graph. The Eq. 2.18 is for error, e, which is:

$$e = y - a_0 - a_1 x \tag{2.18}$$

Each of the *k'*s represents as slope. This equation of linear regression will follow the Dincer and Kargi (2000) Eq. 2.16 in which it will be changed to reciprocal equation:

(2.19)

$$\frac{1}{U}=\frac{\emptyset_H X}{(N_0-N)}=\frac{1}{k}+\frac{K_N}{k}\frac{1}{N}$$

Where, the Eq. 2.17, Eq. 2.18 and Eq. 2.19 were used for determination of kinetic parameter which was plotted as $\frac{1}{U}$ versus $\frac{1}{N}$ in which will give a result line with a slope of $\frac{K_N}{k}$ and intercept of $\frac{1}{k}$. From the slope and intercept of the best – fit line, the following values of k and K_N (Dincer and Kargi, 2000) is determined by Linear Regression method where this equation can be performed for both the UMP soil mixed culture (UMPC) and poultry farm soil mixed culture (PFC). After the determination of k and K_N, a new equation in which Eq. 2.20 is formed by definition of ammonia-N reduction that is:

$$\mu=\frac{1}{\emptyset_C}=\frac{Y K_N}{K_{N+N}}-b=YU-b \qquad (2.20)$$

Where, μ is specific growth rate of organisms (d^{-1}); θc is poultry farm wastewater (d); Y is the growth yield coefficient (gX/gN), and b is the ammonia-nitrogen reducing constant (d^{-1}). A plot of $1/\theta c$ versus U results in a line with a slope of Y and intercepts of b. Thus the reducing in ammonia-N concentration in PFW can be determined. The kinetic parameter can be solving using Linear Regression method by Excel Solver.

Based on White and Reddy (2003), this linear regression method can used in order to determine the kinetic parameter k and K_N for nitrification process and reduction of ammonia-N. Other than that, the researchers of Verchot et al. (2002) and Wlodarczyk et al. (2004) claim that by using the Linear Regression Method can help in more accurate results and can help in the determination of the ammonia-N reduction in contaminated soil and wastewater.

CHAPTER 3

METHODOLOGY

3.1 Process Flow Diagram

Methodology is an important part of this study. To accomplish this study, the following flow chart shows the process of reduction of ammonia-N in poultry farm wastewater (PFW) by nitrification process using Linear Regression Method for determining the kinetic study. There are four major steps to be used for this research in which will be shown in Figure 3.1.

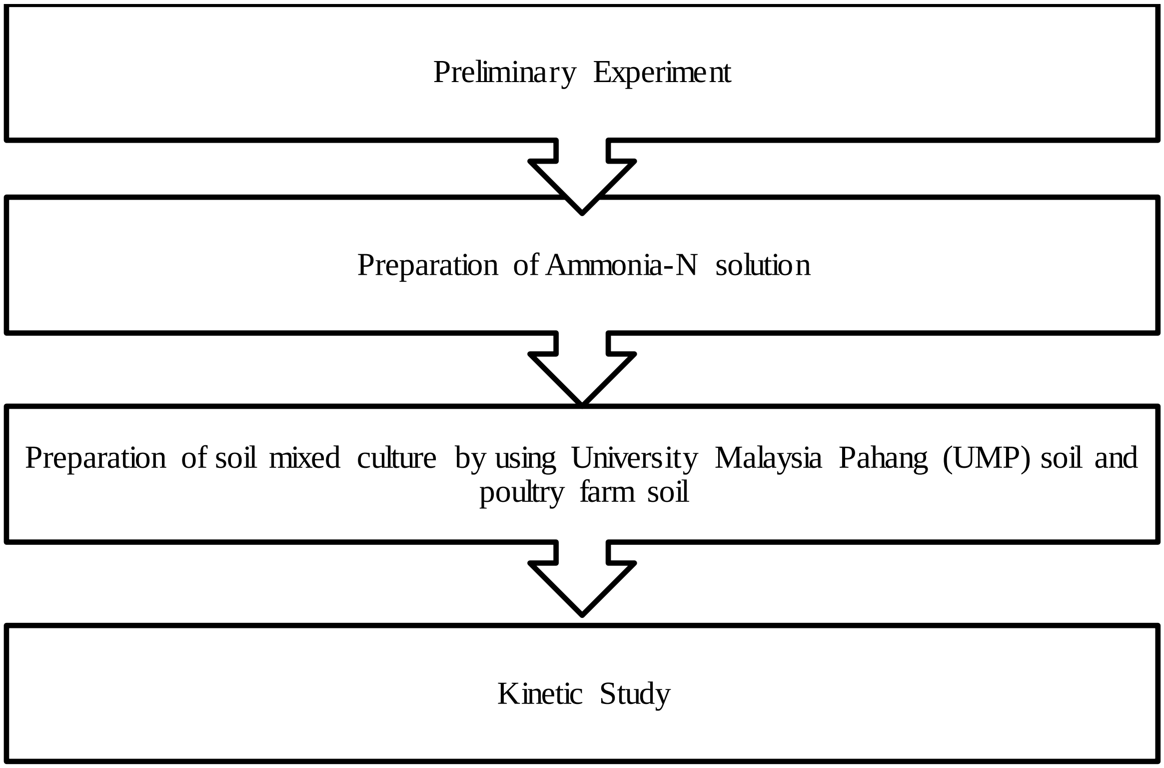

Figure 3.1 Four major steps to be used in the research

3.2 Preliminary Experiment

Preliminary experiment was important in order to find the suitable time of reaction for reducing ammonia-N concentration. For identifying the suitable time of reaction, several runs were conducted in this experiment. There were four sets of runs which were by combining ammonium nitrate solution with UMP soil, ammonium nitrate solution with soil from poultry farm, poultry farm wastewater (PFW) with UMP soil and PFW with soil from poultry farm. The reaction time for reducing ammonia-N is shown as Table 3.1.

