AMMONIA-N REMOVAL USING SOIL MIXED CULTURE: FACTORIAL ANALYSIS

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the requirements for the award of the degree of
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SUPERVISOR'S DECLARATION

I hereby declare that I have read this thesis and in my opinion this thesis has fulfilled the qualities and requirements for the award of Degree of Bachelor of Chemical Engineering (Biotechnology)

Signature : ……...

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Date : 18 FEBRUARY 2013

STUDENT'S DECLARATION

I hereby declare that this thesis entitled "*Ammonia-N Removal Using Soil Mixed Culture: Factorial Analysis*" 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.

Signature : ……...

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Special dedication of this grateful feeling to my parents,
Abd Ghalib bin Daud and Faudziah binti Wahab,
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For All Your Care, Support, Best Wishes and Believe in Me.

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AMMONIA-N REMOVAL USING SOIL MIXED CULTURE:

FACTORIAL ANALYSIS

ABSTRACT

Ammonia-N content in the soil around poultry farm can harm on the poultry itself because it can cause diseases and the worst effect is mortality; causes the breeders to have a great number of losses. This research studied the factors that influence ammonia-N removal using soil mixed culture in order reduce the ammonia-N content in soil around the poultry farm. The ammonia-N solution treated with soil mixed culture that believed containing high density of soil mixed culture microbes. These microbes undergone some reactions that reduced the ammonia-N concentration. The parameters of this research were agitation, reaction time, type of soil, temperature, and soil to water ratio. Preliminary experiments conducted to find the suitable reaction time, were done by treating ammonia-N solution with UMP soil. The five factors were applied for 32 runs experiment, involving poultry farm soil, UMP soil and ammonia-N solution. The experimental design was done by Design Expert software. The single factor that gave highest contribution was type temperature (16.84%) while for interaction factor, type of soil-temperature showed the most significant removal with 9.1%. Verification experiments done using poultry farm wastewater treated with UMP and PF soil. Run 3 (no agitation, 5-hours reaction time, 1:6, PF soil, 25^0C), Run 4 (no agitation, 5-hours reaction time, 1:6, UMP soil, 25^0C) and Run 7 (no agitation, 2-hours reaction time, 1:1, PF soil, 30^0C) removed 48.28%, 48.28% and 41.38% of the actual ammonia-N concentration in poultry farm wastewater accordingly. The error of predicted and actual percentages removal of Run 3, 4 and 7 were 1.25%, 0.71% and 2.39% respectively. The coefficient r^2 of this model for factorial analysis was 0.8383. Optimization on the factors in nitrification process is highly recommended.

PENYINGKIRAN AMMONIA-N DENGAN MENGGUNAKAN KULTUR CAMPURAN TANAH: ANALISIS FAKTORIAL

ABSTRAK

Kandungan ammonia-N dalam tanah di sekitar kawasan ternakan ayam boleh mendatangkan bahaya kepada ternakan kerana boleh menyebabkan pelbagai penyakit dan yang paling buruk adalah kematian yang boleh menyebabkan penternak kerugian. Jadi, kajian ini mengkaji faktor-faktor yang mempengaruhi penyingkiran ammonia-N dengan menggunakan kultur campuran tanah untuk menyingkir ammonia-N di sekitar kawasan ternakan. Cecair ammonia-N dirawat dengan menggunakan tanah yang dipercayai mengandungi kultur campuran tanah yang tinggi. Bakteria-bakteria ini menjalankan rantaian reaksi yang mengurangkan kepekatan ammonia-N. Beberapa pendekatan dijalankan iaitu agitasi, tempoh reaksi, jenis tanah, suhu, dan nisbah tanah kepada air. Uji kaji awalan dijalankan untuk mengkaji tempoh reaksi yang sesuai, telah dilakukan dengan merawat cecair ammonia-N dengan tanah UMP. Lima faktor diaplikasikan dalam 32 uji kaji, melibatkan tanah di sekitar kawasan ternakan, tanah UMP dan cecair ammonia-N. Reka bentuk uji kaji dilakukan dengan menggunakan perisian Design Expert. Faktor tunggal yang menyumbangkan peratusan terbesar adalah suhu (16.84%) sementara untuk faktor interaksi, jenis tanah-suhu menunjukkan kesan paling ketara dengan peratusan sebanyak 9.1%. Ujian pengesahan dilakukan dengan menggunakan sisa kumbahan kawasan sekitar ternakan, dirawat dengan tanah UMP dan tanah sekitar kawasan ternakan. Uji Kaji 3 (tanpa agitasi, 5-jam tempoh reaksi, 1:6, tanah PF, 25^0C), Uji Kaji 4 (tanpa agitasi, 5-jam tempoh reaksi, 1:6, tanah UMP, 25^0C) dan Uji Kaji 7 (tanpa agitasi, 2-jam tempoh reaksi, 1:1, tanah PF, 30^0C) masing-masing mengurangkan 48.28%, 48.28% dan 41.38% dari kepekatan asal cecair ammonia-N dalam sisa kumbahan ternakan. Ralat nilai penyingkiran ramalan dan sebenar untuk Uji Kaji 3, 4 dan 7 masing-masing adalah 1.25%,0.71%, dan 2.39% . Pemalar r^2 dalam model ini untuk analisis faktorial adalah 0.8383. Pengoptimalan faktor-faktor dalam proses nitrifikasi adalah sangat digalakkan.

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LIST OF SYMBOLS

^{0}C	Degree Celcius
%	Percentage
NH_3	Ammonia
NH_4^+	Ammonium Ion
NO_2^-	Nitrite Ion
NO_3^-	Nitrate Ion
NH_3-N	Ammonia-N
O_2	Oxygen

LIST OF ABBREVIATIONS

UMP	Universiti Malaysia Pahang
PF	Poultry Farm
Hr	Hour
Min	minute
TKN	Total Kejaldah Nitrogen
SS	Suspended Solid
VSS	Volatile Suspended Solid
TSS	Total Suspended Soild
MLSS	Mixed Liquor Suspende Solid
COD	Chemical Oxygen Demand
BOD	Biochemical Oxygen Demand
AOB	Ammonia Oxidizer Bacteria
NOB	Nitrite Oxidizer Bacteria
AMO	Ammonia Monooxygenase
HAO	Hydroxylamine Oxide
NOR	Nitrite Oxide Reductase
DM	Dry Matter
OM	Organic Matter
N_{org}	Organic Nitrogen
ATP	Adenosine Triphosphate

SCM	Sheep and Chicken Manure
HCM	Horse and Chicken Manure
Rpm	Revolution per Minute
Ppm	Part per Million
Mg/L	Miligram per Liter

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Recently, so many paper works had reported that, in poultry industry, the poultry breeders facing losses because a number of their poultry cannot be sold due to health problem. The health problems among the poultry is mainly due to the high concentration ammonia-N, in which is excreted from the poultry itself in the form of nitrogen. This ammonia-N concentration must be reduced as low as possible in order to maintain the poultry health and the workers as well. But the solution of this problem seems not well evolved. If the nitrification-denitrification process is fully utilized and manipulated, this

poultry problem can be resolved. This fact can be proved because the first substrate consumed in nitrification process are ammonia, NH_3 and ammonium, NH_4^+. If this biological process is utilized wisely, the ammonia-N will be used up and its concentration around the farm will be reduced as much as possible. Thus the losses problem in poultry industry can be solved. By using Design Expert, this study will find the effectiveness of the soil mixed culture in reducing ammonia-N concentration to the possible lowest value and analyze the interaction between the factors of the experiment.

1.2 Problem Statement

In poultry industry, due to some health problems, a number of the poultry cannot be sold. This causes the poultry breeders to have a great number of losses. The poultry has some health problems due to the high concentration of ammonia in the surrounding environment of poultry farm. At certain level of ppm or mg/L, ammonia-N content will causes decrease in appetite, loss in weight of poultry and the worst case is mortality. This concentration must be reduced as low as possible to avoid losses in poultry industry. So, the aim of this study is to minimize the ammonia-N concentration by using soil mixed culture. The soil is believed to contain high density of mixed culture microbes that involved in nitrification process. Since nitrification process consume ammonia as the first substrate to proceed, this research brings good news to poultry industry because the poultry's health can be improved.

1.3 Research Objective

The main objective of this research is:

i. To study the factors that influence ammonia-N removal using soil mixed culture

1.4 Scope of Study

The scopes of this study are:

i. To do preliminary experiments to find the suitable reaction time with using shake flask.
ii. To use soil mixed culture to reduce ammonia-N concentration in poultry farm wastewater
iii. To compare the effectiveness of two types of soil mixed culture in reducing ammonia concentration.
iv. To do screening two level of factorial by Design Expert software.
v. To analyze the interaction of the factors by using Design Expert software.

1.5 Significance of Study

The factorial analysis on ratio of soil to water composition in water-soil solution, temperature condition, agitation speed, reaction time and type of soil that believed affecting the ammonia-N removal in ammonia-N solution using soil mixed culture will be studied in this research. The interaction between these factors will be analyzed by utilizing Design Expert software. Then model equation for two factorial analysis can be obtained. Verification of this model will be done in validation tests involving poultry farm wastewater is to, thus allowed the prediction of the removal rate.

CHAPTER 2

LITERATURE REVIEW`

2.1 Introduction

This chapter reviews characteristics of ammonia, effects of ammonia-N toward poultry, the process of nitrification, factors affecting the nitrification, and two level of factorial. The study done on 2004 by Ritz *et al.* shows that, the poultry consume the nitrogen supply from their feed like corn, soy, wheat and grain, that contain amino acid.

The consumed nitrogen then excreted in large amount. This is because, first, usually the feed composition is not well balanced in term of amino acids ratio. Second, from the consumed amino acids, only about 33% are digested and the remaining amino acid is being excreted. Wong (2005) adding that, the excretion product from poultry are in the uric acid form. The uric acid then converted into urea before further converted to ammonia by hydrolysis, mineralization process.

2.2 Characteristics of Ammonia

Ammonia has chemical formula of NH_3 or in ionized form, is NH_4^+ and has molecular weight of 17g/mol. Shakhashiri (2008) and Brigden and Stringer (2000) support that, the boiling point of ammonia is around -33 to -35^{0}C. Because ammonia easily liquefied, it useful as in refrigeration system while its volatile alkaline make it suitable for household cleaning agent (Brendel *et al.*, 2000). According to Auburn University (2002), Moghadassi *et al.* (2008), Shakhashiri (2008), Ritz *et al.* (2004), ammonia is a colorless irritant, corrosive, reactive and toxic gas with sharp pungent smell. Ammonia is used to make fertilizers, explosive, plastics and fuels (Wisconsin Department of Health Services, Bureau of Environmental Health, 2012; and Shakhashiri, 2008).

2.3 Ammonia-N in Sewage

Ammonia-N is the analyte of ammonia quatitated based on the trivalent nitrogen concentration (analyte: a compound or element tested for by the method referenced in this research) (U.S. Environmental Protection Agency Office of Water, 2001). Sewage is water-carried wastes, either in the form of solution or suspension, intended to flow away from a community after has been fouled by a variety of uses. Sewage, which is also known as wastewater flow, is used water supply of the community. It contains more than 99.9% pure water and is characterized by its volume or its physical flow rate condition, chemical constituents and the bacteriological organism that it contains. Depending on their origin, wastewater can be classified as sanitary, commercial, industrial, agricultural, runoff (Sanamdikar and Harne, 2012), residential, and municipal wastewater (Gross, 2005).

2.4 Composition of Ammonia-N in Wastewater

A considerable amount of literature has been published on properties of municipal wastewater. Based on the study done by Yusof *et al.* (2010), the municipal solid water (MSW) from Kuala Lumpur city was mainly generated from the household

around this city. Based on the data of their study, MSW from Kuala Lumpur around March until May 2008 consist of following parameter as stated in Table 2.1.

Table 2.1 Characteristic of Stabilized Leachate (March-May 2008)
(Source: Yusof *et al.*, 2010)

Parameter (mg/L)	Range	Mean values (Standard Deviation)
$N\text{-}NH_4^+$	500-2450	1452 (872)
$N\text{-}NO_3^-$	0-16.2	5.6 (7.9)
alkalinity	2300-10900	6618 (3730)

Siripong and Rittmann (2007) also had studied on municipal wastewater. Their samples were taken from seven WRPs of the Metropolitan Water Reclamation District of Great Chicago. The samples were taken in winter season and summer season. The set of data were as Table 2.2.

Table 2.2 Characteristic of Water from Seven WRPs of the Metropolitan Water Reclamation District of Great Chicago (Source: Siripong and Rittmann, 2007)

	WRP	Source of Influent	Inf TKN(mg/L)	Eff NH_3-N (mg/L)	Eff NO_2^- + NO_3^- (mgN/L)
Winter set	Calumet	industrial and residential	27	0.22	9.7
	Egan	residential	35.7	0.13	13.5
	Hanover Park	residential	37.1	0.8	12
	Kirie	industrial and residential	32.8	0.19	5.4
	Lemont	residential	34.4	0.09	20.6
	North Side	industrial and residential	24.5	1.9	6.6
	Stickney	industrial and residential	41.7	0.7	12.4
Summer set	Calumet	industrial and residential	21.1	0.11	6
	Egan	residential	28.4	1.3	6.1
	Hanover Park	residential	41.3	0.29	12.2
	Kirie	industrial and residential	25.7	0.38	7.1
	Lemont	residential	26.2	0.17	13.9
	North Side	industrial and residential	19.5	0.75	7.7
	Stickney	industrial and residential	33.6	0.56	8

According to Table 2.3, seed sludge from the aeration tank at Tai Ping Sewage Plant in Harbin, China had about 28.2-44.6 mg/L of ammonium-N concentration.

Table 2.3 The Characteristic of Sample from Aeration Tank at Tai Ping Sewage Plant in Harbin, China
(Source: Wang *et al.*, 2012)

Characteristic	Concentration (mg/L)
COD	274-396
TN	30.6-45.9
NH_4^+-N	28.2-44.6
SS	2850
VSS	1980

ABS factory is located at Tainan County, Taiwan, studied by Popuri *et al.* (2011) had TKN of 340-670 and ammonium-N about 103-400mg/L and the other characteristics were presented in Table 2.4

Table 2.4 Characteristic of Wastewater from ABS Factory
(Source: Popuri *et al.*, 2011)

Characteristic Items	Range	Mean values (Std Dev) mg/L
TSS (mg/L)	131-152	142 (8)
COD (mg/L)	2200-4700	3970 (1320)
BOD_5 (mg/L)	800-2400	1440 (490)
TKN (mg/L)	340-670	540(120)
NH_4^+-N (mg/L)	103-400	320 (75)
NO_2^--N (mg/L)	0-1.8	0.64 (0.5)
NO_3^--N (mg/L)	0-2.9	1.45 (1.0)

Aiyuk *et al.* (2004) had collected domestic wastewater samples from somewhere in Belgium for 450 days period, and the samples detected to have 40mg/L of Total Kjeldahl Nitrogen (TKN) and 24±11mg/L of ammonia-N concentration. In Australia, Blackburne *et al.* (2007) found that domestic wastewater that they collected weekly after on-site primary sedimentation and predenitrification treating contained no ammnia nitrogen. Domestic wastewater choosen by Feng *et al.* (2008) from China have 70 mg/L of TKN and 40mg/L of ammonia-N. Samples collected from septic tank by Oladoja and Ademoroti (2006) (from Nigeria) and Wu *et al.* (2007) (from China) have ammonia nitrogen concentration of 13 and 79mg/L respectively.

Rodríguez *et al.* (2011) had done study on the wastewater from a meat product processing company, which the first was washing water, generated from washing process of the interior of vehicles, packages, cans and equipment for storage of raw material and the second one was obtained from the process of transforming raw material into meal to make meat extract using the cooker batch and supercooker process.

Table 2.5 Average Composition of Wastewater From Meat Product Processing Company
(Source: Rodríguez *et al.*, 2011)

Parameter (Mg/L)	Washing Water Average (Std Dev)	Condensate Average (Std Dev)
COD_T	8308.33 (1823.38)	1381.14 (483.64)
COD_F	3922.44 (1539.83)	822.47 (215.33)
BOD_5	2684.54 (1686.49)	563.42 (219.39)
TSS	1710.70 (973.84)	7.19 (3.12)
VSS	1242.15 (817.43)	6.13 (2.91)
NH_4^+-N	365.14 (85.66)	615.54 (129.39)
BOD_5/NH_4^+-N	7.45 (4.59)	0.94 (0.36)

Swine waste water was studied by Lim *et al.* (2012) and Cheng and Liu (2002) in different location. Swine waste water usually washed out of the slaughtering house, treated in aerobic lagoon and used as nutrient for cropland. Some of the treated water in anaerobic lagoon normally recycled and used to remove swine waste from the slaughtering house. Nitrogen removal from anaerobically treated swine waste is difficult due to its low COD/N ratio. The challenge in nitrification process for swine wastewater is the oxidizing of ammonium ion would cause the pH drop during nitrification process taking in place. Unfortunately, nitrifying bacteria is very sensitive toward pH changes, and they may inhibited by this condition. The pH drop may insignificant for nitrifying bacteria of municipal wastewater treatment because of lower ammonium ion presence, but not for swine wastewater, because of high ammonium concentration in the swine waste. The following information was obtained from Cheng and Liu (2002) data.

