REMOVAL OF AMMONIUM-NITROGEN (NH_4-N) BY MICRO FLORA IN DRAIN

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JUDUL **REMOVAL OF AMMONIUM-NITROGEN (NH_4-N) BY MICRO FLORA IN DRAIN.**

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ROBIATUN BINTI ISMAIL

A thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering

Faculty of Chemical & Natural Resources Engineering
Universiti Malaysia Pahang

May, 2008

DECLARATION

I declare that this thesis entitled "Removal of ammonium-nitrogen (NH_4-N) by micro flora in drain" 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."

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DEDICATION

Dedicated to God;

He makes everything possible and

Confirms once again His grace is sufficient…

And also Special Dedication to my family members that always love me,

My friends and all faculty members…

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ABSTRACT

Nitrogen is a one of the important nutrient for aquatic plants and algae. Some of the nitrogen can get from the ammonium cation. However, if there are excessive of nutrients (contains nitrogen) it will stimulate the aquatic plant and algal growth. The uncontrolled release of nitrogen to the environment will cause to serious pollution problems. In order to prevent this problems become more serious, we should handled the nitrogen as a nutrient resource rather than a pollutant that only has to dispose off. Biological treatment is one of the potential treatment alternatives to reduce the nitrogen, where the nitrogen is used as nutrient to the microorganisms. In this study, the sequence batch reactor (SBR) system was used. Different organic loading rate (0.4, 1.3, 2.2, 3.1 and 4.0 mg/l/day) was studied in SBR system to compare the treatment efficiency in terms of water quality parameters (chemical oxygen demand, COD), suspended solid and the changes in NH_4-N concentration. The treated wastewater was analyzed for COD, NH_4-N concentration and suspended solid by using HACH equipment. From this experiment the result shows that the organic loading rate to get maximum percentage COD reduction is 0.4 mg/l/day with 47.05% COD reduction and 124.13 mg/l suspended solid. Meanwhile the value of organic loading rate to get the maximum percentage concentration reduction is also 0.4 mg/l/day with 36.71% concentration reduction and 124.13 mg/l suspended solid. For maximum percentage COD reduction and maximum percentage concentration reduction the optimum value organic loading rate that need is 3.59 mg/l/day with 38% COD reduction, 24.9% concentration reduction and 208.49 mg/l suspended solid. All this results are base on Design of Expert. As a conclusion, this study showed that microflora in drain was efficient in removing the wastewater that contains ammonium-nitrogen.

ABSTRAK

Nitrogen merupakan salah satu nutrien yang penting bagi tumbuhan akuatik dan rumpai air. Sejumlah nitrogen boleh didapati dari ammonium kation. Walau bagaimanapun, jika terdapat terlalu banyak nutrien (yang mengandungi nitrogen) ia akan merangsang pertumbuhan tumbuhan akuatik dan rumpai air. Pembebasan nitrogen yang tidak terkawal akan menyebabkan masalah pencemaran yang serius. Dalam rangka untuk mencegah masalah ini daripada menjadi lebih serius, lebih elok jika kita mengawal nitrogen sebagai sumber nutrien daripada bahan pencemar yang hanya dibuang. Rawatan biologi adalah salah satu rawatan alternatif yang berkeupayaan untuk mengurangkan nitrogen, dimana nitrogen digunakan sebagai nutrien kepada mikroorganisma. Dalam kajian ini, sistem reaktor yang berturutan (SBR) akan digunakan. Kadar beban organik yang berbeza (0.4, 1.3, 2.2, 3.1 and 4.0 mg/l/hari) telah dikaji di dalam sistem SBR untuk dibandingkan kecekapan rawatan dari segi parameter kualiti air (keperluan kimia oksigen, COD) dan perubahan kepekatan NH_4-N . Air sisa yang dirawat telah dianalisis untuk COD, kepekatan NH_4-N dan pepejal yang terampai dengan menggunakan peralatan HACH. Dari eksperimen ini keputusan menunjukkan kadar beban organik untuk mendapat maksimum peratus pengurangan COD ialah 0.4 mg/l/hari dengan 47.05% pengurangan COD dan 124.13 mg/l pepejal terampai. Sementara itu nilai kadar beban organik untuk mendapat maksimum peratus pengurangan kepekatan juga adalah 0.4 mg/l/hari dengan 36.71% pengurangan kepekatan dan 124.13 mg/l pepejal terampai. Untuk maksimum peratus pengurangan COD dan maksimum peratus pengurangan kepekatan, nilai optimum kadar beban organik yang diperlukan adalah 3.59 mg/l/hari dengan 38% pengurangan COD, 24.9% pengurangan kepekatan dan 208.49 mg/l pepejal terampai. Kesemua keputusan ini adalah berdasarkan Design of Expert. Sebagai kesimpulan, kajian ini menunjukkan mikroflora didalam longkang adalah efisien dalam menyingkirkan air sisa yang mengandungi ammonium-nitrogen.