Table 3.1 The reaction time of preliminary experiment for all sets of run (set 1 to 4)

Experiment		Set							
		1		2		3		4	
		Time (h)		Time (h)		Time (h)		Time (h)	
		0	5	0	5	0	5	0	5
Concentration (mg/L)	Initial	95.0	95.0	95.0	95.0	280.0	280.0	280.0	280.0
	Final 1	57.5	60.0	57.5	55.0	192.5	220.0	210.0	232.5
	Final 2	55.0	55.0	50.0	52.5	190.0	220.0	215.0	232.5
	Final 3	62.5	55.0	47.5	52.5	195.0	220.0	222.5	237.5
	Average	58.3	56.7	51.7	53.3	192.5	220.0	215.8	234.2

Where set 1 is the ammonia-N with UMP soil, set 2 is the ammonia-N with poultry farm soil, set 3 is the PFW with UMP soil and lastly is set 4 in which PFW with poultry farm soil.

From the Table 3.1, the reduction of ammonia-N from 0 to 5 hour is for UMP soil with ammonia-N whereas a slight increase of ammonia-N concentration from 0 to 5 hour is for poultry farm soil with ammonia-N. These two sets has a better reduction than the other two sets due to the ammonia-N in other two sets increases a lot from 0 to 5 hours. Thus, set 1 and 2 has been chosen for this research.

3.3 Preparation of Ammonia-N Solution

Pure ammonia-N was prepared as a stock solution for the whole experiment. The process of this preparation is by using ammonia-N as the solute. This ammonia-N was diluted with water in order to get the stock solution. Eq. 3.1 was used for dilution of ammonia-N.

$$\frac{Ammonia - N\ concentration\ \times\ molecular\ weight\ ammonia - N\ nitrate}{molecular\ weight\ of\ ammonia - N} \quad (3.1)$$

The diluted ammonia-N was then used in the spectrophotometer HACH DR/2800 model. The concentration of ammonia-N preparation is 120 mg/L. This concentration will then combine with the soil mixed culture and there will be further discussed for reduction of ammonia-N.

3.4 Preparation of Soil Mixed Culture by Using University Malaysia Pahang (UMP) Soil and Poultry Farm Soil

The soil mixed culture was prepared in this experiment in order to determine the effect of soil reaction towards ammonia-N. This solution was prepared by mixing two main components that are dry soil and distilled water which the ratio of this solution is

1:6 where 100 mL of soil is added with 600 mL of distilled water. This soil was taken from two different places that were University Malaysia Pahang (UMP) soil and poultry farm soil.

3.5 Kinetic Study

The experimental equation will be conducted using the Monod Equation which its function is to determine ammonia-N removal by using soil mixed culture from UMP and soil mixed culture from poultry farm. 100 mL of soil mixed culture prepared from the part 3.3 for UMP soil will be mixed with 150 mL of ammonia-N stock solution. From the 250 mL of mixed solution of ammonia-N and UMP soil, only 5 mL of solution is taken to be diluted in a 1245 mL of deionized water (1250 mL conical flask). After dilution, 10 mL of the diluted solution is taken in which it will be added with Ammonia Salicylate Reagent Powder Pillow where it will be shaking for three minutes. After three minutes, the solution is added with Ammonia Cyanurate Reagent Powder Pillow in which it will be shaking for 15 minutes. After 15 minutes, the solution will be placed inside the HACH DR/2800 equipment for the ammonia-N concentration result. This experiment will be conducted for an interval of 30 minutes or ½ hour until reaches 5 hours. After finish, the steps for the UMP soil mixed culture (UMPC) with ammonia-N will be repeated but the soil will be changed from UMP to poultry farm soil mixed culture (PFC). From these result, the graph is being plotted from data (Table B.2 and B.5) that obtained by the experimental time versus concentration.

Experimental data that was obtained at a different concentration of ammonia-N after the nitrification process occurred was used to determine the kinetic parameters (Table B.3 and B.6). These kinetic parameters were calculated by using the Kinetic Modeling for nitrification process and it was shown in the graph which has been plotted by following the nitrification kinetic:

a) Nitrification kinetic

The nitrification balance is constructed as below:

$$U = \frac{Q(N_0 - N)}{VX} = \frac{kN}{K_N + N} \tag{3.2}$$

Then create a reciprocal equation from Eq. 3.1 to get:

$$\frac{1}{U} = \frac{\emptyset_H X}{(N_0 - N)} = \frac{1}{k} + \frac{K_N}{k}\frac{1}{N} \tag{3.3}$$

From the slope and intercept of the best – fit line, the following values of k and K_N (Dincer and Kargi, 2000) was determined by Linear Regression method where this

Eq. 3.3 can be performed for both the UMPC and PFC. After k and K_N was determined, a new Eq. 3.4 was formed by definiton of ammonia-N reduction that was:

$$\mu = \frac{1}{\emptyset_C} = \frac{YkN}{K_{N+N}} - b = YU - b \tag{3.4}$$

A plot of $1/\theta c$ versus U results in a line with a slope of Y and intercept of b. Thus the reduction of ammonia-N concentration was being determined by this graph. The kinetic parameter for both soils is the same that is k and K_N .Then, kinetic parameter can be solve using Linear Regression method by Excel Solver.

The analysis method used in this comparison of soil mixed culture form UMP and poultry farm in reducing ammonia-N by using Linear Regression method with Microsoft Office Excel Solver Program (Table B.4 and B.7).

CHAPTER 4

RESULT AND DISCUSSION

4.1 Kinetic Parameter Result

The result of calculated kinetic parameter of poultry farm soil mixed culture (PFC) and University Malaysia Pahang soil mixed culture (UMPC) are presented in Appendix A, while the values got from the calculation for the k and K_N are presented in each part of UMPC and PFC. The data obtained from the experiment were presented in the form of line chart and tables for each parts of soil mixed culture.

4.2 Kinetic Determination by Nitrification Process

Nitrification process is one of the most important biological processes in order to remove the excess ammonia-N in any soil or places. There is evidence that nitrification process is important source of N_2O in both soils and aquatic systems (Goreau, Kaplan, Wofsy, McElroy, Valois, & Watson, 1980). According to Kutty, Isa & Leong (2011), Lang & Elliot (1997) and Krist, Davide, Peter, & Willy (1999), the nitrification process undergoes two-step process in which it will occurred when the ammonia (NH_3) is oxidized to nitrite (NO_2^-) and nitrate (NO_3^-) by aerobic autotroph bacteria and it will finally have an output of nitrogen gas (N_2). In this process, the important factor that needs to be considered is determination of kinetic parameter of nitrification. Based on Juliana, Simone, Marcio, Benedito, & Fernando (2011), they stated that in order to know the nitrifying activity, the kinetic parameters must be known. The kinetic parameter will determine whether the soil can be a good reducing agent for nitrification process or it is not suitable. In order to determine this kinetic parameter, there were two types of soil that was used which were the UMPC and PFC.