Table 2.6 Characteristic of Swine Wastewater Sample in the Tank Treating Anaerobically Swine Wastewater
(Source: Cheng and Liu, 2002)

Parameter (Mg/L)	Influent Average (Std Dev)
NH_4^+-N	221 (42.0)
COD	697 (155.0)
TSS	364 (65.5)
VSS	302 (97.1)

The following data were obtained by Rodriguez and Mesa (2009); 562.10mgN-NH_4^+/L, 8980 mg COD/L and 6028mg BOD_5/L. They did the experiment on the pet food company.

It is estimated globally, during 1990s the ammonia emission was about 5 300 000 tan N from animals other than cattle and pig, and this number including from poultry excretion (Bouwman *et al.*, 1997). Experiment on poultry manure shows that majority of the nitrogen, about 60-70%, is in the form of uric acid and urea (Nahm, 2003). Other animal manure was studied by Arriaga *et al.* (2011). They had categorized two type of manure which were first, sheep and poultry manure (SCM) and horse and poultry manure (HCM). Their data are as presented in Table 2.7. The total manure comprised of 43.4% and 42.5% of dry matter for SCM and HCM respectively.

Table 2.7: Properties of Manure
(Source: Arriaga *et al.*, 2011)

	OM (%)	N (%)	NH_4^+-N (%)	NO_3^--N (%)	Norg (%)	C/N
SCM	45.4	3.2	0.37	0.06	2.77	8.1
HCM	45.4	1.7	0.29	0.06	1.35	15.3

In the study of Pei *et al.* (2010), the waste used was a portion of the Fuhe River, China, which mainly composed of urban sewage and treated wastewater from Baoding city. Fuhe River discharges nitrogen into the Baiyangdian Lake, which the largest natural fresh water in Northern China Plain.

Table 2.8 Characteristic of Sample from Fuhe River
(Source: Pei *et al.*, 2010)

Site	Depth (cm)	TON (g /kg DW)	NH_4^+-N (mg/kg DW)
1	0	1.15 (0.34)	44.87 (2.32)
2	25	2.02 (0.78)	145.22 (4.57)
3	50	3.69 (0.69)	144.73 (6.71)
4	75	2.75 (0.45)	101.06 (6.72)
5	100	3.41 (0.22)	121.30 (7.83)

Six full-scale wastewater treatment plants were performed by Daigger and Littleton (2000), using staged, closed loop bioreactor to determine the simultaneous biological nutrient removal degree. Their samples were taken from plant around USA and presented in Table 2.9.

Table 2.9 Characteristic of Plant Around USA
(Source: Daigger and Littleton, 2000)

Plants	Location	Sludge Processing	Average Flow (m_3/day)	Organic Loading (kg/m_3/day)	SRT (days)	MLSS (mg/L)	Operating Period	TKN (mg/L N)		NH_3-N (mg/L N)		NO_3^-N (mg/L)
								in	out	in	out	out
Elmwood WWTP	Evesham, NJ, USA	Aerobic Digestion, Belt Filter Press Dewatering	7100	0.15	33	3175	1/98-9/98	32.5	2	25	1.1	1.13
Hartford WWTP	Mount Laurel, NJ, USA	Aerobic Digestion, Belt Filter Press Dewatering	15000	0.16	30	3500	1/98-9/98	-	-	-	0.12	-
Hammonton WWTP	Hammonton, NJ, USA	Aerobic Digestion, Belt Filter Press Dewatering	3400	0.18	20	2200	7/94-6/95	37	2.1	-	0.24	2.93
Chalfont WWTP	New Britain, NJ, USA	Aerobic Digestion, Belt Filter Press Dewatering	11400	0.2	24	4000	1/94-12/94	-	-	- 15.8	1.03	5.5

Table 2.9 Characteristic of Plant Around USA
(Source: Daigger and Littleton, 2000)
(continued)

Plants	Location	Sludge Processing	Average Flow (m_3/day)	Organic Loading (kg/m_3/day)	SRT (days)	MLSS (mg/L)	Operating Period	TKN		NH_3-N (mg/L N)		NO_3^-N (mg/L)
								in	out	in	out	out
weetwater Creek Wwtp	Gwinnett County, Ga, Usa	Aerobic Digstion	40500	0.46	07-Okt	3411	1/94-12/94	-	-	13	0.14	4.5
Lake Geneva Wwtp	Lake Geneva, Wi, Usa	Thickening , Aerobic Digestion	5700	0.28	22	4000	1/94-12/94	-	1.3	-	-	2.62

2.5 Effects of Ammonia-N toward Poultry

According to research done by Wisconsin Department of Health Services (2012).), ammonia concentration in mg/L or in convenient work, part per million (ppm), with value of 1 does not give any effect in health problem. Holland *et al.* (n.d) suggests that at 60ppm of ammonia, the poultry will experience eye keratoconjunctivis. In contrast, Ritz *et al.* (2004) said that, eye keratoconjuctivis will happen when the ambient ammonia concentration approaches 46-102 ppm. Estevez (2002) said that in her paper, at around 25-50 ppm, air sac inflammation will happens in poultry, and at 100 ppm, mortality in poultry will increased. She adding that, at 10 ppm, trachea irritation will occur. Holland *et al.* (n.d) support that, if the poultry is left to be in 10 ppm ambient ammonia for 49 days, poultry will be in state of excessive mucous production, matted cilia, normal muciciliary fall-off. According to Ritz *et al.* (2004), the cilia loss and increasing number of cell that functioning in secreting mucous was found in poultry exposed to 75-100 ppm of ammonia. Based on Ritz *et al.* (2004), Auburn University (2002) and Estevez (2002)'s paper, the poultry will feels Newcastle-bronchitis challenge and damage in respiratory tract, at 20 ppm ammonia concentration, paralysis in cilia, decreased in appetite, reduction in body weight, at 25 ppm. It is believed, at 50 ppm, small number of cilia will be destroyed with that ammonia concentration based on research done by Auburn University (2002). And Holland *et al.* (n.d) agree with the opinion, and adding that, respiratory infection with secondary infection for 50 ppm for a long period of time.

2.6 Nitrification

2.6.1 Nitrification Process

Nitrification is defined by Sumner (2000), Lee and Lin (2007), Liu and Lipták (1997), and Jie *et al.* (2009), as the process of nitrogen in the form of NH_3 or NH_4^+ is being converted into nitrite (NO_2^-) and nitrate (NO_3^-) by the help from microbes with characteristic of autotroph. Based on the research done by Lee and Lin (1999) and Chen *et al.* (2011), nitrification is a process that undergone in aerobic condition where oxygen should be present. The Water Planet Company (n.d) and Corbitt (1999) says that the dissolved oxygen (DO) should be at least 1.0mg/L or more in order the nitrification to proceed efficiently. Nitrification process was firstly observed by Schlesinger and Muntz around 1877. Winogradsky found that ammonia oxidation process could supply energy and the bacteria that involved in nitrification process could synthesize carbon through the reduction of carbon dioxide instead of carbon compound (Gilhawley, 2008).

Autotroph (from the Greek *autos*= self and *trophe*= nutrition) is the organism that obtained their energy and material from inorganic source (Jacobson, 2002; Francis, 2008) and their carbon source is mainly from carbon dioxide- an inorganic compound source (Jacobson, 2012). Autotrophic bacteria are obligate aerobic and cannot gain adenosine triphosphate (ATP- if the third phosphate from this ATP is removed by hydrolysis, a substantial amount of free energy is released) (Manser *et al.*, 2006). Some species of archaea thought to have ability to undergo ammonia oxidation (Loescher *et al*., 2012).

Overally, the process of nitrification is a microbial process where aerobic process is carried out by Gram-negative non-spore forming, autotrophs, in which ammonium is oxidized to nitrite (NO_2^-) by ammonia oxidizing bacteria (AOB). Enzyme ammonia mono-oxygenase (AMO) wills transforms hydroxylamine ammonium in the cytoplasm, which is then converted to nitrite by enzyme hydroxylamine oxide reductase (HAO) in the periplasm (Offre *et al*., 2009). Subsequently nitrite is oxidized to nitrate (NO_3^-) using nitrite oxidizing bacteria (NOB) through the enzyme nitrite oxide reductase (NOR). The AOB and NOB use carbon dioxide or inorganic carbon as carbon source for the synthesis of cellular material and ammonia or nitrite as an energy source (Rodríguez *et al.*, 2011).

The nitrite oxidizing bacteria are chemolithothroph with the members are capable of autotrophic, mixotrophic or even heterotroph growth. Some species can grow under various condition, including aerobic/litotrophic, aerobic/mixotrophic or anoxic/heterotrophic. The primary source of energy for oxizing bacteria is the aerobic oxidation of NO_2^- to NO_3^-, which catalyzed by enzyme nitrite oxidoreductase with oxygen supplied by water and oxygen acting as the electron acceptor (Sumner, 2000).

If NH_3 is the substrate, then the ammonia oxidizing will function the process is as Equation (2.6.1).

$$NH_3 + O_2 + 2H^+ + 2e^- \rightarrow NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^- \quad (2.6.1)$$

The nitrite oxidizing will convert the nitrite as in Equation (2.6.2).

$$NO_2^- + H_2O \rightarrow NO_3^- + 2H^+ + 2e^- \quad (2.6.2)$$

No bacteria have been found which can convert NH_3 to NO_2^- directly (Sumner, 2000).

If the NH_4 is the substrate, then the reaction will be like Equation (2.6.3) and nitrite oxidation proceeded as in Equation (2.6.4) while the overall equation as stated in Equation (2.6.5).

Ammonia oxidation:

$$NH_4^+ + 1.5O_2 + 2HCO_3^- \rightarrow NO_2^- + 2H_2CO_3 + H_2O \quad (2.6.3)$$

Nitrite oxidation:

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad (2.6.4)$$

Overall reaction:

$$NH_4^+ + 2O_2 + 2HCO_3^- \rightarrow NO_3^- + 2H_2CO_3 + H_2O \quad (2.6.5)$$

(Lee and Lin, 2007; Lee and Lin, 1999).

Nitrification is not necessarily carried out by autotroph bacteria. Autotrophic nitrifier consume ammonium and produce nitrate under aerobic condition while heterotrophic nitrifier consume ammonium and organic carbon to produce nitrate (Strom *et al*., 2004) and nitrite (Dworkin, 2006). The condition is aerobic condition and this can be done by numerous bacteria and fungi (Gilhawley, 2008). But the rates of NO_2^- and NO_3 production by heterotrophic is much lower than the production by aututrophic microorganism. Some heterotrophic nitrifier is capable of denitrification (Sumner, 2000).

2.6.2 Microbes of Nitrification

The researches done found that, the genus that may work as the ammonia oxidizing bacteria are come from *Nitrosomonas* and *Nitrospira* (Water Planet Company, n.d; Qin *et al.*, 2006; Jung *et al*., 2011) Other genus that involved in aerobic ammonia oxidation are *Nitrosovibrio* (Gilhawley, 2008), *Nitrococcus* and *Nitrosolobus* (Carvalho *et al.*, 2006; Rodriguez and Mesa, 2009; Allen, 2009). Vymazal (2007) reported that genera *Nitrosospira, Nitrosovibrio, Nitrosolobus, Nitrosococcus* and *Nitrosomonas* are strictly chemolithotrophic (aerobic). Among the *Nitrosomonas* genus, the commonly used are *Nitrosomonas europaea* that originally from soil and water sewage, have the straight rod of cell shape (Sumner, 2000; Yusof *et al.,* 2010), *N. eutropha* from sewage also have straight rod (Sumner, 2000; Jie *et al.*, 2009), *N. oligotropha* from fresh water, straight rod (Sumner, 2000; Manser *et al.*, 2006), *N.marina* and *N. cryotolerans* from marine environment with straight rod shape (Sumner, 2000). *Nitrospira briensis* (Sumner, 2000; Yusof *et al.,* 2010), *Nitrosolobus multiformis* (Sumner, 2000; Yusof *et al.,* 2010; Pei *et al.,* 2010), *and Nitrsovibrio tenuis* (Sumner, 2000; Yusof *et al.,* 2010) are originate from soil but have cell shape of spirals, lobate and curved rod respectively. Species of *Nitrococcus* that have same cell shape; spheres, are *oceanus* (from marine environment) (Sumner, 2000), *halophilus* (from salt lakes) and *mobilis* (from brackish) (Sumner, 2000; Yusof *et al.,* 2010; Manser *et al.,* 2006; Carvalho *et al.,* 2006). *Nitrosomonas communis* used in the study done by (Manser *et al.,* 2006; Chang *et al.,* 2011) while *Nitrosomonas nitrosa* was used in Chang *et al.* (2011)'s study. *Nitrosopumilus maritimus* (phylogenetic group: Thaumarchaeota), *Nitrosococcus oceania* (*Gammaproteobacteria*), *Nitrosomonas marina* (*Betaproteobacteria*) and

Nitrosomonas cryotolerans (*Betaproteobacteria*) also believed posses the characteristic of ammonia-oxidizing based on the research done.

Yusof *et al.* (2010) stated that all species from *Nitrospira* genus can function as nitrite oxidizer. The species that mostly used are, *Nitrobacter winogradsky,* (from soil and water with pleomorphic rods), *N. hamburgenesis* (from soil with pleomorphic rod). *Nitrospira marina, Nitrospira gracilis* and *Nitrococcus mobilis* are from marine nature with spiral, spender rod and sphere shape respectively (Sumner, 2000). Gilhawley (2008) had proposed that *Nitrosomonas ureaea* also can demonstrate as nitrifier. In research done by Loescher *et al*. (2012) and Offre *et al.* (2009) found that *Nitsosopumilus maritimus*, an archaea also capable of ammonia oxidizer.

According to Sumner (2000), heterotrophic nitrifiers also can undergo nitrification process, but the rate of nitrite and nitrate production is much lower compared to autotrophic microbes. The main genera that can conduct heterotrophic nitrification are *Staphylococcus, Micrococcus, Streptococcus, Pseudomonas*, and *Bacillus.* Yang *et al.* (2011) used *Bacillus subtilis* A1 as the microbe to do both ammonia and nitrite oxidation but *Microvirgula aerodenitrificans, Pseudomonas stutzeri, Alcaligenes faecalis, Pseudomonas putida, Acinetobacter calcoaceticus* also can be used. Taylor *et al.* (2009) mentioned in their study, *Providencia rettgeri* YL, *Paracoccus denitrificans* or also known as *Thiosphaera pantotropha*, *Pseudomonas stutzeri* and *Alicaligenes faecalis* may act as heterotrophic nitrifier, while *Thauera mechernichensis* was used in the reaction of Chang *et al.* (2011)s' study. Gram negative, non-motile and rod-shaped, bacterium *Providencia rettgeri* YL was found Taylor *et al.* (2009), to exhibit the ability of heterotrophically nitrifying various concentration of ammonia-N. The characteristic of heterotrophic nitrifiers also

possessed by yeast *Williopsis californica* (Wheatley *et al.*, 2001). In study done by Urakawa *et al.* (2012), *Staphylococcus aureus, Klebsiella pneumonia, Serratia marcescens* and *Bacillus megaterium* were mentioned and tested because they are believed acquired the quality to be categorized as heterotrophic bacteria. *Pseudomonas fluorescens, Pseudomonas aeruginosa, Bacillus licheniformis, Enterobacter aerogenes, Enterocloacae, Klebsiella pneumonia, Klebsiella planticola, Alcaligens faecalis, Achromobacter denitrificans, Serratia marcescens* and *Paracoccus denitrificans* showed the positive responses towards ammonium and nitrate ions with using ammonium nitrate (Zhou *et al.*, 2007).

2.6.3 Factors Affecting Nitrification

2.6.3.1 Temperature

From the study of Chen *et al*. (2011), the rate of ammonia removal was tested at 20, 25 and 30^0C. And the result showed that, nitrification rate was the best at 30^0C. The Water Planet Company (n.d) claims that, nitrification rate reaches maximum rate at the temperature between 30-35^0C. At temperature of 40^0C and higher, nitrification rate falls near to zero. At below than 20^0C, nitrification rate is slow, but it still continues until 10^0C. But once the nitrification stops, it will only resume when the temperature get over 10^0C. Corbitt (1999) suggest that both *Nitrosomonas* and *Notrobacter* are effectively work in 30-36^0C. The reaction done by Artiga *et al.* (2005) was performed in 35^0C, while in research done by Taylor *et al.* (2009), the

temperature was ranged from 10-40^{0}C, and the result showed that ammonia removal rate was best at between 20-30^{0}C. All the experiments done by Manser *et al*. (2006), Jubany *et al.* (2009) were conducted in ambient temperature which between 20^{0}C and 25^{0}C respectively to get the best ammonia removal reading.

For mutant bacterium *Nitrosomonas sp*. TN0632, the reported suitable, optimum temperature for growth was between 27-29^{0}C (Mizoguchi *et al.*, 1998). The optimum temperature for other specific bacteria are: *Nitrosovibrio sp* TYM9; 20^{0}C (Tokuyama *et al*., 1997), *Nitrobacter agilis* ATCC14123; 30-35^{0}C, *Nitrosolobus multiformis* ATCC25196; 25-30^{0}C (Takahashi *et al.*, 2001), and *Nitrosomonas europaea a*nd *N.eutropha* at 25^{0}C (Siripong and Rittman, 2007). For soil mixed culture obtained from three conifer forest, Pedersen *et al.* (1999) had done the incubation in 25^{0}C.

2.6.3.2 Reaction Time

Reaction time may give influence in term of stability reading (Pansu *et al*., 2001) This is proven by a number of researches done with consideration about the reaction time factor. In the experiments done by Smith, reviewed from Papen and Butterbach-Bahl (1999) three different moistures were tested; 40%, 50% and 60% in every 2-hours. The nitrate and nitrite concentration accumulation in term of time were observed. For all moisture content, fluctuation pattern data were collected over time. The reading of nitrite and nitrate concentration were increased, but at t=6hr, the concentration will decreased somehow for every moisture content. Jie *et al.* (2009) has done experiments where effects of ratio of organic carbon to inorganic nitrogen

source were tested, and the changes in ammonia, nitrite and nitrate concentration over time were observed. The time interval was 4-hours. For ratio C\N=0, 0.5, 1.0, 2.0 and 4.0, the ammonia removal rate were decreasing fluently, but for C\N ratio=8 and 16, fluctuation pattern vividly observed. While the nitrite concentrations were fluctuated over time for all ratios. Yan Chen *et al.* (2011) observed nitrite concentration in term of time (hr) in every one-hour for 150, 300, 450, 600 and 750mg/L of influent CODCr. The nitrite concentration also fluctuated (the reading increased, but at some time, the reading decreased) over time like in case of Jie *et al.* (2009). Nitrite and nitrate concentration in every 30minutes were observed by Lotito *et al.* (2012) for effect of nitrite addition (15mg/L) and the result were nitrite concentration will decreased while nitrate concentration increased in every 30minutes until infinity (saturated reading) values was approached.

The data obtained by López-Fiuza *et al.* (2002) showed that, at every 1-hour, the ammonia-N concentration was decreased for different cell density in gel, gel thickness and cell density in bioreactor. Cheng and Liu (2002) has done experiment, investigated the ammonia-N, nitrite-n and nitrate-n concentration for every 5-hours with different ammonia-N concentration (56, 101, 251mg/L). The ammonia-N concentration, just like other research, decreased in every 5-hours, while the nitrate-n concentration increased continuously and nitrite-n increased at t=5.5 (56mg/L), t=5 and 7 for 101mg/L and t=27h for 251mg/L.

2.6.3.3 Agitation Speed

The common reasons of agitation employment are to harmonize the solution mixture and for aeration condition. For Rodríguez *et al.* (2011) agitation system was for aeration function in sequencing bath reactor (SBR) and suspension of *Scenedsmus* cell respectively. In the concentrated suspension of nitrifying and denitrifying cells together with mixture of 10% polyvinyl alcohol (PVA) and 2% sodium alginate, stirring was by Cao *et al*. (2002) got the uniformized mixture. No experiment that specified on the effect of agitation speed. In study done by Pei *et al.* (2010), agitation was employed for mixing function in the sample that containing solid fresh sediment and sterile isotonic solution, $(NH_4)_2SO_4$.

In the sequential researches involving nitrification organism, *Nitrosomonas* and *Nitrobacter*, Uygur and Kargi (2002, 2003, 2004a, 2004b), agitation was varied from 25 and the reason of agitation in these studies were to provide mixing. The same goes for Gilhawley (2008) where in the operational control, the agitation was included to ensure a homogenous sample.

Taylor *et al.* (2009) had investigated the effect of agitation speed on ammonia removal by *Providencia rettgeri* YL. To observe the effects of dissolved oxygen concentration on ammonia removal, shaking speed was varied at 60, 80 and 120rpm. At 30^0C, ammonia removal detected was efficiently at 120rpm compared to 60 and 8-rpm at the same temperature. At 30^0C, 120rpm condition yielded 99-100% ammonium-n removal while 80 rpm and 60 rpm gave 51% and 33% ammonium-n removal respectively.

In study of Zhou *et al.* (2007) with 100rpm, *P. aeruginosa* reduced the ammonium and nitrate ions (from ammonium nitrate solution) completely while in 140rpm, the remaining ammonium and nitrate ions were 3% and 80% respectively. At t=6 -hours, the ammonium and nitrate ions were completely utilized at 70rpm and 140rpm with the same composition of media. This data suggest that, the utilization of ammonium ions was accomplished by controlling the agitation speed.

2.6.3.4 Ratio of Soil to Water Composition

The solid/liquid (S/L) ratio used by Chimenos *et al.* (2003) was 12, 16, 20, 24, 30 and 34 g/L. Hamid *et al.* (2004) maintained the water content in soil at 20%. Bottomley *et al.* (2004) made 1:10 w/v of soil slurry for their study.

2.7 Two Level of Factorial Analysis.

Factorial design is a tool that allows factors of experiment to be done simultaneously (Anderson and Whitcomb, n.d). Screening design with 2 level of factorial is used to detect the factors or independent variables, such as temperature (Tumpang, 2009) that have high impact on the responsive variables (Ramírez *et al.*, 2001) because it provides contrast of averages, hence provide statistical power to estimate the effects of factors (Anderson and Whitcomb, n.d). Besides that, it is used

to identify the interaction between two or more factors as compared to one-factor-at-time technique. Traditionally, the conditions that believed will affect the production were varied one-at-a-time, meaning that one variable kept varying while other factors were kept constant. The idea of this strategy is very simple and easily to be done, but on the other side, this method is a time, manpower and chemical consuming (Tumpang, 2009). So, factorial design provide the advantages over the disadvantages of one-factor-at-a-time, where it will reveals the interaction of factors and as the more factors included, the more pronounced the factorial design (Anderson and Whitcomb, n.d). Besides, it required less number of experiments, thus, save time, glassware, chemical and manpower. It is also well-organized approach for collection and analyzing the information. The choosing factors influenced by mathematical modelunder consideration (Tumpang, 2009).

Factorial analysis has two approaches which are full or partial/fractional (FFD). Both are good approaches (Ehlen *et al.* (2005). Experimental design with two levels of factorial has formula of 2^k where k is number of factors. Then, the most significant parameters will be selected to form a central point (Januszkiewicz *et al*., 2008) and (Ramírez *et al.*, 2001).The factors of the experiment either categorical or numerical (it can be adjusted to any level). The full factorial design allows the estimation all the factors interaction (Anderson and Whitcomb, n.d). A number of tests in the center of the field needed to control the validity and stability of the factorial design (Ramírez *et al.*, 2001; Januszkiewicz *et al*., 2008; Anderson and Whitcomb, n.d; Ehlen *et al.*, 2005). In the screening process, all the factors are studied at two levels (this level can be either presence or absence, or two acceptable values of the factors) which used to identify the important factors, rather than

identify the optimum value of each factors (Chakraborty *et al.*, 2009). The combination of randomized factors will be done by Design Expert software.

Guo *et al.* (2007) had used two level of factorial in their research involving the ammonia-oxidizing bacteria. The considered factors in medium composition, were calcium source (coral sand and calcium carbonate (solid)), volume of 1.47% KH_2PO_4 (none and 140ml), volume of 6.4% of $NaHCO_3$ (none and 2ml) and volume of distilled water (none, 2, 2.14 and 0.14ml). Lang and Elliott (1997) investigated the effects of medium (MM220 or MM350), planting (absence or presence) and ratio of ammonia-N to nitrate-n fertilizer (1:3 or 3:1) on the medium pH and most probable number (MPN) counts. The effects of concentration of ammonium nitrogen (2 or 4mg/ml) in the influent water to the nitrification reactors and percentages of carrier element filling of the nitrification reactors (25 or 50%) were done by Björnsdotter (2005).

CHAPTER 3

METHODOLOGY

3.0 Flowchart of Research Methodology

The brief method for this study is depicted as Figure 3.1.

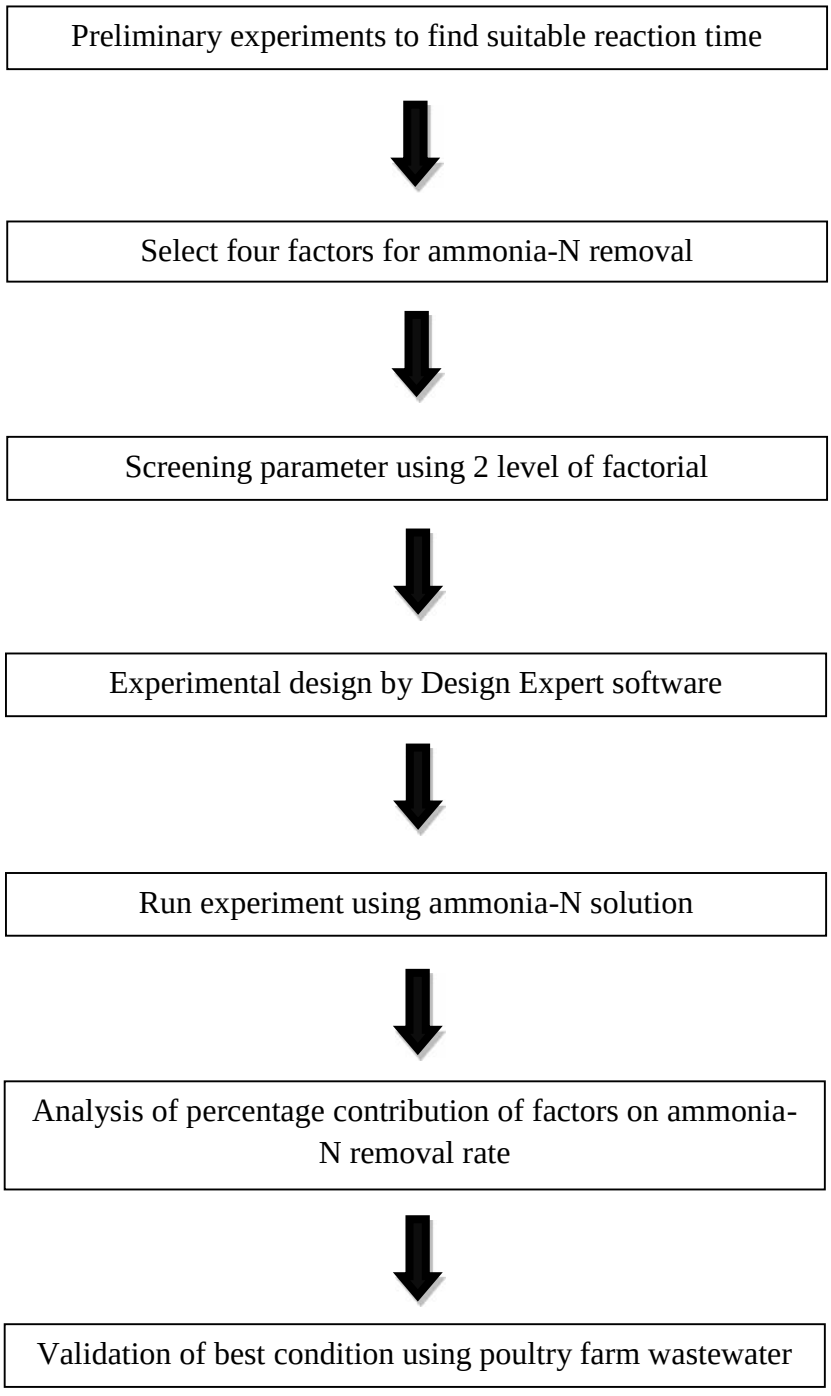

Figure 3.1 Process Flow Chart

3.1 Preparation of Materials

The ammonia-N solution is made by dissolving about 533.6mg of ammonium nitrate salt in deionized water until the solution become one liter. Then, for water-soil solution (containing soil mixed culture) was made by dissolving the soil content of soil from Universiti Malaysia Pahang (UMP) into deionized water in ratio of 1 part of soil and 6 parts of deionized water. This ratio is also applicable for from a poultry farm's (PF).

3.2 Preliminary Experiment to Find Suitable Reaction Time

Preliminary experiments were important to identify the suitable reaction time in which yield the stable removal reading. In order to find the suitable reaction time, several runs were conducted using the UMP soil and ammonia-N solution. From the removal rate results, hours of reaction time showed and gave a stable ammonia-N removal reading were selected. The series of preliminary experiments to determine the suitable reaction time as done as in Figure 3.2 until Figure 3.7 and Table 3.1. The selected suitable reaction time were after 2 -hours and 5 -hours of treatment. Run 1 until Run 6 were using the ammonia-N solution treated with UMP soil, while the Run 7 and Run 8 were using ammonia-N solution treated with UMP and PF soil.

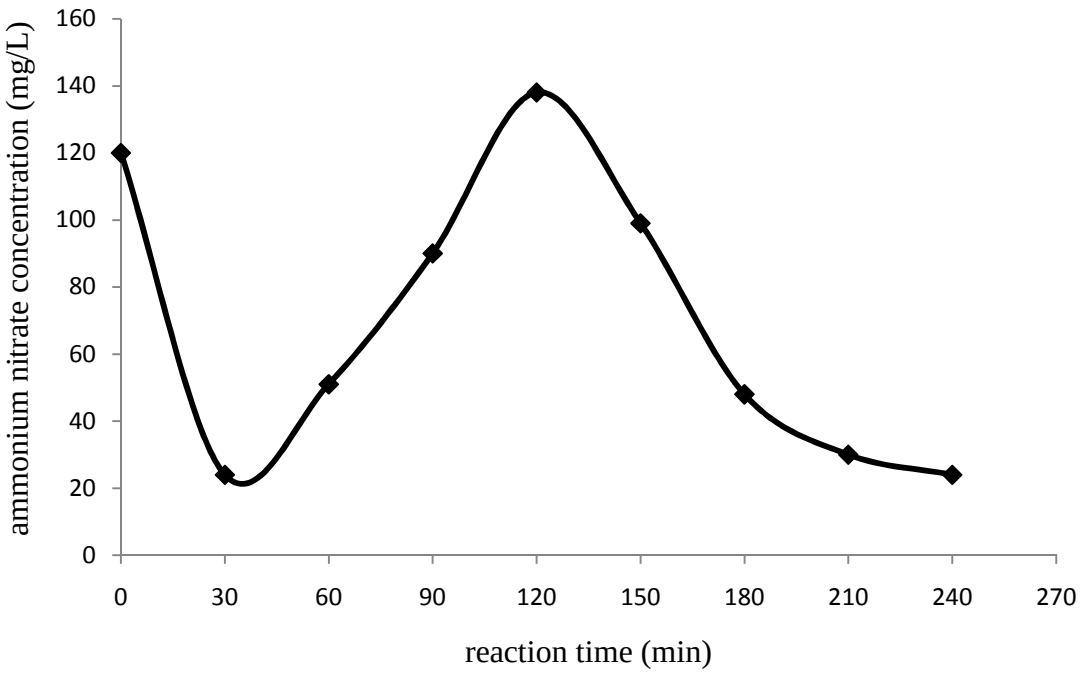

Figure 3.2 Graph of Ammonia-N Concentration (Mg/L) versus Reaction Time (Min) for Preliminary Run 1

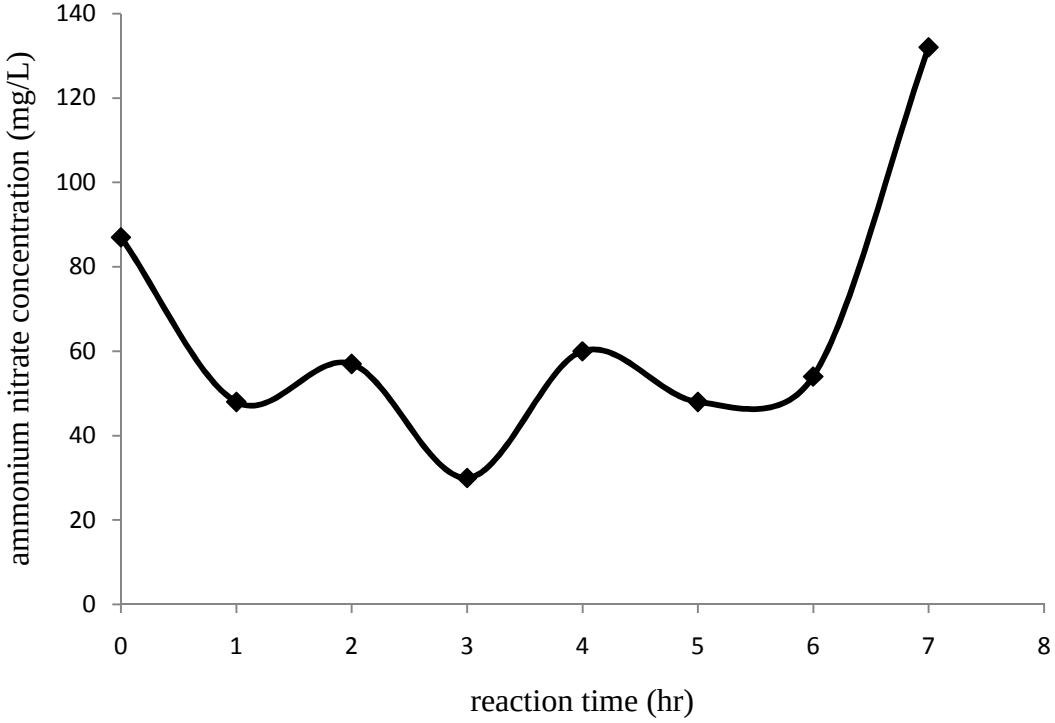

Figure 3.3 Graph of Ammonia-N Concentration (Mg/L) versus Reaction Time (Min) for Preliminary Run 2