4.3 Soil Mixed Culture

The determination of kinetic parameter must have the following data of U, N_o, N, X, $\frac{1}{U}$ and $\frac{1}{N}$. These coefficients are described as the specific rate of nitrification for U, N_o and N are the feed and effluent NH_4-N concentrations (mg/L), X is the concentration of soil mixed culture from UMP and poultry farm (mg/L), k (h^{-1}) and K_N (mg/L) are the maximum nitrification rate and saturation constants, respectively that are presented in the Table of B.3 and B.6. These two tables show the value for each coefficient that needed for determination of kinetic parameter of soil mixed culture for both UMP soil and poultry farm soil will be further discussed.

The data from Table B.3 and B.6 are use in order to determine the kinetic constants for each of the soil mixed culture in which it is then used for plotting the graph of ammonia-N concentration versus time. Experimental data from the graph obtained at different concentration and time are plotted as $\frac{1}{U}$ versus $\frac{1}{N}$ result in a line with a slope of $\frac{K_N}{k}$ and intercept of $\frac{1}{k}$. From the slope of the graph where obtained and intercept of the best – fit line, the following values of k and K_N (Dincer and Kargi, 2000) are determine by Linear Regression method. The plotted graph of concentration versus time is shown in Figure 4.1 and 4.3 whereas for the graph of $\frac{1}{U}$ versus $\frac{1}{N}$ is plotted in Figure 4.2 and 4.4. Figures 4.1 and 4.3 depict the graph of interaction between soil mixed culture and ammonia-N concentrations with time whereas for Figures 4.2 and 4.4 depict the graph of the nitrification process of the ammonia-N concentration and soil mixed culture for this research.

4.3.1 University Malaysia Pahang Soil Mixed Culture (UMPC)

The analysis of Figure 4.1 reveals that the effectiveness of UMPC was more pronounced in the first 2.5 h and after this period, its effectiveness towards reducing the ammonia-N concentration decreases slightly. This proves that UMPC has reduced the ammonia-N concentration gradually with time. According to the figure, it can be shown that the longer the time, the more the ammonia-N concentration decreases in fluctuating manner with suitable soil used or treatment. Based on Norton & Stark (2011), the rates of nitrification generally respond quickly to ammonium additions whereas the increase in ammonia-N oxidizer population causes a significant lag to nitrification process.

Nitrification process is used to reduce the ammonia-N concentration inside the poultry farm wastewater (PFW) so it can reduce the diseases and mortality to the poultry industries. At the first time set which was 0 sec, the ammonia-N concentration that obtained was about 63 mg/L. After half an hour, the sample was tested again for the ammonia-N concentration and discovers that it reduces to about 55 mg/L. The next half an hour after that, it was tested again and obtained that it increases the concentration of ammonia-N to 58 mg/L.

From these times, it signify that it has a slight increase in ammonia-N concentration this is cause due to the bacteria produced the ammonia-N in order to reprocess the reaction. After that, it will be decreased again because the nitrification process has begun to react again. As from Figure 4.1, it shows that the reaction inside the ammonia-N solution occurred in nitrification and reduction of nitrification. Thus the graph will fluctuate until it will get a slightly straight line where majority of ammonia-N concentration has been used up for this nitrification process. Thus, the graph will have a slight fluctuate of increase and decrease of ammonia-N. The graph that can be used is the graph which follows the R^2 that is higher value than the presume R^2 which is 0.8. Since the R^2 value is much higher than the presume R^2 which is 0.8542, thus, the data from the graph can be used to plot a new graph of $\frac{1}{U}$ versus $\frac{1}{N}$. This will determine the coefficient of the kinetic parameter which is k and K_N.

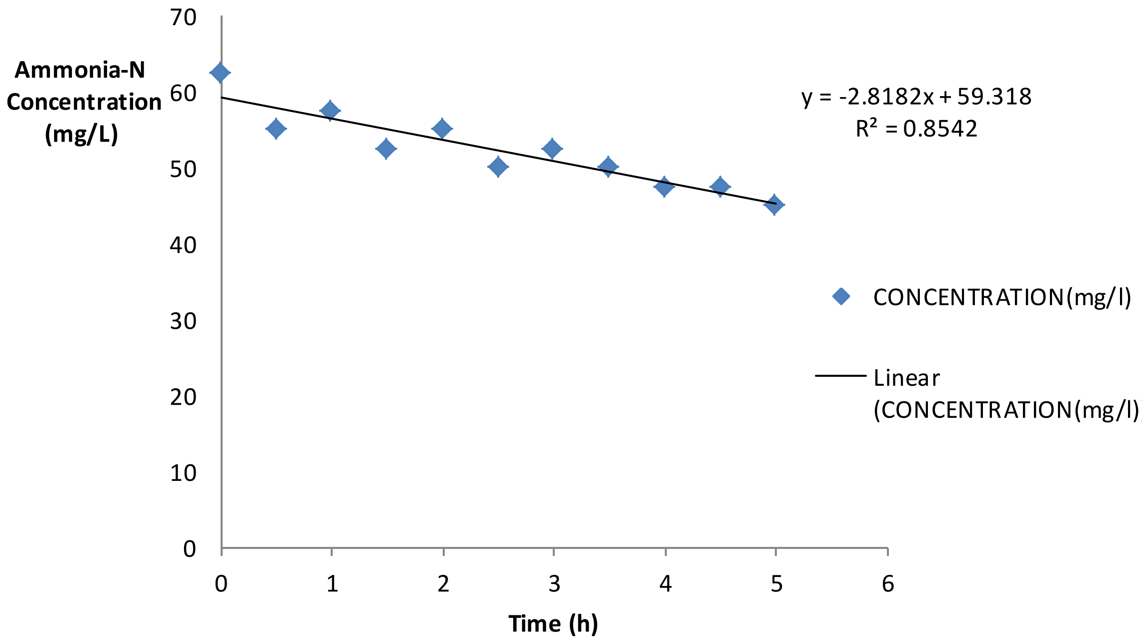

Figure 4.1 Ammonia-N concentrations versus time for UMPC

Based on the analysis of Figure 4.2, the required value was obtained for the kinetic parameter calculation that is 0.8149. This value is taken from the y-intercept where it is the most important value for the calculation. From this value, the kinetic parameter of k and K_N for UMPC got is by using the following equation $\frac{1}{U} = \frac{\emptyset_H X}{(N_0 - N)} = \frac{1}{k} + \frac{K_N}{k}\frac{1}{N}$. The data obtained from the calculation is as shown:

$$k = 1.227\ \text{h}^{-1}$$

$$K_N = 67.609\ \text{mg/L}$$

The kinetic parameter gains from the calculation of data taken from the graph for UMPC are k = 1.227 h^{-1} and K_N = 67.609 mg/L. The kinetic parameter obtain determines the nitrification reaction of ammonia-N reduction is either a slow or a fast reaction. The lower the value of the kinetic parameter K_N, the slower the nitrification reaction due to this will slow down the chemical process of ammonia-N reduction. And also the higher the k value, the slower the reaction due to this kinetic parameter shows the time taken for the reaction to finish one nitrification process. In this case, the value that obtain from the calculation is an average value which shows that the UMPC have some effect to the ammonia-N concentration but it has a slower reaction due to the bacteria does not adapted to this kind of situation (Li, Irvin, & Baker, 2006). This concentration of ammonia-N will get affected but it will take a longer time for the process of nitrification and reduction of ammonia-N concentration.

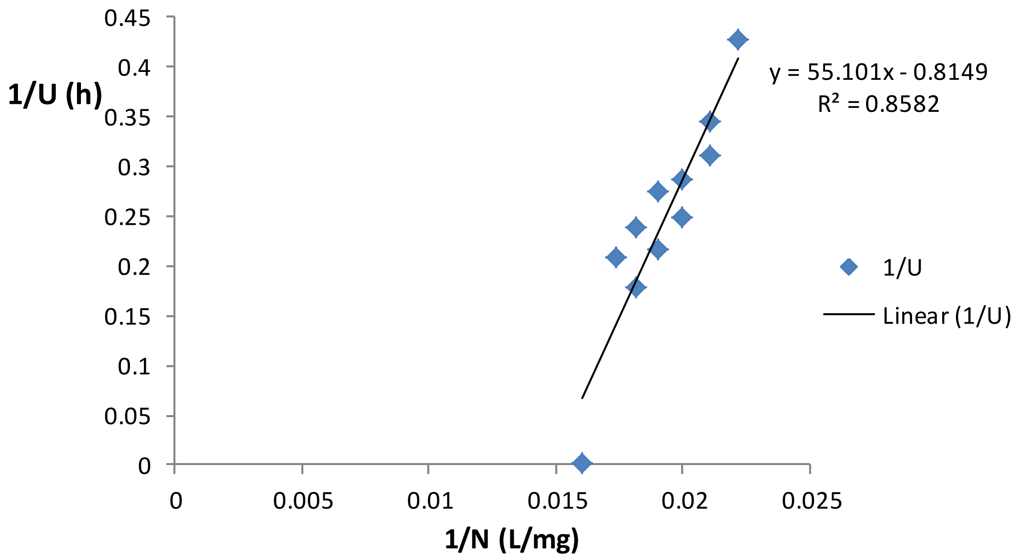

Figure 4.2 $\frac{1}{U}$ versus $\frac{1}{N}$ for UMPC

4.3.2 Poultry Farm Soil Mixed Culture (PFC)

Based on the analysis of Figure 4.3, the poultry farm soil mixed culture (PFC) has affected the ammonia-N concentration that can be used in many industrial for ammonia-N reduction. From the Figure 4.3, it signifies that the PFC affects the ammonia-N concentration where the longer the time, the more the ammonia-N concentration decreases. For the first set time that was 0 sec, the ammonia-N concentration obtained was about 60 mg/L. After half an hour, the sample was tested for the ammonia-N concentration and discovers that the value is reduced to 50 mg/L. The next half an hour after that, the value then increases the concentration of ammonia-N to 52 mg/L.

The process that occurred at this state was nitrification and reduction of nitrification where the decrease and increase of ammonia-N will occur until it reaches zero according to Weaver (2011). This zero indicates that all ammonia-N has been used up and get almost a constant line graph. The R^2 constant value for this graph is shown to be 0.818 which is higher than the presume value of 0.8. Thus, it can be used in this graph to develop a new graph of $\frac{1}{U}$ versus $\frac{1}{N}$. This graph will be used in determination of the kinetic coefficient of k and K_N.

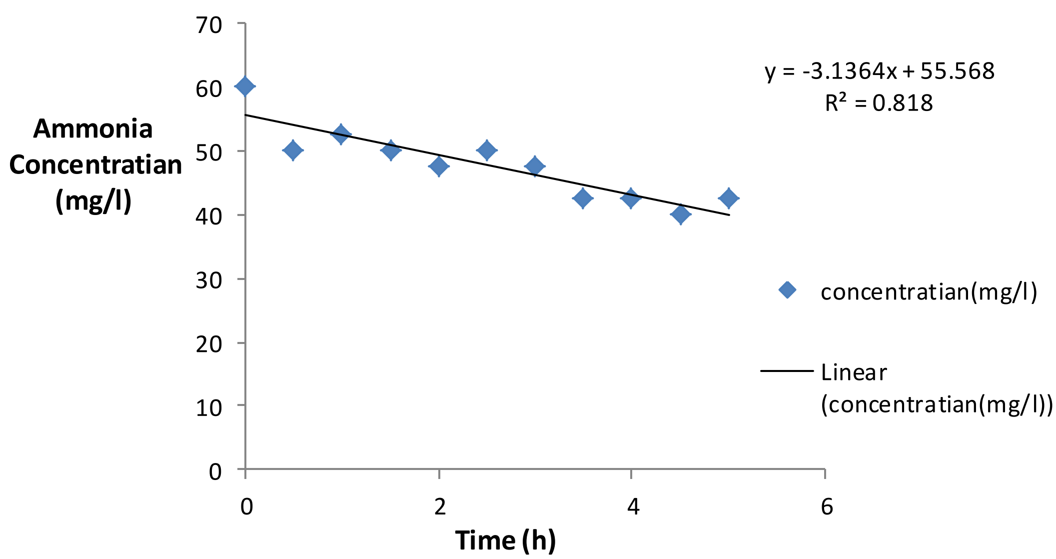

Figure 4.3 Ammonia-N concentrations versus time for PFC

Based on the analyzing of Figure 4.4, the required value for the determination of the kinetic parameter for this PFC is 0.9177. This value is inserted into the equation at UMPC and being calculated. The value that obtained from the calculation as shown:

$$k = 1.090\ \text{h}^{-1}$$

$$K_N = 68.454\ \text{mg/L}$$

From the data, the value for the parameter for k and K_N are 1.090 h^{-1} and 68.454 mg/L. This K_N value determines whether the reaction can reduce the ammonia-N concentration based on the k value. This result shows that the k value is 1.090 h^{-1} which in this time, it can reduce the ammonia-N concentration to the K_N value which is 68.454 mg/L. Thus, this reaction is quite fast for higher reduction of ammonia-N concentration. This shows that the bacteria inside the PFC have been able to adapt to this kind of situation and able to reduce the ammonia-N concentration to almost zero. This indicates that PFC can be used in this situation to reduce the ammonia-N concentration until zero.

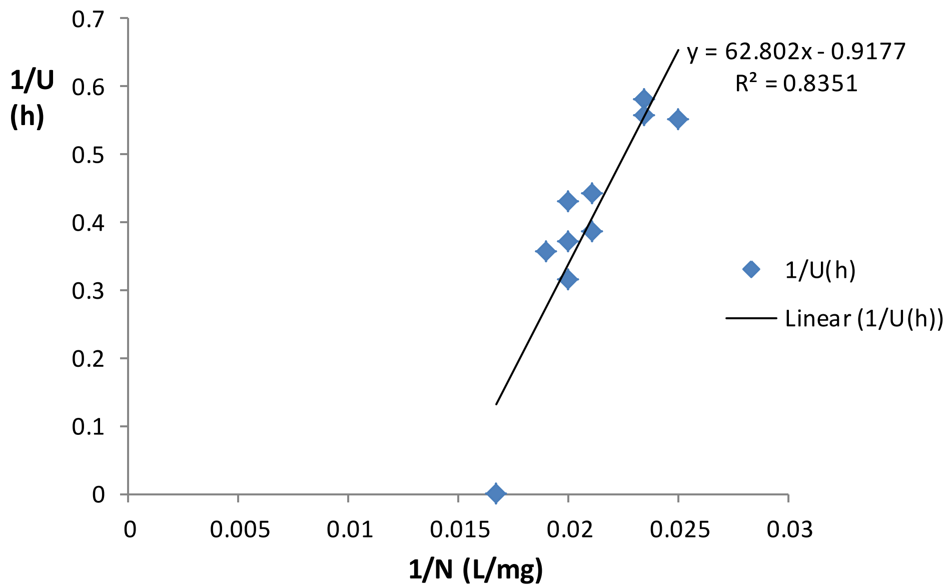

Figure 4.4 $\frac{1}{U}$ versus $\frac{1}{N}$ for PFC

4.4 Comparison of Kinetic Parameters

4.4.1 Comparison between Poultry Farm Soil Mixed Culture (PFC) and University Malaysia Pahang Soil Mixed Culture (UMPC)

In this section, the comparison between the PFC and UMPC for the reduction of ammonia-N concentration based on the obtained data and value and both compared to other research for ammonia-N reduction will be discussed.

From the results on part of 4.3, it shows that the UMPC is less suitable in reducing the ammonia-N concentration. This is because the soil mixed culture has the bacteria needed but it cannot perform to the fullest due to the condition at the UMP ground and poultry farm soil is different. The data shows that the UMP soil has a longer time to reduce the ammonia-N concentration than the poultry farm soil mixed culture. This can be compared as the value of *k* for UMP soil is 1.227 h^{-1} and poultry farm soil is 1.090 h^{-1}. The poultry farm soil has a faster nitrification reaction time to reduce the ammonia-N concentration than the UMP soil due to the bacteria contains inside the PFC has a higher population than UMPC. Based on Brion & Billen (1999), they found out that the lower or smaller the population of the *Nitrosomonas* and *Nitrobacter* sp., the slower the robust reaction of the bacteria in reducing the ammonia-N concentration. This proves that the bacteria in poultry farm soil have higher population than the UMP soil. Thus, it can reduce ammonia-N concentration for a lesser time in any ammonia-N contaminated area such as poultry farm wastewater.

Furthermore, the kinetic parameter K_N got from the poultry farm is much higher than the UMP soil. This shows that poultry farm soil can reduce much more ammonia-N which is 68.454 mg/L rather than the UMP soil that is 67.609 mg/L. The higher reduction of ammonia-N depends on the bacteria conditions inside the soil mixed culture. In this case, the poultry farm soil has a suitable condition than UMP soil. Based on the researchers of Hunt & Vanotti (1995), they found out that higher chemical present of NH_3, oxygen and carbon dioxide needed for the bacteria (suitable condition) can increase the effectiveness of the reduction of ammonia-N concentration. This research has proven that the PFC has the most suitable conditions for the reduction of ammonia-N rather than UMPC.

In comparison with these two kinetic parameters between UMPC and PFC, the poultry farm soil is much greater since it can reduce the greater amount of ammonia-N concentration within a shorter period of time than UMP soil. This UMP soil reduces less ammonia-N concentration with a longer time period. The value for comparison between this two soils mixed culture is for UMP soil, the time taken that is 1.227 h^{-1} for reducing the ammonia-N concentration is 67.609 mg/L whereas for the poultry farm soil, it takes about 1.090 h^{-1} to reduce the ammonia-N concentration that is 68.454 mg/L.