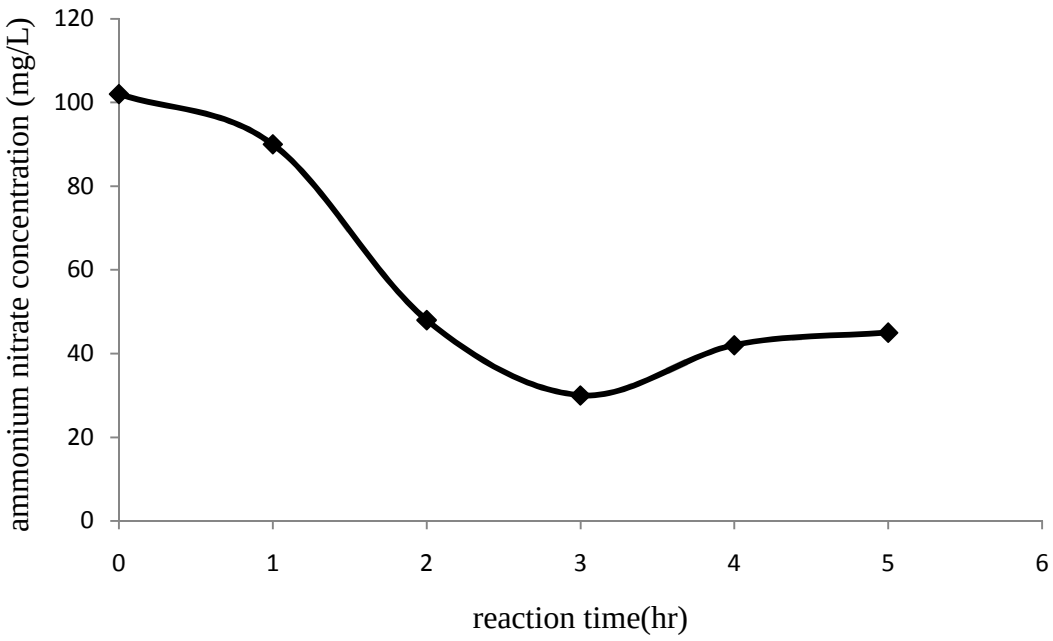

Figure 3.4 Graph of Ammonia-N Concentration (Mg/L) versus Reaction Time (Min) for Preliminary Run 3

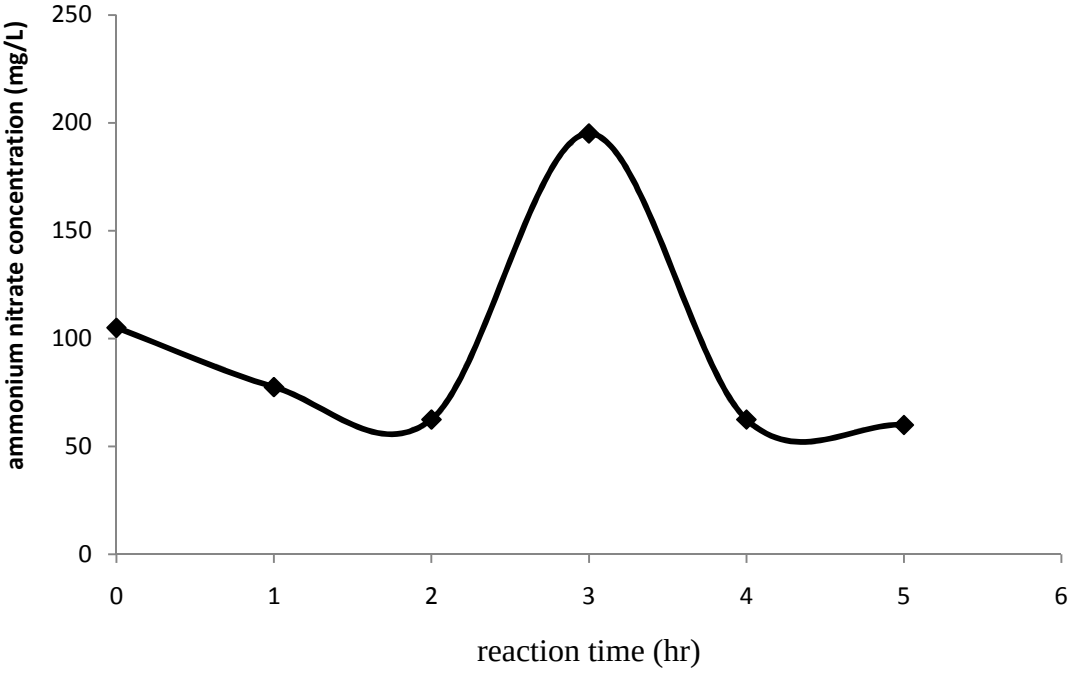

Figure 3.5 Graph of Ammonia-N Concentration (Mg/L) versus Reaction Time (Min) for Preliminary Run 4

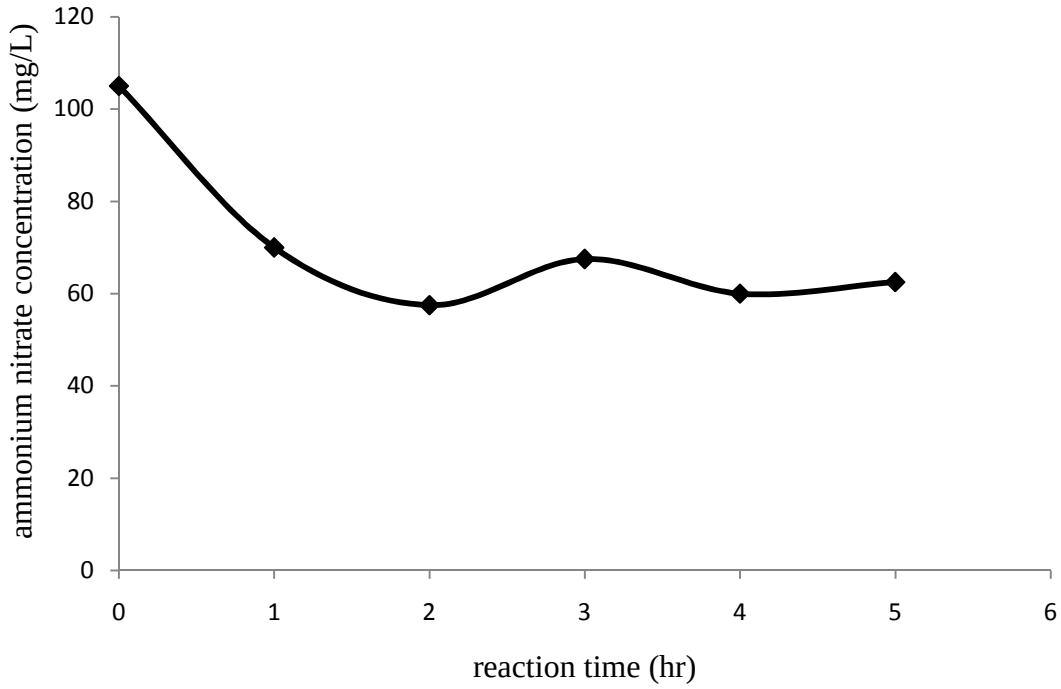

Figure 3.6 Graph of Ammonia-N Concentration (Mg/L) versus Reaction Time (Min) for Preliminary Run 5

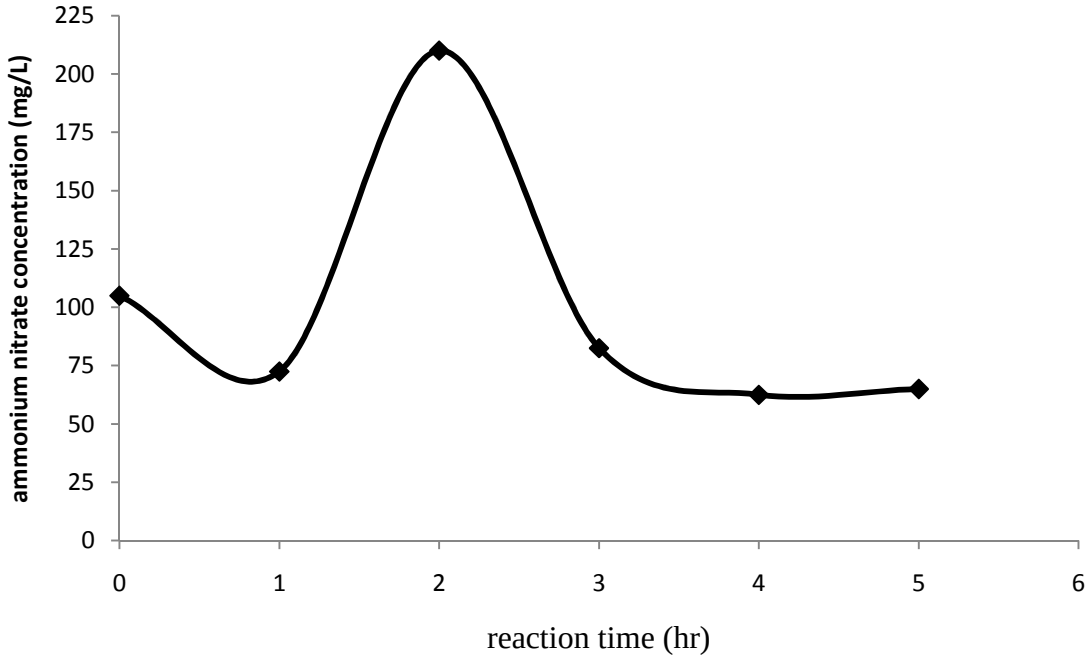

Figure 3.7 Graph of Ammonia-N Concentration (Mg/L) versus Reaction Time (Min) for Preliminary Run 6

Table 3.1 The Concentration of Ammonia-N Solution before and after Treatment of UMP and PF Soil for Preliminary Run 7 and Preliminary Run 8

	Ammonia-N Concentration Preliminary Run 7		Ammonia-N Concentration Preliminary Run 8	
Time (hr)	UMP	PF	UMP	PF
0	92.5	92.5	347.5	347.5
5	60.825	60.075	231.67	239.17

3.3 Selected Factors for Ammonia-N Removal

After the suitable reaction time was identified, the another four factors that have been proposed were implemented in experiments involving of soil mixed culture from both soil type and ammonia-N solution. The factors were type of soil, ratio of soil to water composition, surrounding temperature and agitation speed.

3.3.1 Type of Soil

There were two types of soil that used to treat the ammonia-N solution, which were soil that obtained around the poultry farm and ordinary soil that obtained from around UMP. Poultry Farm (PF) soil was functioned in treating the ammonia-N and poultry farm wastewater solution by reducing their ammonia concentration. It was believed, the soil around the poultry farm has high density of mixed culture microbes

that involved in nitrification process where the microbes consumed the ammonium ion thus reducing the ammonia concentration. while UMP soil was believed containing an amount of soil mixed culture microbes density, but much lower compared to the soil obtained around the poultry farm. This soil was functioned as the reference in comparing the effectiveness of both soils mixed culture in reducing ammonia concentration in ammonia-N solution. The difference between soil from UMP and soil from the poultry farm was, the UMP soil believed to contain lower mixed culture microbes compared to the soil from poultry farm, thus the rate of ammonia removal was lower.

3.3.2 Ratio of Soil to Water Composition

The ratios of soil to water composition were 1: 1 and 1: 6. The lower the ratio, the more concentrated the water-soil solution. It was believed that the lower ratio, the higher the mixed culture microbes in the solution. Thus, yield higher rate of ammonia removal. These ratios have to differ greatly in order to get significant different removal values.

3.3.3 Surrounding Temperature

The proposed temperatures were 25^0C and 30^0C. The surrounding laboratory temperature was 25^0C. For runs with 30^0C, hot plate was used to increase the temperature. The reason of choosing these temperatures was because some sientists said 30^0C was the optimum temperature for nitrification while 25^0C is the temperature of laboratory.

3.3.4 Agitation Speed

Experiments were conducted with stirring and without stirring to study how does the stirring will affect the rate of ammonia-N removal. For with stirring part, agitation speed that used was 200rpm by using orbital shaker.

3.3.5 Reaction Time

From the preliminary experiments, the suitable reaction times to measure the removal rate were t= 2-hours and 5-hours.

The ranges of factors selected were presented in Table 3.2.

Table 3.2 The Ranges of Selected Factors

Factor (s)	Ranges
Reaction Time	t= 2-hours and 5-hours
Type of Soil	UMP and PF soil
Ratio of Soil to Water Composition	1 : 1 and 1 : 6
Temperature	25^0C and 30^0C
Agitation Speed	0 rpm and 200 rpm

3.4 Screening Parameter Using 2 Level of Factorial

The proposed factors earlier that gave some influence the rate of ammonia-N removal were screened using 2-level of factorial created by Design Expert software. The five factors; type of soil, ratio of soil to water composition, surrounding temperature, agitation speed and reaction time were expected to affect the ammonia removal rate. The designs had 32 trials experiment with randomized values of factors, and several replicates at the center points to determine the possible occurred error.

The design of experiments was performed by Design Expert software where all the factors were randomized and there were several runs with all the parameters controlled. There were 32 runs in total and the parameters of each experiments were as in Table D1 in Appendix D.

After the experimental condition were designed by Design Expert, the experiments conducted using the ammonia-N solution treated with UMP and PF soil. The experiment were 32 runs in total.

The identifying and analyzing factors that really gave influence on ammonia-N removal based on the 32 data of experiments were done using Design Expert software.

3.5 Validation of Best Condition Using Poultry Farm Wastewater

After the equation and coefficient of equation r^2 obtained based on 32 runs, the validation tests were conducted (3 runs) using poultry farm wastewater treated with UMP and PF soil mixed culture. The poultry farm wastewater was made by dissolving them in deionized water with the ratio of 1:1. The prediction removal rate based on equation obtained from 32 runs. This prediction then, compared with the actual values (removal rate). The condition of the 3 runs were conducted as in Table 3.3.

Table 3.3 The Conditions for Run 3, 4 and 7

Run	Agitation (rpm)	Reaction time (hour)	Type of soil	Temperature (^{0}C)	Soil : water ratio
3	0	5	PF	25	1 : 6
4	0	5	UMP	25	1 : 6
7	0	2	PF	30	1 : 1

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Factorial Analysis Result

The result of 32 runs using the ammonia-N solution treated with soil mixed cultures were showed in Table D2 in Appendix, while 3 runs using the wastewater from chicken farm were presented in Table 4.3. The results obtained from the experiments were presented in percentage contribution of every factor toward the ammonia-N removal.

4.2 Factors Affecting Ammonia-N Removal Process

The five factors that were tested in this ammonia-N removal research were agitation (factor A), reaction time (factor B), type of soil (factor C), temperature condition (factor D) and ratio of water to soil (factor E). In this research, the single factor that most affecting the ammonia-N removal was temperature (Figure 4.1 and 4.2). Most of investigators employed temperature effect either as the optimal temperature of microbes just like what Mulder *et al*. (2006), Chimenos *et al*. (2003) and Yoon *et al.* (2008) did, where they only used one fixed temperature value to find the other optimal parameters. Chen *et al*. (2011), Chung *et al.* (2005), Sajuni *et al.* (2010), Andersson *et al*. (2001), Yoon *et al*. (2008), Breisha and Winter (2010) and Jiang *et al.* (2011), in other hand, conducted experiments in wide rough range of temperature to find the optimum temperature for their specified microorganisms.

The example that shows how deeply temperature can influence the nitrification rate was described by Malolo (2011) and Henry and Jones (1972). According to Malolo (2011), the temperature play crucial role on the chemical reaction, reaction rate, and microorganism's life. The increasing temperature can causes climbing number of bacteria in reactor which consequently causing increased of nitrification rate. In certain range of temperature, the number of microbes decreased dramatically. This declining pattern is due to endogenous respiration, but the main reason was the growing of protozoan population that reduces numbers of the desired microorganism. Besides that, temperature is important controlling variable because activities of nitrification increase with temperature but then stop at certain point due to the denaturation of microbes.

Mulder *et al.* (2006) stated that, biological processes will produce heat, and consequently, heat production is very significant in wastewater. For every 10^0C, 1 gram of ammonia per liter is produced via nitrification. They claimed in their paper, wastewater temperature can be said as one of the most dominant factor for nitrification in nature. Blackmer *et al.* (2008) suggested that the temperature condition should be warm enough to encourage a high degree of microorganism respiration and reproduction. Jones and Morita (1985) supported that nitrifying bacteria is very sensitive to low temperature thus nitrification rate can be inhibited by low temperature, as the low temperature can narrowing the microorganism's activity. Very low temperature can deactivate them while high temperature can kill them. At suitable temperature, ammonia oxidizer have significant higher growth rate (Mulder *et al.*, 2006). To demonstrate the strong influence of temperature on nitrification rate, Chen *et al.* (2011) had done the experiment ammonia removal with respect to temperature, and the highest percentage of ammonia removal can be achieved within 12-hours was 97.48%.

The nitrification temperature should be controlled because high temperature leading to the increase of free ammonia level in the suspended solution (Yoon *et al.* 2008; EPA, 1993; Ford, 1980; Aktan *et al.*, 2012) which later will inhibit both ammonia and nitrite oxidizers (Kim *et al.*, 2006; Sun *et al.*, 2008; Anthonisen *et al.*, 1976; Yang *et al.*, 2004). This inhibition resulting the reduction of ammonia-N removal efficiency. Brendel *et al.* (2000) support that, the relative abundance of free ammonia and ionized ammonium ion is primarily a function of temperature and pH. Given a static pH value, any increasing of temperature will bring about the increasing of fraction of free ammonia molecule concentration per total ammonia and ammonium ion concentration while decreasing temperature causes the increasing fraction of ammonium ion per total

ammonia and ammonium ion concentration $\left(\frac{NH_4^+}{NH_4^+ + NH_3} \right)$. High ammonium ($NH_4^+$) ion concentration does not a matter because have no effect/ harm the chain reaction, in fact, ammonium ion is the substrate for of ammnonia oxidizer (Schmidt *et al*., 2004), but differed for free ammonia (NH_3) molecule, because it can become an inhibitor for *Nitrospira* (Kim *et al.*, 2008) and *Nitrosmonas* (Paredes *et al.*, 2007, Anthonisen *et al.*, 1976,Villaverde *et al.*, 2000, Kim *et al.*, 2006; Park and Bae, 2009; Villaverde *et al.*, 1997).

Whatever concentration of free ammonia is, that free ammonia molecule being able to inhibit the nitrification rate is due the ability of ammonia that can easily penetrate through the cell membrane and dissolve in lipid. This fact was investigated by Speece (1996) and Gallert and Winter (1997). The uncharged and lipid soluble ammonia molecule causes imbalance of proton and it ends up with a kind of specific enzyme inhibition (Sprott and Patel, 1986). Because of this facts, it is important that the temperature should be controlled not too high or low. Based on the equilibrium balance ammonia or ammonium per total ammonia and ammonium, because pH testing was not conducted, so the actual amount ammonia and ammonium ion existed in the ammonia-N solution. Although ammonia can be inhibitor to *Nitrosomonas* but the reaction acts at certain concentration limit, plus there are still other ammonia oxidizers exist in the soil mixed culture that can consume ammonia. The ratio of *Nitrosomonas sp* and other ammonia oxidizer (i.e *Nitrospira sp.*) idea triggered by the research done by Boyle-Yarwood *et al.* (2008) that studied the ratio of ammonia oxidizing bacteria and archea

composition in soil and research of Pedersen *et al.* (1999) on heterotrophic and autotrophic nitrifier ratio in conifer forest soil.

The soil to water ratio contribute 5.87% for removal. As claimed by Camberato (2001) the water content have little effect on nitrification rate. Reaction time contributes the lowest percentage for the removal rate. Sometimes, in certain biological, reaction time factor does not play crucial role to contribute toward the reaction conducted. This proved by observing the slow rate of ammonia-N removal cited by other authors as presented in Table 4.1. Their data have been standardized and expressed as average value in mg/L/day. The lesser the value give the meaning that reaction time has no effect on the removal rate. The theory is, insignificant amount of ammonia-N is being removed as compared to the initial concentration after 24-hours.

Table 4.1 The Removal Rate of Ammonia-N Cited by Authors

Authors	mg/ L/ day
Chen *et al*. (2011)	1.8-4.5
Jamieson *et al.* (2003)	2.2-5.8
Wett (n.d.)	32.7
Bao *et al*. (2011)	22-25
Figueroa *et al.* (2012)	±4.5
Mohammed *et al.* (2008)	1.215-3.349

Comparing to this study, the ammonia-N removal treatment varied from 5.5-20.4165mg/L/h. Converting this unit into mg/L/d, thus the removal rate ranged from 132 to 490 mg/L/d. These values were taken and calculated from the 32 runs treatment as stated in Table D1 and D2 in Appendix D.

In this study, the combination of agitation-temperature and type of soil-temperature were the factors contribute the lowest and highest percentages toward the ammonia-N removal rate in term of factor interaction category. The importance of type of soil and temperature used towards the nitrification process has been emphasized by Webster and Hopskins (1996) and Godde and Conrad (1999, 2000) where the removal rate varied depends on these factors based on their evident result .

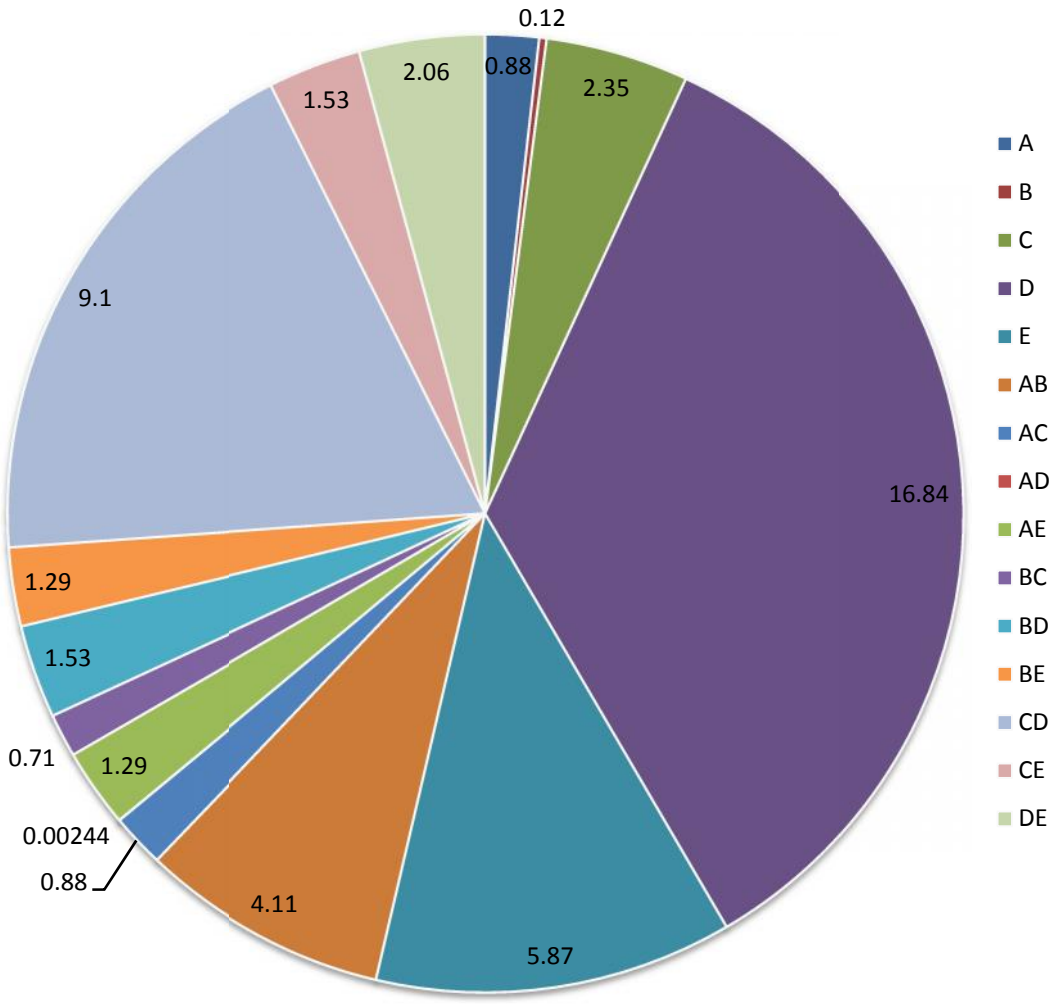

Figure 4.1 Percentage Contribution of every Single Factor and Interaction between Factors Affecting Ammonia-N Removal [A=agitation; B=reaction time; C=type of soil; D= temperature; E= ratio of water to soil; AB= agitation-reaction time; AC= agitation-type of soil; AD= agitation-temperature; AE= agitation-soil to water ratio; BC= reaction time-type of soil; BD= reaction time-temperature; BE= reaction time-soil to water ratio; CD= type of soil-temperature; CE= type of soil-soil to water ratio; DE= temperature-soil to water ratio]

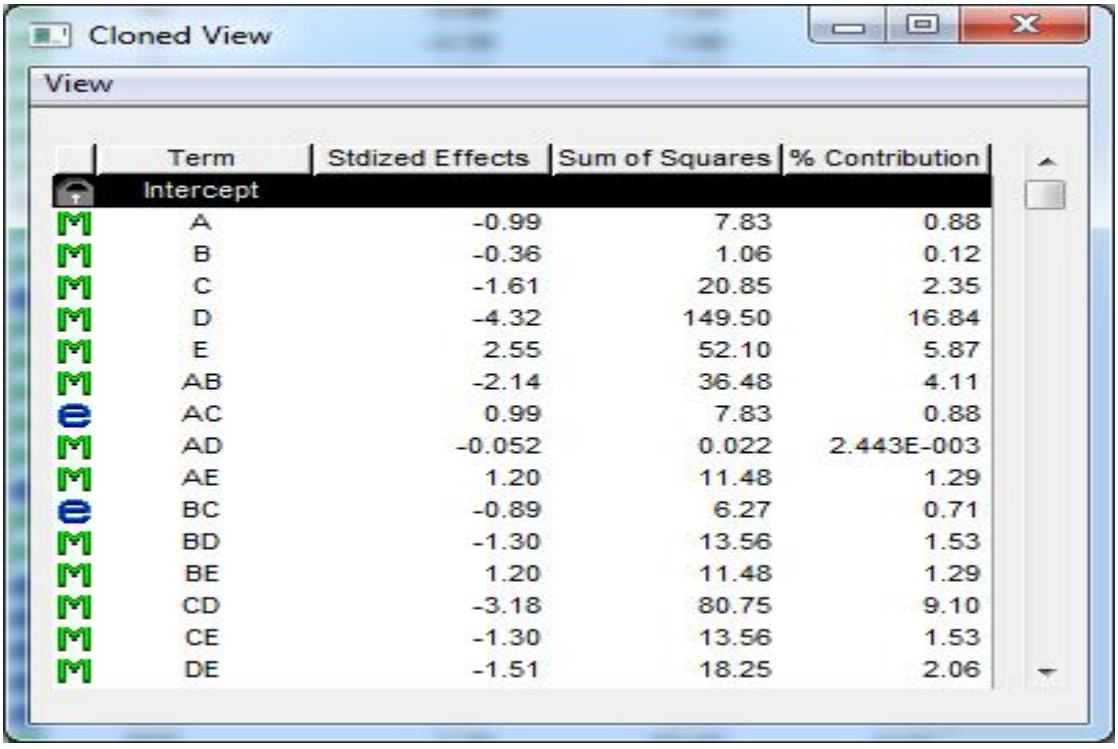

Figure 4.2 The Percentages of Influential of Factors. [A= agitation, B= reaction time, C= type of soil, D= temperature, E= ratio of water to soil]

4.3 Interaction between Factors

Every parameter have own contribution toward the ammonia-N removal. But interaction between these parameters can be utilized to yield higher ammonia-N removal. There were ten pairs of interaction combination of factors in total. The three combinations of factors from Figure 4.1 and Figure 4.2 that showed highest contribution toward the removal rate (CD, AB, and DE) will be further discussed in detail.

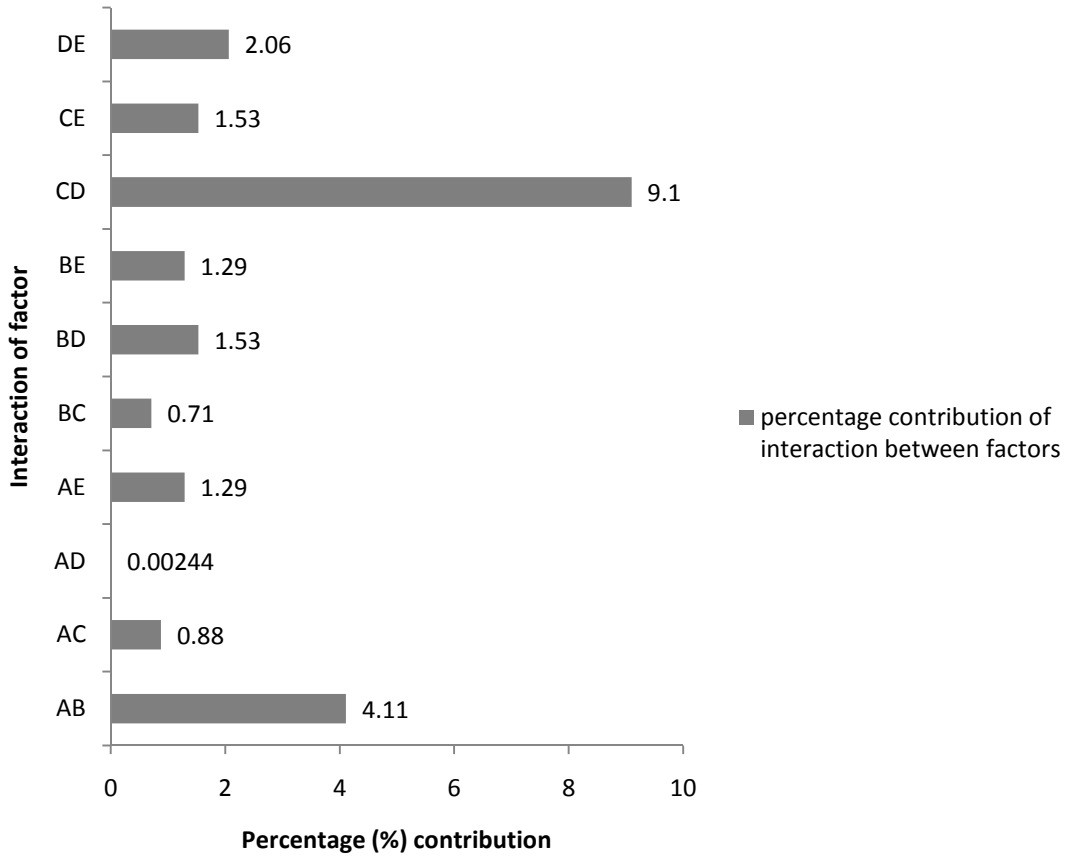

Figure 4.3 Percentage Contribution of every Interaction between Factors Affecting Ammonia-N Removal [AB= agitation-reaction time; AC= agitation-type of soil; AD= agitation-temperature; AE= agitation-soil to water ratio; BC= reaction time-type of soil; BD= reaction time-temperature; BE= reaction time-soil to water ratio; CD= type of soil-temperature; CE= type of soil-soil to water ratio; DE= temperature-soil to water ratio]

4.3.1 Interaction between Reaction Time and Agitation

Figure 4.4 until 4.6 depict wide relationship between factors with each single figure can be translated in many ways. Figure 4.4 showing the interaction between factor A (agitation) and B (reaction time). The line of 2-h reaction time and 5-h reaction time intercepted on the same agitation speed point which is about 200rpm. Following the 2-h line, as the agitation speed increase, the efficiency of ammonia-N removal declined. The

percentage ammonia-N removal of non-agitated 2-hours reaction time › non-agitated 5-hours reaction time. The removal from non-agitated 5-hours reaction time, agitated 5-hours and 2-hours reaction time has insignificantly differences When using 5-hours as the reaction time, whether with or without agitation, the ammonia-N removal rate has no significant differences.

Fast agitation could damage the cell (Schneider *et al.*, 1995). The mechanical stirring used by Artiga *et al.* (2005) for their nitrifying sludge was 150rpm only. Ammonia removal test done by Yoon et al. (2008) was 60rpm while Taylor *et al.* (2009) used shaking speed was varied at 60, 80 and 120rpm, Zhou *et al.* (2007) varied the speed at 70-140 rpm and Pedersen *et al.* (1999) at 150rpm.