Soil contains a wide range of microorganism inside them that they can be in large group or small group. This group depends on the carbon sources that presented in the soil. According to Hoorman & Islam (2010), they reported that soil microorganisms exist in large numbers in soil as long as there is a carbon source and other sources for energy. The microorganisms can only recycle about 20-30% of carbon which causes them to have a low life span. Any changes to the environment of the soil can cause the microorganism to be disturbed and become inferior or having a lower life span. Based on the research of Muntean (2010), this researcher stated that changes occurred to the soil such as food supply, temperature, moisture, oxygen supply, and etc. can greatly affect the dominant microbes to become inferior microbes due to lack supplies of energy source. The type of microbes that follows this condition is nitrifiers' bacteria in which *Nitrosomonas* sp. and *Nitrobacter* sp. Since UMPC has a lower supply of nitrogen and carbon source, it causes the nitrifiers bacteria to become inferior species which the bacteria become a small group of microbes in the soil. This proves that UMP soil has a smaller value of K_N in which reduce the ammonia-N lesser than the poultry farm soil.

4.4.2 Comparison with Other Researchers

Table 4.1 shows the experimental values of K_N and k obtained by different researcher for kinetic parameter which can be observed that the numerical values obtained by different authors have used different experimental method and also different wastewater as a source of contaminant which compare with University Malaysia Pahang soil mixed culture (UMPC) and poultry farm soil mixed culture (PFC).

Table 4.1 Values of K_N and k which determined by various author with UMPC and PFC

Author	K_N (mg/L)	k (d^{-1})	Type of Bacteria Present
Dincer and Kargi (2000)	5.14	1.15	*Nitrosococcus* sp. *Nitrococcus* sp.
Juliana et al (2011)	84.32	5.48	*Nitrosomonas* sp. *and Nitrobacter* sp.
Kapagiannidis et al (2005)	4.10	1.46	*Nitrosococcus* sp. *Nitrococcus* sp.
Author	K_N (mg/L)	k (h^{-1})	Type of Bacteria Present
This Study (UMP Soil) (2012)	67.609	1.227	*Nitrosomonas* sp. *and Nitrobacter* sp.
This Study Poultry farm soil (2012)	68.454	1.090	*Nitrosomonas* sp. *and Nitrobacter* sp.

This subsection will explain about the different value get for the parameter from UMPC, PFC and other research data. It will also be explained about the type of bacteria present inside the soil the researchers use. Based on the research Juliana et al. (2011), they obtained the results for the values of k and K_N that is 5.48 d^{-1} and 84.32 mg/L. The researcher's value of time reduction for ammonia, k, got is smaller than the value that was determined from the experiment by using UMPC that is k = 1.227 h^{-1} and PFC that is k = 1.090 h^{-1} but have a higher reduction of ammonia-N concentration which for UMP soil is K_N = 67.609 mg/L and poultry farm soil is K_N = 68.454 mg/L. The researchers of Juliana et al. (2011) use a type of soil that have a lot of active bacteria of

Nitrosomonas sp. and *Nitrobacter* sp. for the reduction of ammonia-N than UMPC and PFC.

From the data obtained from the researchers, it can be seen that the kinetic parameter from this experiment can be used for future references to get the calculation for nitrification process. The reaction of poultry farm soil is much faster than UMP soil and other researchers where it can reduce the ammonia-N concentration in a shorter period of time. These indicate that the bacteria present inside the soil mixed culture of these researchers have a smaller colony than PFC. Due to the lack of bacteria inside the soil mixed culture they used, the reaction time needed for the bacteria to reduce the ammonia-N concentration is slower. Furthermore, the value obtains from the research of Juliana et al. (2011) has higher reduction of ammonia-N concentration whereas for the other researchers of Dincer and Kargi (2000), and Kapagiannidis et al. (2005) has a lower reduction of ammonia-N in their soil mixed culture used. This higher reduction of ammonia-N concentration shows that the bacteria inside the soil mixed culture used by these researchers have a high suitability for the bacteria to adept and to multiply whereas for lower reduction of ammonia-N dignifies that the bacteria have a lower sustainability. The soil mixed culture for researchers of Juliana et al. (2011) used has quite high in NH_3, oxygen and carbon dioxide for the bacteria to live comfortable and dependent whereas for other researchers, they have a lower sustainability to the bacteria in which they cannot live comfortable and the dependent. For PFC and UMPC, these soils can considerably lessen the ammonia-N concentration due to the high nutrient present inside these soils. This has been prove by the research of Hunt & Vanotti (1995), where they found out that higher chemical present of NH_3, oxygen and carbon dioxide needed for the bacteria (suitable condition) can increase the effectiveness of the soil mixed culture in reducing ammonia-N concentration. The PFC has higher suitability to reduce the ammonia-N concentration than the UMP soil, researcher of Dincer and Kargi (2000), and Kapagiannidis et al. (2005) but lower than Juliana et al. (2011).

The reduction of ammonia-N concentration and the period also depends on the bacteria present inside the soil mixed culture. The bacteria present inside the UMP, poultry farm and Juliana et al. (2011) soil mixed culture are *Nitrosomonas* sp. and *Nitrobacter* sp. whereas Kapagiannidis et al. (2005) and Dincer and Kargi (2000) contains *Nitrosococcus* sp. *and Nitrococcus* sp.. Since the present of different bacteria can increase the effectiveness of nitrification thus, the three researches can reduce a significantly amount of ammonia-N concentration due to using the *Nitrosomonas* sp. and *Nitrobacter* sp.. Other than that, the soil taken at different places have different colonies of nitrifying bacteria due to the different in physical and chemical properties of the soil (Yuan, Ran, Shen, & Wang, 2005). Based on Mota, Ridenoure, Cheng & Reyes III (2005), they stated that the number and activity of nitrifying bacteria are considered the limiting reaction in removing the ammonia-N concentration in swine waste water. Thus, different colonies of bacteria give a different nitrification and reduction of ammonia-N concentration. Based on Yuan et al. (2005), they stated that the *Nitrosomonas* sp. and *Nitrobacter* sp. can reduce higher reduction of ammonia-N rather than the *Nitrosococcus* sp. *and Nitrococcus* sp. due to the *Nitrosomonas* sp. and *Nitrobacter* sp. are more suitable in the nitrification situation in which can react to almost all of the ammonia-N in contaminated soil and wastewater.