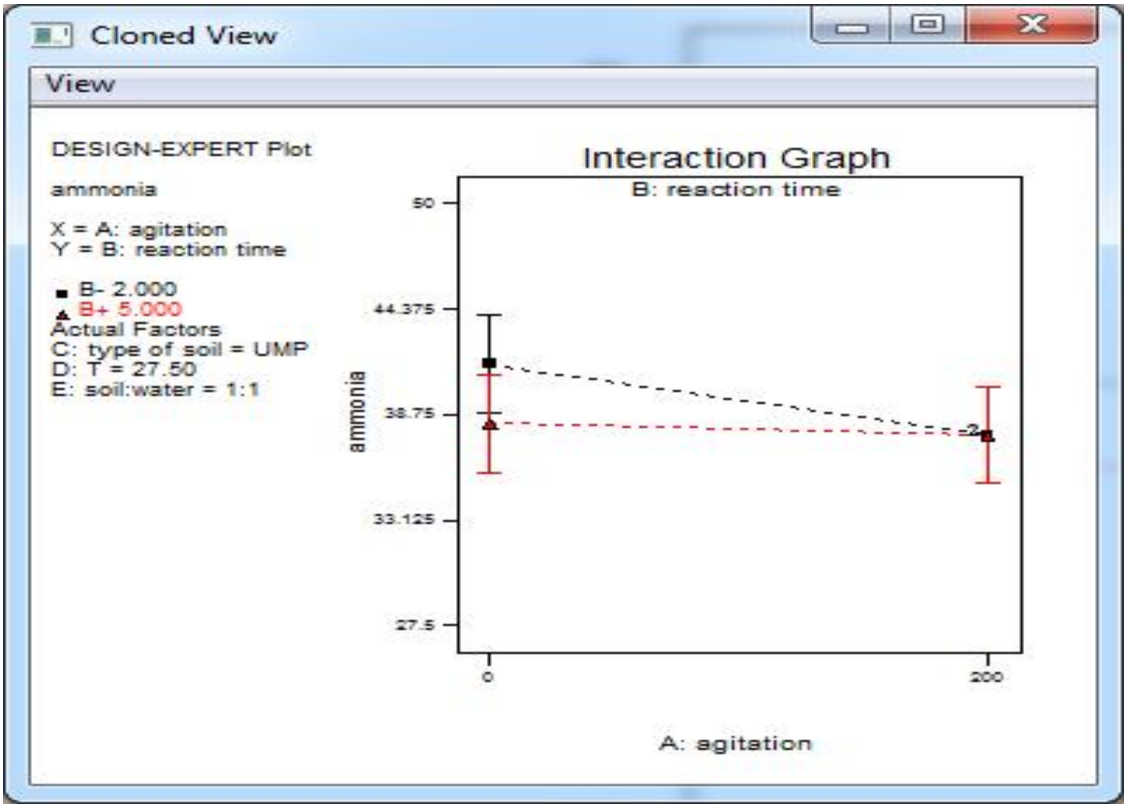

Figure 4.4 Interaction Graph between Agitation and Reaction Time

4.3.2 Interaction between Type of Soil and Temperature

From the result stated in Figure 4.5, there is an interaction between type of soil and temperature condition. The interaction seems to lies exactly on the UMP soil point. Along the 25^0C- line, the ammonia removal with PF soil treatment is greater than UMP soil treatment. But the pattern is somewhat contrast for the case of 30^0C temperature condition. The percentage of ammonia-N removal quite good in combination of 25^0C and PF soil compared to 25^0C of UMP soil. While in condition of UMP soil and 30^0C, the ammonia-N removal was better than PF soil condition of the same temperature.

Soil contains a wide range of microorganism described as “black box” (Shukla and Varma, 2011). Nannipieri *et al.* (2003) reported that the living population inhabiting soil includes macrofauna, mesofauna, microfauna and microflora. There is link between the microbial biodiversity and soil function. Soil maintains biogeochemical cycles because microorganisms living in the soil degrade. Soil particle mainly made up of clay-organic matter, and microorganism in soil are closely associated with these particles (Shukla and Varma, 2011). The activity and interaction of these microbes with other microbes, larger microorganism and the soil particles primarily depend on the condition of microhabitat level which differs among the microhabitats even in very small distance (Wieland *et al.*, 2001). Fertility of soil depends not only on its chemical composition but also on the qualitative and quantitative nature of microorganism inhabiting it (Giri *et al.*, 2005). So, these facts cause the every biological and chemical reactions that happened in soil are differed in every microhabitat. Even in some distance of the same microhabitat, the reaction still differed.

In a given type of soil, many kinds of microbes may inhabit. For example, it was reported that acidic soil may be a home for community of *Nitrosomonas europaea*-like organism (Carnol *et al.*, 2002), *Nitrospira*-like organism (Stephen *et al*., 1996). At the same time that *Nitrospira* dominant in neutral agricultural soils (Stephen *et al*., 1998), neutral grasslands (Kowalchuk *et al.* 2000a, b). The result from experiment done by Shaw *et al*. (2006) revealed that *Nitrospira* sp strain also inhabitated clay loam soil. What can conclude from these discoveries is that, all microbes, not restricted only to *Nitrospira* species, can evolve and adapted to the different environment. It is not impossible that, during the evolution and adaptation of microbes to the surrounding,

their optimal temperature may also being altered, not bound to one fixed temperature, but depending to their nature.

Lishman *et al.* (1999) had used the temperature range of 20^0C in experiment involving species of *Pseudomonas* and *Paracoccus*. Priha and Smolander (1994) used forest soil (with nitrogen as nutrient limiting tree growth), to investigate effect of different temperature on the nitrification rate. In experimental soil treated with CaN at 14^0C and 21^0C, the total nitrate and nitrite content had become 25 µg/cm^3 and 210µg/cm^3 respectively from initial value of 0.2µg/cm^3. The optimal temperature of *Arthrobacter oxydans* CH8 microbes in experiment of Chung *et al.* (2005) lies $\pm 26^0$C. Higher than that range will causes the loss of efficiency. Experiment involved wastewater that used by Sajuni *et al.* (2010) to study the temperature impact on ammonia-N removal, had employed 25-35^0C temperature condition. At high temperature, the ammonia removed smoothly (about 93.5%) and when at lower temperature, the rate declined as the temperature decreased. Low temperature normally has drastic effect on bacterial process rate. Below 15^0C, the nitrification rate drop, 12^0C causes the rate to be reduced 50%.

Microbes inhabiting the soil depends on soil environment, and these microbes have own optimal temperature to carry their tasks. Taking the fact, microbes existed in the PF and UMP soil also may differ. Because of that, in the present study, the soils that taken in two place have different optimal temperatures.

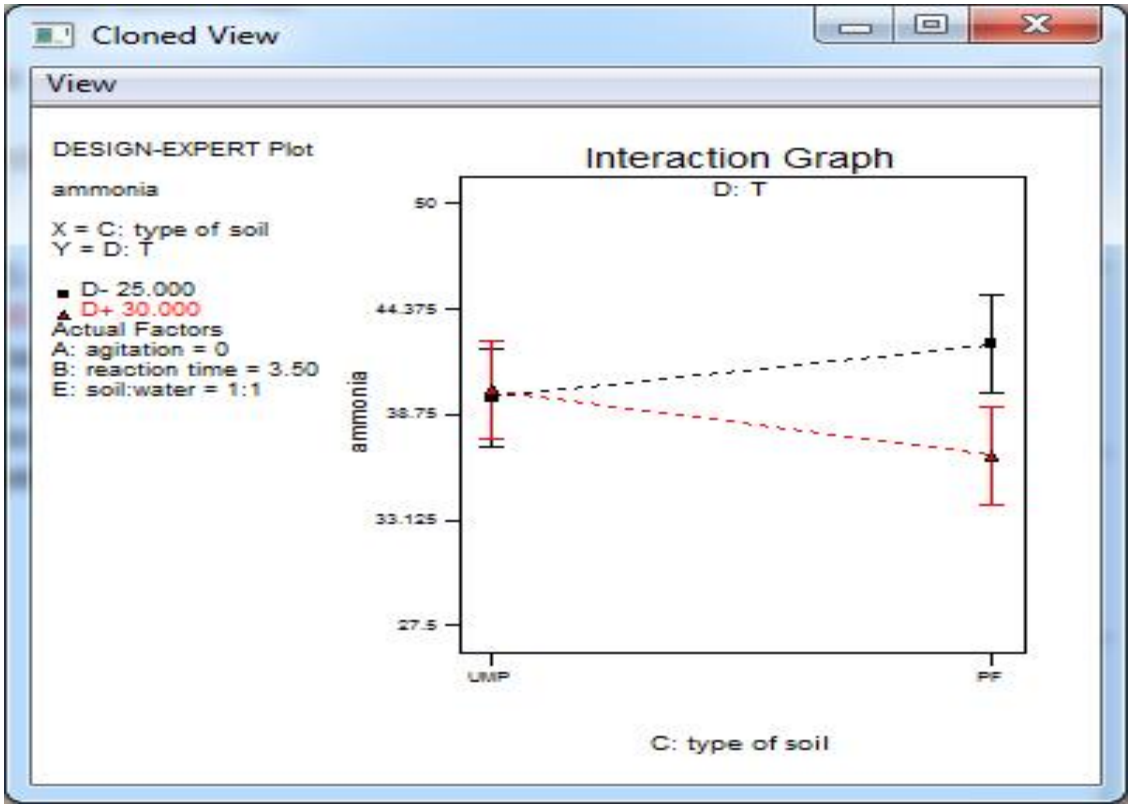

Figure 4.5 Interaction Graph between type of Soil and Temperature

4.3.3 Interaction between Soil to Water Ratio and Temperature

At 25^0C, 1 : 6 of soil to water ratio performed better than 1 : 1. No differences in removal rate can be seen at temperature condition of 30^0C. Theoretically and logically, the ratio that performed better should be 1 : 1 because the more concentrated of the soil-mixed culture solution, the more chances of microbes of nitrification lived in that solution. Within 1:1 of soil to water ratio, the removal at 30^0C was better than 25^0C. This result obey the statement from Wong (2005) that stated that ammonia emission should be higher in spring and summer that has higher ambient and soil temperature. While in 1: 6, the removal of 25^0C was better than at 30^0C.

Ammonium-N and ammonia-N which serves as energy source for microorganisms responsible for its oxidation, can inhibit the biological activity if free ammonia exist above the restricted amount. Free ammonia can function of temperature, pH and ammonium-N concentration. Free ammonia ion will increase in condition of high pH (Gerardi, 2003). Wastewater temperature may inhibt nitrification by increasing free ammonia concentration at high temperature (Kim *et al*., 2006). Among the type of inhibitor, the inhibition that caused by free ammonia concentration is considered as the most influencing factor compared to ammonium-N and nitrite ion (Chen *et al*., 2006).

In any biological pathway, second substrate of a certain reaction should be proceeded to third product before the first substrate can be further catalyzed into the second substrate. If second substrate not catalyzed to third product, then the second substrate may inhibit the reaction until the normal reaction takes place (Robert and King, 1987). This kind of inhibition falls into non-competitive inhibitor. Non competitive inhibitor controls the series of enzymatic-catalyzed reaction. When this substance reached certain concentration it turns into inhibitor. It binds to allosteric site of enzymes and prevents the reaction series from proceeding. The reaction started over again when the concentration of this substance falls to low level (Kent, 2000). Means that the ammonia oxidation may stucked if the nitrite oxidation is blocked. For nitrification mechanism, various processes may lead to nitrite accumulation. First is the inhibition of nitrite oxidation. Second is a sudden increase of ammonia oxidation rate without a parallel increase in subsequent nitrite oxidation rate. Ammonia oxidation and nitrite oxidation are dependence each other. Burns *et al.* (1996) concluded that, from their experiments, the rate of ammonia oxidation was only required to be slightly in excess of

nitrite oxidation in order to simulate nitrite accumulation. This imbalance rate would occur if the activity of nitrite-oxidizer was inhibited to a greater extent than that of ammonia oxidizer (Smith *et al.*, 1997). Soil with high ammonium-N ion may resulting selectively inhibitory of nitrite oxidizer, causes the accumulation of nitrite.

Nitrite oxidizing bacteria is more sensitive to free ammonia than ammonia oxidizer. Ammonia concentration has shown to slow down the rate of nitrite oxidization, sufficiently to cause nitrite accumulation (Monaghan and Barraclough, 1992; Jones and Schwab, 1993; Burns *et al*., 1995). Once the inhibitory effect has been eliminated, the nitrite oxidizer will activate once again, and the rate of nitrite-oxidization to nitrate increase (Burns *et al.*, 1996).

Free ammonia will inhibit nitrite-oxidoreductase which located in *Nitrobacter* cell membrane (Yang and Alleman, 1992). Alleman (1984) had shown that nitrite oxidizer is two order of magnitude more sensitive to free ammonia inhibition than ammonia oxidizer. And Stein and Arp (1998) had studies that this nitrite concentration or accumulation of nitrite could caused the specific loss of ammonia oxidizing activity in *Nitrosomonas*, which thus causes the accumulation of ammonia concentration in solution media.

At 25^0C and 30^0C, the ratio of 1 : 6 showed higher removal rate than 1 : 1 at both temperature. It is assumed that 1 : 1 have higher microbes number than 1 : 6 since 1 : 6 has very dilute microbes concentration per liter of solution. High bacterial numbers do not always reflect high activity. Yanai *et al.* (2004) reported that *Nitrosomonas europaea* ATCC 25978, have tendency to be inhibited by higher concentration of

ammonia-N in liquid media. This phenomenon suggesting that as the microbes number increase, the higher chances of inhibition occured.

The simple message was, at high temperature, the ammonia-N concentration is higher over the ammonium-N ion concentration in solution medium. This ammonia-N concentration may inhibit the ammonia and nitrite oxidation rate. Since the nitrite oxidizer is more sensitive than ammonia oxidizer, so it may inhibited badly. The inhibition of nitrite oxidation, consequently can cause the inhibition of ammonia oxidizer because it turned into non-competitive inhibitor, thus increase magnitude of inhibition of ammonia oxidizer. In other hand, inhibition of ammonia oxidizer by free ammonia already take places. This may explained why ammonia removal rate decreased with temperature of this present study. The second matter was in the case of inhibition happened, as the number of microbes increased, the higher the tendency of being inhibited.

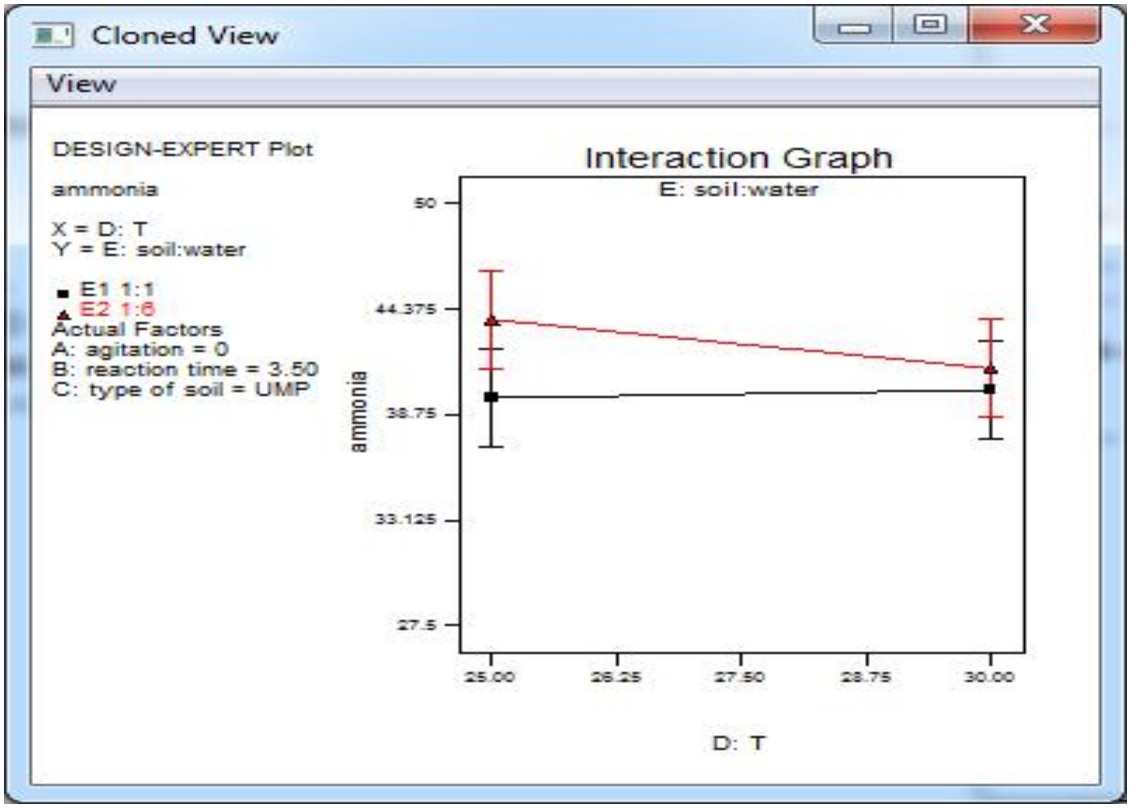

Figure 4.6 Interaction Graph between Soil to Water Ratio and Temperature

4.4 Best Conditions using Two Level Factorial Analysis.

In year of 2011 research done by Chen *et al.*, the highest and lowest percentage ammonium-N successfully removed under different dissolved oxygen condition were 97.48 and 38.95% respectively. Ammonium-N removed from wastewater obtained from meat product processing company used by Rodŕiguez *et al.* (2011) ranged from 19 to71% depending on day of operation. Along the 16 days of operation, the ammonia removed from the continuous ammonia concentration supply done by Chung *et al.* (2005) at least 75%. The highest achievement almost hit 100% removal. The same goes

for Sajuni *et al*. (2010)'s experiment. The treatment involves samples from freshwater fish water. 90-100% removal achieved during 5 until 30-days of operation. Percentage removal below than 10% was done by Andersson *et al*. (2001). But the highest removal achieved by the same authors was almost 90% efficiency. At different solid to liquid ratio, 54-96% of initial ammonia content succeeds removed from carmine lake process wastewater (Chimenos *et al*., 2003).

From 700mg/L of ammonia concentration, the final concentration of the solution was 420mg/L which means the lowest removal (40%) while the highest removal was 100% (0mg/L at the outlet concentration. This achievement accomplished by different pH, aeration rate, temperature and headspace aeration (Yoon *et al*., 2008). Through experiments done by Ilies and Mavinic (2001), the ammonia removed ranged 48-100% while Jiang *et al*. (2011) got 15-100%. The ammonia removal percentages cited by other authors were as depicted in Table 4.2.

Table 4.2 Ammonia Percentage Removal Cited by Other Authors

References	Range of ammonia percentage removal (%)	Biological treatment
Jie *at al.* (2009)	33.5-100	*Nitrosomonas europaea*, *Pseudomonas nitroreducens* isolate DB9b, *Acidovorax avenae* subsp. Citrulli, *Pseudoxanthomonas byssovorax*, *Nitrobacter* sp. PBAB10, *Leifsonia* sp. PTX1, *Sinorhizobium* sp. R-24605, *Pseudomonas* sp. iCTE22, *Acinetobacter junii*, *Acidovorax* sp. PD-10, *Comamonas* sp. JS-6, *Nitrosomonas eutropha* isolate F6, *Nitrosomonas* sp. WH-2, *Comamonas* sp.Y14B, *Nitrobacter* sp. PBAB10, Uncultured *Nitrobacter* sp. clone BL017B39, Uncultured *Nitrobacter* sp. clone VAS9
Jamieson *et al.* (2003)	51-93	Greenhouse wetland using *Typha sp.*(a plant species) using three wetlands cell in series
Wett (n.d.)	89.3±1.2	Full-scale single sludge system
Kunz *et al.* (n.d.)	30-95	Utilization of Anaerobic Ammonia Oxidation (Anammox) reactor
Welander *et al.* (1998)	2-100	Pilot scale suspended carrier biofilm reactor. Biofilm grow on the inner surface of carrier made of combination polyethylene, lime, and compounds. Two different models were used.
Bao *et al.* (2011)	75	Microbes grown on carbon foam, a packing media for biological aerated filter
Kimochi *et al.* (1998)	49.0-97	Wastewater treatment with aerobic and anoxic condition
Uemoto and Morita (2010)	95	Use Nitrosomonas europaea and Paracoccus pantotrophus
Figueroa *et al.* (2012)	93	Use autotrophic nitrogen removal over nitrite (CANON) reactor
Vázquez *et al.* (2006)	12-67	Aerobic biodegradation
Patel *et al.* (2006)	>96	Circulating fluidized bed reactor (CFBB) with anoxic and aerobic bed
Mohammed *et al.* (2008)	95.7-99.8	Membrane bioreactor at different operating conditions

Table 4.2 Ammonia Percentage Removal Cited by Other Authors (continued)

References	Range of ammonia percentage removal (%)	Biological treatment
Kim *et al.* (2008)	82-98	Membrane bioreactor combined with nitrification reactor with internal recycle rate varied from 100-500%
Fan *et al.* (2009)	77-97	Pre-anoxic, anaerobic, anoxic, oxic (A^2O) treatment
Sun *et al.* (2012)	20-41	Columns of wetland seeded with microbes (Azoarcus-Thauera-cluster,Hymphomicrobium, Paracoccus genus, Saprospiraceae family)
Deiber *et al.* (1997)	22-91	Aqueous waste treated catalytic with wet air oxidation
Huang *et al.* (2012)	65.5-92	Combination of chemical precipitation followed sequencing batch reactor (SBR)
Ying *et al.* (2011)	81-100	Cyclic activated sludge system (CASS) process
Sun *et al.* (2011)	5-100	Anoxic/aerobic membrane bioreactor (A/O MBR)
Liang *et al*. (2011)	55-60	Vertical submerged biofilm reactor
Hu *et al.* (2012)	76-96	Five anoxic-oxic sequencing batch reactors (A/O SBRs)
Wieβner *et al.* (2005)	82	Microbial biodiversity from *Juncus effusus* plant-laboratory scale
Wiessner *et al.* (2005)	15-90	Microbial community developed as mats in wetland technologies-laboratory scale
Kalyuzhnyi *et al*. (2006)	42-100	DEAMOX (DEnitrifying AMmonium OXidation)-Anaerobic ammonia oxidation coupled to suphide-driven conversion where anaerobic ammonium oxidation reaction with autotrophic denitrifying conditions using suphide as the electron donor within an anaerobic biofilm.
Clifford *et al.* (2010)	95.7	Horizontal flow biofilm reactor (HFBR) with employment of ammonia oxidising bacteria (AOB) and nitrite oxidising bacteria (NOB)
Zhang *et al.* (1998)	90-100	Treatment of anaerobic-anoxic-oxic (A_1-A_2-O) fixed biofilm system

Table 4.2 Ammonia Percentage Removal Cited by Other Authors (continued)

References	Range of ammonia percentage removal (%)	Biological treatment
Lv *et al.* (2010)	98	Lab-scale sequencing batch reactor with domination of aerobic ammonium-oxidizing genus Nitrosomonas, small amount of anaerobic ammonium oxidation bacteria (AnAOB), Nitrospira spp.
Saeed *et al.* (2012)	86	Treatment with pilot-scale hybrid constructed of wetland system (three series)
Vymazal (2001),Vymazal (2005a), Vymazal (2005b), Vymazal (2007)	48.3-84.2	Treatment with various type of constructed wetlands
Wu *et al.* (2008)	76.16–91.83	Constructed mangrove wetland as secondary treatment system

The model equation of two factorial analysis in this study was depicted in Equation (4.1)

$$y = 39.87 - 0.49A - 0.18B - 0.81C - 2.16D + 1.28E - 1.07AB - 0.026AD + 0.60AE - 0.65BD + 0.60BE - 1.59CD - 0.65CE - 0.76DE + 2.21ABD - 1.85ABE + 1.38BDE \quad (4.1)$$

where,

y = ammonia-N removal rate

A= agitation

B= reaction time

C= type of soil

D= temperature

E= ratio of water to soil

The other interaction coefficient excluded in this equation such as AC and BC, because of insignificant coefficient values. R^2 is the relative predictive power of a model and a descriptive measure between 0 and 1 (Atiaa and Handhel, 2009). The closer the value to 1, the better the model and the ability to predict accurately is higher. The r^2 is 0.8383. According to the Figure 4.7 and Table 4.3, the percentage removal that should be achieved from run 3, 4 and 7 of this study were 48.8802, 48.6198 and 42.3698% respectively (as predicted by Design Expert software). But the actual efficiency values achieved through the experiments were 48.27586207, 48.27586207 and 41.37931034% (Table 4.3). These values (values of the expected and actual ammonia-N percentage removal) were quite insignificantly differed because of 1.25, 0.71 and 2.39% error still can be accepted (Table 4.3). The predicted removal values were predicted by using the Equation 4.1. And these runs were done in conditions as presented in Table 3.7. Validation test on PF wastewater showed that the conditions that gave highest removal, were Run 3 and Run 4, about 48%. The condition was treatment of 1 : 6 ratio of UMP soil to water, conducted without agitation at 25^0C where the removal rate measured after 5-hours reaction time. The other treatment yielded the same values was 1 : 6 PF soil to water ratio, without agitation, at 25^0C and 5-hours reaction time.

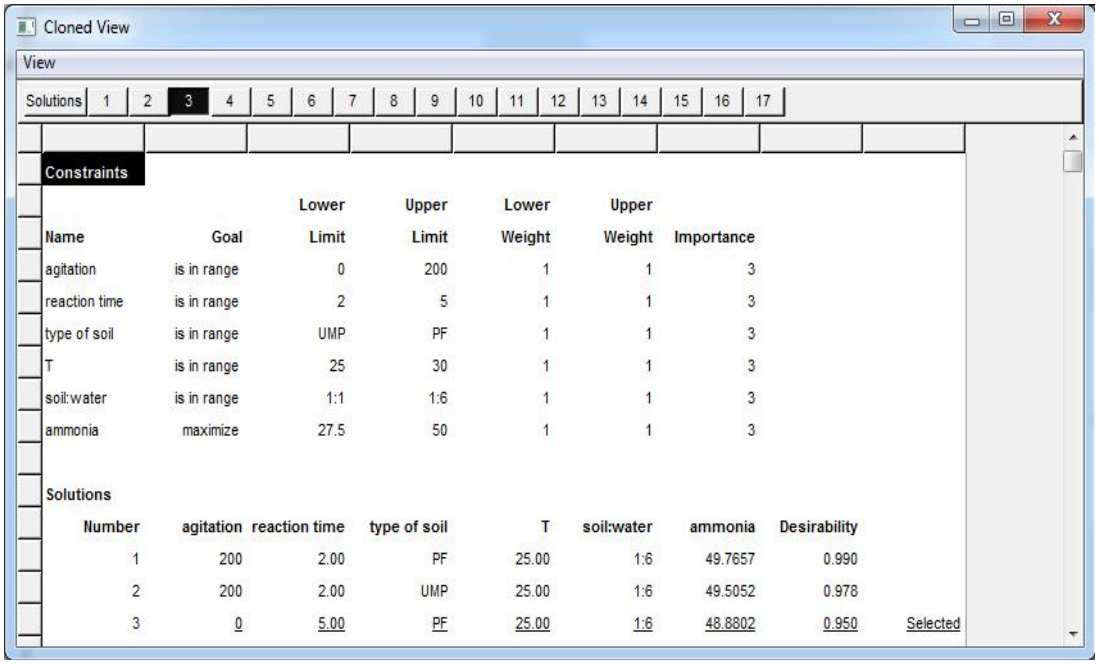

Figure 4.7 The Experimental Designs for Run 3, 4 and 7

Table 4.3 Percentage Removal of Ammonia-N at Certain Conditions

	concentration			percentage removal		percentage error ([(expected-actual)/actual] x 100
run	initial	final	final	predicted	actual	
3	1450	-	750	48.8802	48.2759	1.25
4	1450	-	750	48.8802	48.2759	0.71
7	1450	850		42.3698	41.3793	2.39

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The present study was set out to determine the factors that influence ammonia-N removal. In a nutshell, the objective of this experiment was accomplished. The five factors tested in current study were agitation, reaction time, type of soil, temperature and ratio of water to soil. The analysis of factors was based on two level factorial analysis and the experimental design was done by using Design Expert Software.

The most obvious finding to emerge from this study was that, temperature has the highest percentage contribution towards the removal rate because it has strong influence on the microbes' growth and their activities. Other factors that gave contribution were type of soil (2.35%) and ratio of water to soil (5.87%). In term of interaction between factors, this study has found that combination of type of soil and temperature factor (9.1%) being the most influencing factor followed by agitation-reaction time (4.11%) and temperature-soil to water ratio (2.06%). While combination with the lowest percentage contribution was agitation–temperature.

The results of this research support the idea that there were somehow interaction between agitation-reaction time and type of soil-temperature that can be utilized to give higher ammonia-N removal rate. The empirical findings in this study provide a new understanding of factors enhancing the ammonia-N removal rate. Validation test on PF wastewater showed that the conditions that gave highest removal, were Run 3 and Run 4, about 48%. The condition was treatment of 1 : 6 ratio of UMP soil to water, conducted without agitation at 25^0C where the removal rate measured after 5-hours reaction time. The other treatment yielded the same values was 1 : 6 PF soil to water ratio, without agitation, at 25^0C and 5-hours reaction time. The percentages error were 1.25 and 0.715 for Run 3 and Run 4 respectively. The coefficient r^2 of model for factorial analysis was 0.8383.

5.2 Recommendations

A future study investigating the optimization of removal rate factor would be very interesting since the parameters involved already showed the high contribution on the ammonia-N removal rate.

In optimizing the overall process, it was highly recommended to involve three factors which were temperature in ranged from 25-30^{0}C, ratio of water to soil (1:1-1:6) and on UMP and PF soil. This was to ensure that the process efficient in reducing the ammonia-N concentration in wastewater. With the optimization on these factors, perhaps the removal rate would be more efficient.

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APPENDIX A

Figure A1 until Figure A5 showed the materials used in determining the ammonia-N concentration, using Spectophotometer HACH DR2800

Figure A1 Hach Spectrophotometer DR2800

Figure A2 UMP Soil

Figure A3 Ammonia-N Solution

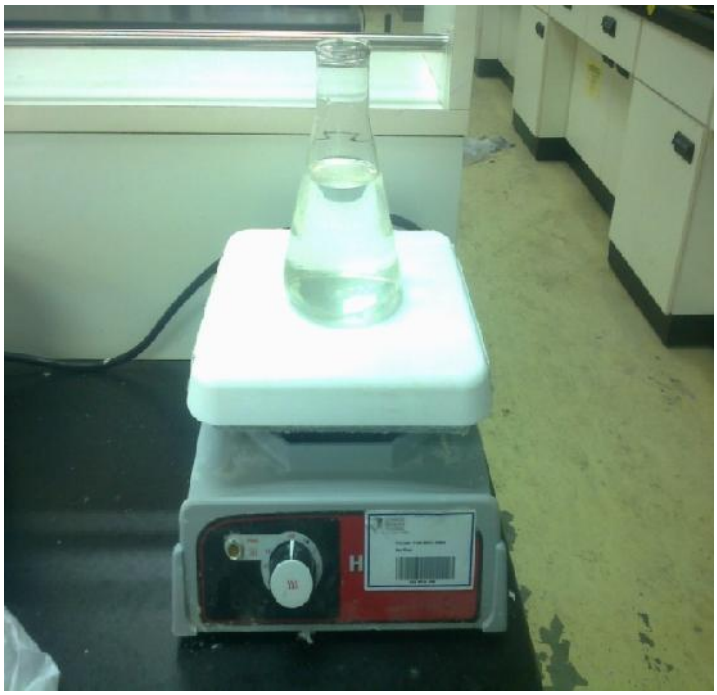


Figure A4 Mixture of Soil Mixed Culture and Ammonia-N Solution

Figure A5 Sample (Soil Mixed Culture and Ammonia-N Solution) and Deionized Water (Blank)