Comparing between these five researches and the value got from the experiment are that the research of Juliana et al. (2011) has a higher nitrification rate that can reduce ammonia-N concentration but PFC has a faster period for the reduction of ammonia-N. This can be explained that bacteria inside the soil mixed culture in the poultry farm soil has higher nitrifying bacteria colonies but lower sustainability compared to Juliana et al. (2011). The soils that other researchers have are low and different species of nitrifying bacteria inside their soil mixed culture and this causes less sustainability. This nitrifying bacterium is an important process due to it causes the reduction reaction to be either fast or slow reaction or takes a longer time to react. It also determines that the nitrifying bacteria that they use did not have the suitable condition for the bacteria to perform the reaction at the required time whereas for the poultry farm culture, it has a suitable condition and has adapted to this condition that helps in the faster reaction and reduction of ammonia-N.

From the comparison, it shows that the soil that described from this research is a better solution for reducing the ammonia-N concentration in many places such as contaminated area and wastewater. The soil that used also has been identified by the kinetic parameter which can help in the nitrification process in reducing the ammonia-N concentration. This statement can be supported by the research of *Allemanand Preston, 2000 where* this researcher stated that a good nitrification process is depend on the nitrifiers and heterotrophs of the bacteria inside the soil that can give a different growth and substrate uptake patterns. As for all of the bacteria, their kinetic rates depend on substrate availability (Li et al., 2006).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

In conclusion, the objective of this experiment is to determine the kinetic parameters of poultry farm soil mixed culture (PFC) and University Malaysia Pahang soil mixed culture (UMPC) to reduce the ammonia-N concentration was successfully achieved. The coefficients that were tested were k and K_N in which the k is for the time taken to reduce the ammonia-N concentration and K_N is for amount of reduction of ammonia-N concentration. These two values of coefficient will determine the best solution in reducing ammonia-N concentration. The analysis of coefficient was based on time, soil mixed culture and the experimental design was done by using Linear Regression Method and Microsoft Office Excel Solver Program.

The most distinct finding to be discovered from this study is the k value which was the time. This k value indicates a fast nitrification reaction that can determine which soil mixed culture is suitable in reducing the ammonia-N concentration. The determination of reduction of ammonia-N concentration also depends on the K_N. The kinetic parameters of the reduction ammonia-N for soil mixed culture were as follows: $k = 1.227\ h^{-1}$ and $K_N = 67.609$ mg/L for University Malaysia Pahang (UMP) soil and $k = 1.090\ h^{-1}$ and $K_N = 68.454$ mg/L for poultry farm soil. The calculated value got can be used due to the R^2 was exceeding the required value of R^2 which was 0.8. For comparison between other researchers, the value of k and K_N got by these researchers is smaller than UMP and poultry farm soil except for Juliana et al. (2011) in which the K_N is much higher than UMP and poultry farm soil. Even though Juliana et al. (2011) has higher K_N, the poultry farm soil mixed culture is much more suitable due to the time taken to reduce the ammonia-N concentration is faster than other researchers.

The results of this research support the idea that the kinetic equation and kinetic parameters were important and it can be utilized to create a new source of soil mixed culture in reducing the ammonia-N concentrations in many fields mainly for poultry farm industry.

5.2 Recommendations

In this study, the ammonia-N reduction by using UMP and Poultry Farm SMC is quite high. However, there are several ways to increase the ammonia-N reduction.

Firstly, the kinetic parameter that has been used should be optimized in order it can be used to determine the effectiveness of the soil used. The optimize condition for the kinetic parameter used in nitrification process is to understand and know the type of nitrifying bacteria colony present inside the soil mixed culture. This is due to the higher the colony, the higher the reduction of ammonia-N (Brion & Billen, 2000). Other than that, scaling up the design of this equation is recommended in order to get a more accurate reading for reducing a large scale of ammonia-N concentration reduction in larger industry.

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APPENDIX A

A.1 Calculation in obtaining the k and K_N for both University Malaysia Pahang soil mixed culture (UMPC) and poultry farm soil mixed culture (PFC)

UMP Soil:

$$\frac{1}{k} = 0.8149$$

$$k = 1.227\ \mathrm{h}^{-1}$$

$$\frac{K_N}{K} = 55.101$$

$$K_N = 67.609\ \mathrm{mg/L}$$

Poultry farm Soil:

$$\frac{1}{k} = 0.9177$$

$$k = 1.090\ \mathrm{h}^{-1}$$

$$\frac{K_N}{K} = 62.802$$

$$K_N = 68.454\ \mathrm{mg/L}$$

APPENDIX B

B.1 Series of experiment data in order to determine the kinetic parameter

UMP soil mixed culture (UMPC)

Table B.1 Data that obtained from the experiment for UMPC

Time (h)	Concentration (mg/L)
0	62.5
0.5	55
1	57.5
1.5	52.5
2	55
2.5	50
3	52.5
3.5	50
4	47.5
4.5	47.5
5	45

Table B.2 The data need for kinetic parameter determination of UMPC

$\emptyset_H$ (h) Interval time	$N_O(\frac{mg}{L})$ Ammonia-N feed	N (mg/L) Ammonia-N effluent	X (mg/L) Total solid Ump soil	$\frac{1}{U}$ (h) (y-axis)	$\frac{1}{N}(\frac{L}{mg})$ (x-axis)
0.5	120	62.5	20	0	0.0160
0.5	120	55.0	23	0.1769	0.0182
0.5	120	57.5	26	0.2080	0.0174
0.5	120	52.5	29	0.2148	0.0190
0.5	120	75.5	31	0.2385	0.0182
0.5	120	50.0	34	0.2486	0.0200
0.5	120	52.5	37	0.2741	0.0190
0.5	120	50.0	40	0.2857	0.0200
0.5	120	47.5	45	0.3103	0.0211
0.5	120	47.5	50	0.3448	0.0211
0.5	120	45.0	64	0.4267	0.0222

Table B.3 Calculated data that was used in the $\frac{1}{U}$ versus $\frac{1}{N}$ for UMPC

$\frac{1}{N}$ (L/mg)	$\frac{1}{U}$ (h)
0.016	0
0.0182	0.1769
0.0174	0.208
0.019	0.2148
0.0182	0.2385
0.02	0.2486
0.019	0.2741
0.02	0.2857
0.0211	0.3103
0.0211	0.3448
0.0222	0.4267

Poultry farm soil mixed culture (PFC)