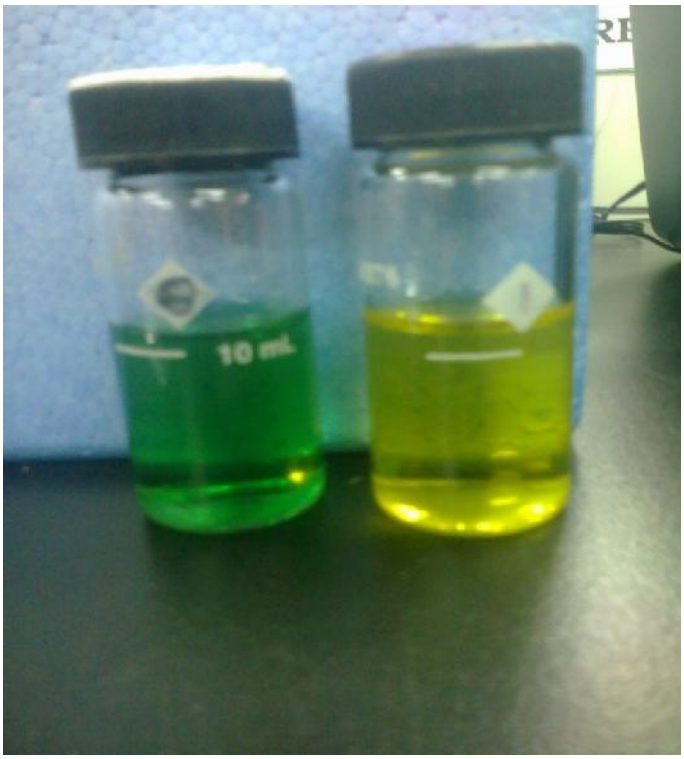
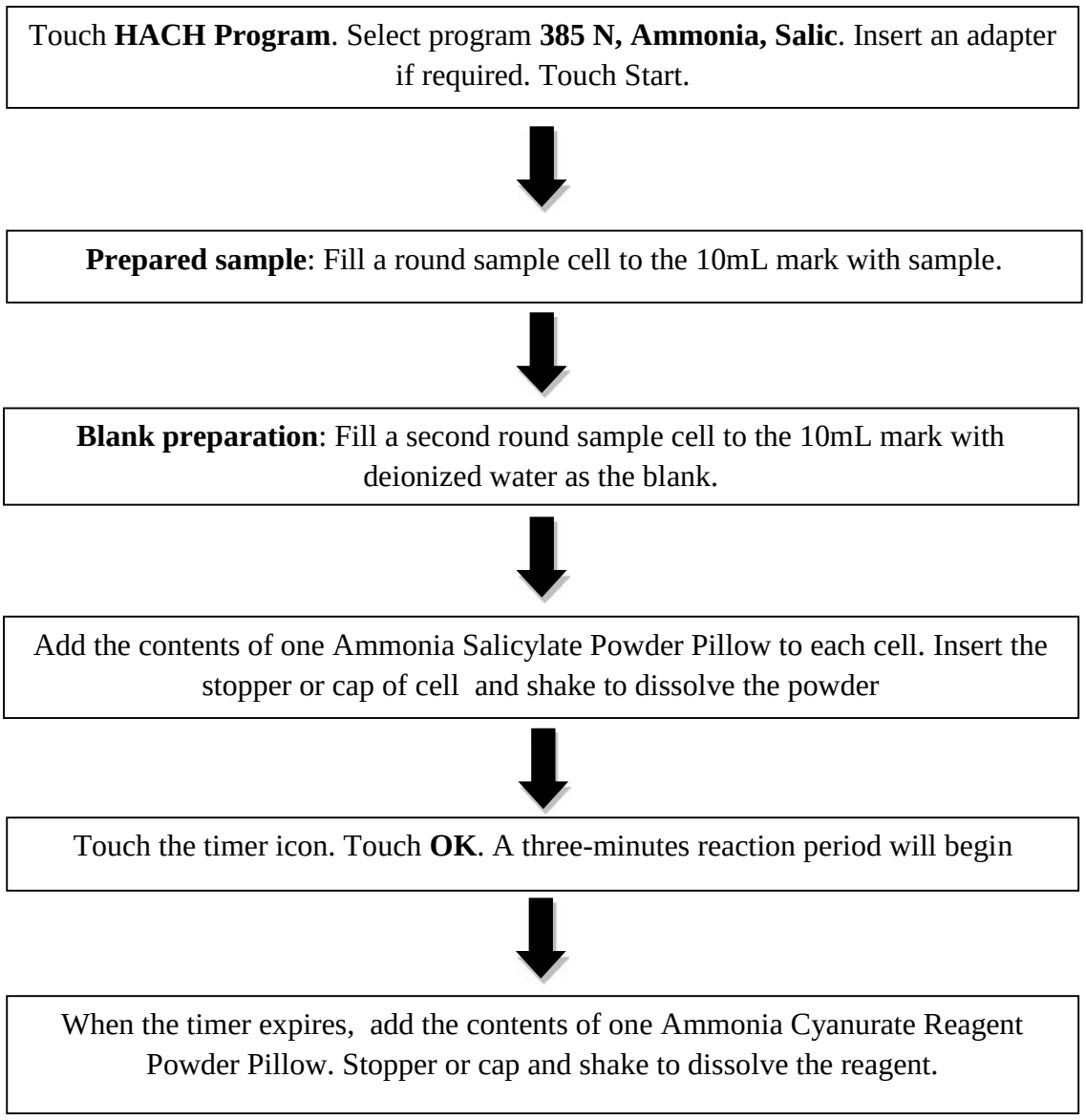


Figure A6 Samples after Some Reaction Time

APPENDIX B

Figure B1 illustrated the procedures of determining the ammonia-N concentration using Spectrophometer DR2800



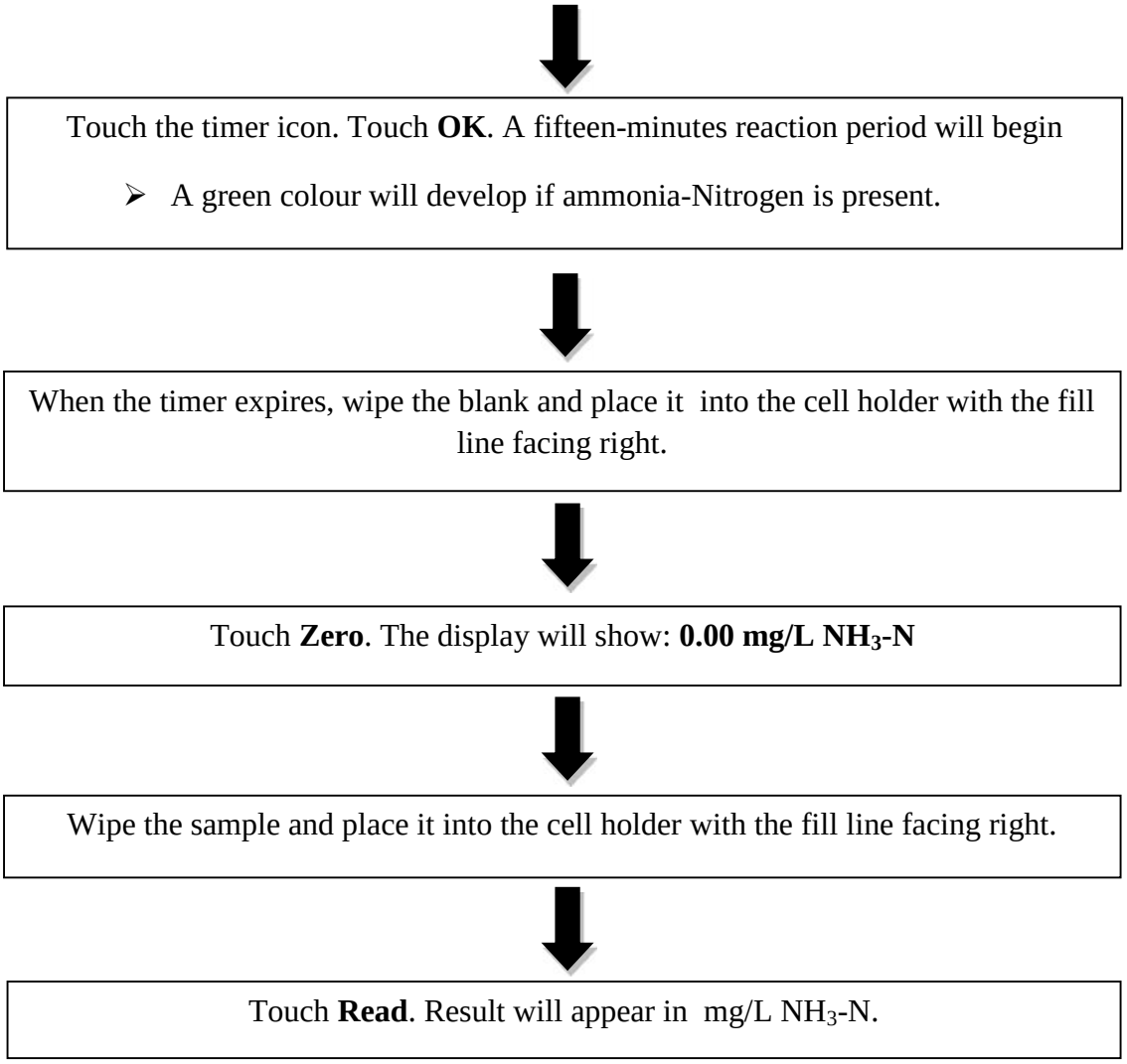


Figure B1 The Procedures Using the DR2800, Method 8155, Program 385

APPENDIX C

Table C1 until Table C4 and Figure C1 and Figure C2 showed the ammonia-N concentration before and after the treatment of UMP soil

Table C1 The Ammonia-N Concentration for Preliminary Run 1

Time (min)	Ammonia-N Concentration (mg/L)
0	120
30	24
60	51
90	90
120	138
150	99
180	48
210	30
240	24

Table C2 The Ammonia-N Concentration for Preliminary Run 2

Time (hr)	Ammonia-N Concentration (mg/L)
0	87
1	48
2	57
3	30
4	60
5	48
6	54
7	132

Table C3 The Ammonia-N Concentration for Preliminary Run 3

Time (hr)	Ammonia-N Concentration (mg/L)
0	102
1	48
2	30
3	42
4	45

Table C4 The Ammonia-N Concentration for Preliminary Run 4, 5 and 6

	Ammonia-N Concentration (mg/L)		
Time (hr)	*Run4	**Run 5	***Run 6
0	105	105	105
1	77.5	70	72.5
2	62.5	57.5	210
3	195	67.5	82.5
4	62.5	60	62.5
5	60	62.5	65

*Run 4: without stirring

**Run 5: without stirring

***Run 6: with stirring

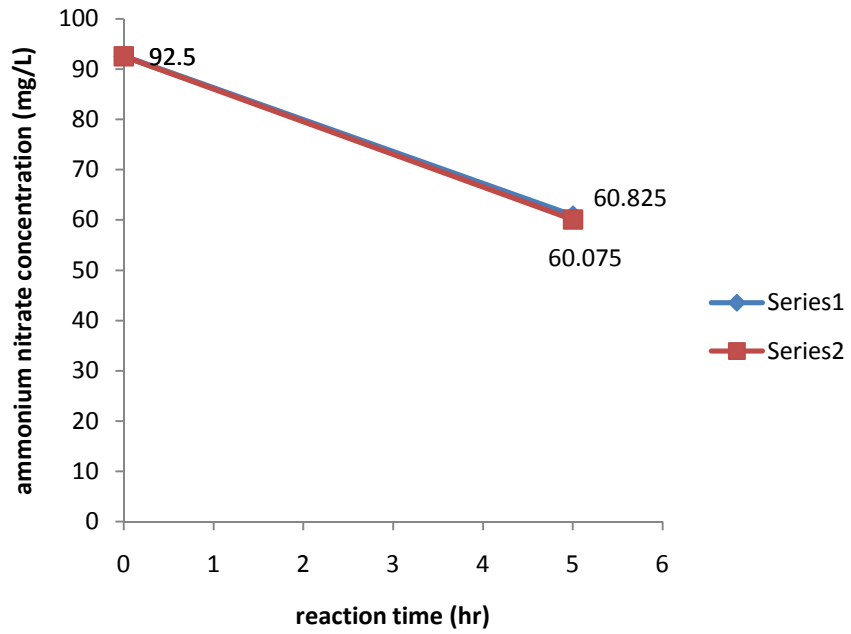


Figure C1 Graph of Ammonia-N Concetration (mg/L) versus Reaction Time (hr) for Preliminary Run 7

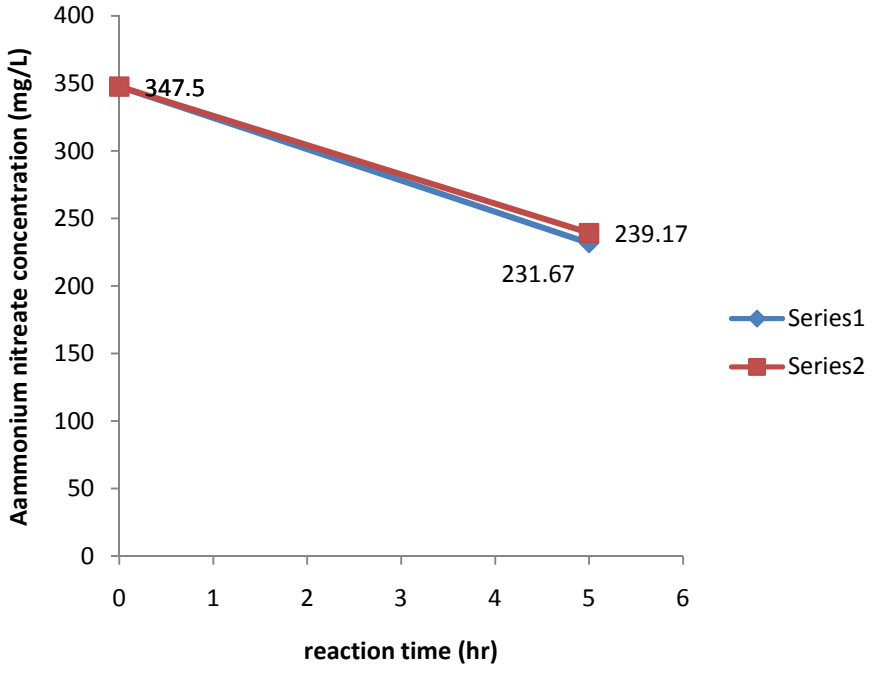


Figure C2 Graph of Ammonia-N Concetration (mg/L) versus Reaction Time (hr) for Preliminary Run 8.

APPENDIX D

Table D1 provide the experimental design of 32 runs while Table D2 showed the percentage removal of every run.

Table D1 The Conditions for 32 Runs Involving Usage of Chemical Ammonia-N Solution Treated with Poultry Farm (PF) Soil and Universiti Malaysia Pahang (UMP) Soil

Std	Run	Block	Factor 1 A: agitation (rpm)	Factor 2 B: reaction time (hour)	Factor 3 C: type of soil	Factor 4 D: temperature (^{0}C)	Factor 5 E: soil: water ratio	Response 1 ammonia
25	1	Block 1	0	2	UMP	30	1:6	3.325
19	2	Block 1	0	5	UMP	25	1:6	7.5
7	3	Block 1	0	5	PF	25	1:1	15
23	4	Block 1	0	5	PF	25	1:6	10
8	5	Block 1	200	5	PF	25	1:1	0
27	6	Block 1	0	5	UMP	30	1:6	12.5
5	7	Block 1	0	2	PF	25	1:1	20
20	8	Block 1	200	5	UMP	25	1:6	-2.5
21	9	Block 1	0	2	PF	25	1:6	7.5
12	10	Block 1	200	5	UMP	30	1:1	0
1	11	Block 1	0	2	UMP	25	1:1	-5
3	12	Block 1	0	5	UMP	25	1:1	-2.5

Table D1 The Conditions for 32 Runs Involving Usage of Chemical Ammonia-N Solution Treated with Poultry Farm (PF) Soil and Universiti Malaysia Pahang (UMP) Soil (continued)

Std	Run	Block	Factor 1 A: agitation (rpm)	Factor 2 B: reaction time (hour)	Factor 3 C: type of soil	Factor 4 D: temperature (^{0}C)	Factor 5 E: soil: water ratio	Response 1 ammonia
10	13	Block 1	200	2	UMP	30	1:1	5
13	14	Block 1	0	2	PF	30	1:1	10
2	15	Block 1	200	2	UMP	25	1:1	7.5
9	16	Block 1	0	2	UMP	30	1:1	7.5
32	17	Block 1	200	5	PF	30	1:6	0
29	18	Block 1	0	2	PF	30	1:6	2.5
24	19	Block 1	200	5	PF	25	1:6	0
6	20	Block 1	200	2	PF	25	1:1	5
17	21	Block 1	0	2	UMP	25	1:6	0
28	22	Block 1	200	5	UMP	30	1:6	-2.5
11	23	Block 1	0	5	UMP	30	1:1	2.5
4	24	Block 1	200	5	UMP	25	1:1	-2.5
30	25	Block 1	200	2	PF	30	1:6	0
16	26	Block 1	200	5	PF	30	1:1	-2.5
31	27	Block 1	0	5	PF	30	1:6	0
22	28	Block 1	200	2	PF	25	1:6	12.5
18	29	Block 1	200	2	UMP	25	1:6	10
14	30	Block 1	200	2	PF	30	1:1	2.5

Table D1 The Conditions for 32 Runs Involving Usage of Chemical Ammonia-N Solution Treated with Poultry Farm (PF) Soil and Universiti Malaysia Pahang (UMP) Soil (continued)

Std	Run	Block	Factor 1 A: agitation (rpm)	Factor 2 B: reaction time (hour)	Factor 3 C: type of soil	Factor 4 D: temperature (^{0}C)	Factor 5 E: soil: water ratio	Response 1 ammonia
26	31	Block 1	200	2	UMP	30	1:6	0
15	32	Block 1	0	5	PF	30	1:1	0

Table D2 The Percentage Removal of 32 runs Involving usage of Chemical Ammonia-N Solution Treated with Poultry Farm (PF) Soil and Universiti Malaysia Pahang (UMP) Soil.

Run	Concentration (mg/L)			Percentage removal (%) [(initial concetration-final concentration)/initil concentration] x 100%
	Initial	Final		
	t=0	t=2jam	t=5jam	
1	100	59.167		40.833
2	100		50	50.00
3	100		50	50.00
4	100		55	45.00
5	100		62.5	37.50
6	100		52.5	47.50
7	100	60		40.00
8	100		60	40.00
9	100	60		40.00
10	100		60	40.00
11	100	62.5		37.50
12	100		60	40.00
13	100	62.5		37.50
14	100	57.5		42.50
15	100	62.5		37.50
16	100	55		45.00
17	100		62.5	37.50
18	100	67.5		32.50
19	100		60	40.00
20	100	60		40.00
21	100	62.5		37.50
22	100		62.5	37.50
23	100		65	35.00
24	100		62.5	37.50
25	100	62.5		37.50
26	100		65	35.00
27	100		65	35.00
28	100	50		50.00
29	100	50		50.00
30	100	65		35.00
31	100	62.5		37.50
32	100		72.5	27.50