Table B.4 Data that obtained from the experiment for PFC

Time (h)	Concentration (mg/L)
0	60
0.5	50
1	52.5
1.5	50
2	47.5
2.5	50
3	47.5
3.5	42.5
4	42.5
4.5	40
5	42.5

Table B.5 The data need for kinetic parameter determination of PFC

$\emptyset_H$ (h) Interval Time	$N_O(\frac{mg}{L})$ Ammonia-N Feed	N (mg/L) Ammonia-N Effluent	X (mg/L) Total Solid Poultry Soil	$\frac{1}{U}$ (h) (Y-Axis)	$\frac{1}{N}(\frac{L}{mg})$ (X-Axis)
0.5	120	60	40	0	0.0167
0.5	120	50	44	0.3143	0.0200
0.5	120	52.5	48	0.3556	0.0190
0.5	120	50	52	0.3714	0.0200
0.5	120	47.5	54	0.3862	0.0211
0.5	120	50	56	0.4286	0.0200
0.5	120	47.5	60	0.4414	0.0211
0.5	120	42.5	90	0.5806	0.0235
0.5	120	42.5	90	0.5806	0.0235
0.5	120	40	88	0.55	0.0250
0.5	120	42.5	86	0.5548	0.0235

Table B.6 Calculated data that was used in the $\frac{1}{U}$ versus $\frac{1}{N}$ for PFC

$\frac{1}{N}$ (L/mg)	$\frac{1}{U}$ (h)
0.0167	0
0.02	0.3143
0.019	0.3556
0.02	0.3714
0.0211	0.3862
0.02	0.4286
0.0211	0.4414
0.0235	0.5806
0.0235	0.5806
0.025	0.55
0.0235	0.5548

APPENDIX C

C.1 Equipment, method of reading and cleaning used during the experiment

Figure C.1 Desiccators is used for Total Solid Analysis

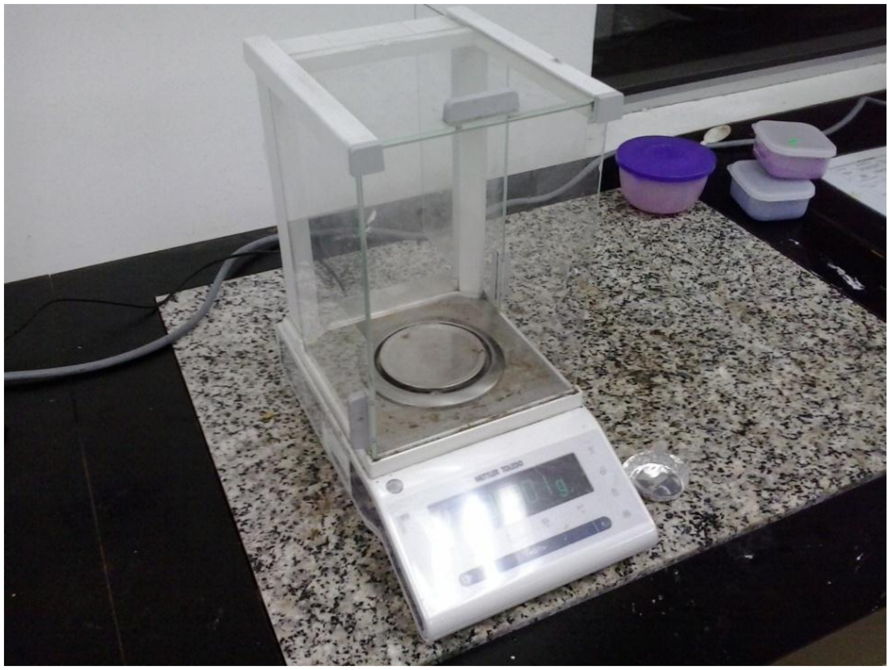

Figure C.2 Weighing balances for weight Total Suspended Solid present (NH_3)

Figure C.3 Stock solution of ammonia-N for use in combining with poultry farm and University Malaysia Pahang soil mixed culture

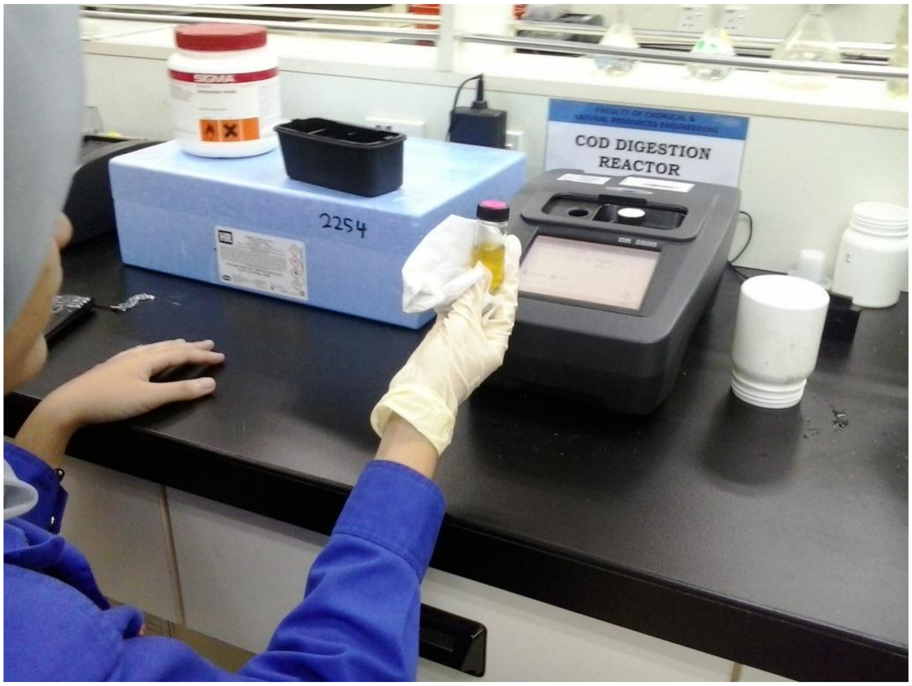

Figure C.4 Testing the ammonia-N concentration by using HACH DR/2800

Figure C.5 Taking the ammonia-N reading for the experiment

Figure C.6 Cleaning the solution of ammonia-N with soil mixed culture after the experiment