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^oC	degree Celsius
^oF	degree Fahrenheit
pH	potential hydrogen
K_0	constant for oxygen
mg	miligram
%	percentage
cm	centimeter
g	gram
L	liter

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CHAPTER 1

INTRODUCTION

1.1 Introduction

The ammonium ion, NH_4^+, is an important member of the group of nitrogen-containing compounds that act as nutrients for aquatic plants and algae. In surface water, most of the ammonia, NH_3, is found in the form of the ammonium ion, NH_4^+. This fact allows us to approximate the concentration of all of the nitrogen in the form of ammonia and ammonium combined, commonly called ammonia nitrogen, by measuring only the concentration of the ammonium ions. Current and impeding legislation for wastewater effluent discharge has necessitated enhanced treatment processes capable of removing higher percentages of chemical oxygen demand (COD), nitrogen, phosphorous, and suspended solids (including pathogenic bacteria, even viruses) (Verstrete, 2002). Nitrogen removal from wastewater has been subject of many studies during the last decade due to increasingly stringent environmental legislation in many countries. The uncontrolled release of nitrogen to the environment is known to cause serious pollution problems. Nitrate pollution in surface water and in groundwater has been attributed to wastewater outfalls and agriculture runoff. High nitrate levels in water can cause infant methaemoglobinaemia. Many rivers now contain more than 10 mg/l NO_3—N and some occasionally exceed 50 mg/l NO_3—N (Horne, 1995). Nitrogen as well as phosphorous plays a major role in eutrophication (Elser *et al*., 1990). Nitrogen in wastewater should be handled as a nutrient resource rather than a pollutant that only has to dispose off (Gijzen and Mulder, 2001). One of the ways to remove nitrogen is by using bioremediation technique, where the nitrogen is used as nutrient to the microorganisms. The approach that has been exploited most consists of stimulation of the soil endogenous microflora by adding an electron acceptor and/or nutriments; in particular nitrogen in the form of ammonium salts (Jamal and Micheal, 1999). This nitrogen source is exploited mainly by microbial biomass for growth and production of degradative enzymes. Some of the ammonium may be transformed into nitrite and nitrate by the nitrification pathway (Prosser, 1989). Ammonium is one of the most important nitrogen compounds in surface waters and other ecosystems for three reasons. First is the preferred nutrient form of nitrogen for most plant species and for autotrophic bacteria; second it is chemically reduced and can therefore be readily oxidized in natural water, resulting in the consumption and decrease of dissolved oxygen; and third is non ionized ammonia is toxic to many forms of aquatic life already at low concentrations more than 0.2 mg/L (Paredes *et al*., 2007). Under aerobic conditions, ammonium is oxidized by microorganisms to nitrate, with nitrite as an intermediate product. Two different groups of bacteria play a role in the nitrification step: ammonium oxidizers and nitrite oxidizers. In the oxidation of ammonia, nitrite is formed as an intermediate product. It has been considered that it can rarely be accumulated in terrestrial and aquatic environments (Paredes *et al*., 2007).

1.2 Problem statement

Nowadays, current mainstream technologies for wastewater treatment, such as the activated sludge process with nitrogen and phosphorous removal, are too costly to provide a satisfactory solution for the growing wastewater problems in developing regions. The treatment of wastewater should be geared towards the effective re-use of nutrients it self (Gijzen and Mulder, 2001). If this nutrients (containing nitrogen in the form of ammonium cation) release to the environment is uncontrolled, it will contribute to the serious pollutions problem and also affect the human health. Some of the disadvantages of ammonium-nitrogen are it is toxic, it can produce eutrophication of surface waters, cause of corrosion and cause of methemoglobinemia in infants (Bitton, 2005). Biological treatment is an environmentally sound approach to reduce nitrogen and phosphorus levels and has been applied for almost 50 years in special reactors termed high-rate ponds (Jing *et al*., 2006). The study shows that there are obvious advantages of eliminating ammonium from wastewater using biological treatment. First, it does not generate secondary pollution by generation of ammonia (NH_3) and second the biomass can be harvested and used as a slow-release fertilizer or soil conditioner (De la Noue *et al*., 1992; Mallick, 2002). Besides the treatment is environmentally approach it is also give low cost of operating.

1.3 Objectives of the Study

The objectives of the study are:

(i) To study the degradation of ammonium-nitrogen by microflora in drain.

(ii) To study the effect of ammonium-nitrogen concentration in the growth of microflora.

(iii) To study the effect of different loading rate in ammonium-nitrogen removal.

1.4 Scope of the study

The scope of study includes the acclimatizing of microflora in drain to treat (degrade) ammonium-nitrogen (NH_4-N) in wastewater. The wastewater that contains NH_4-N was simulated with the appropriate nutrients for microflora. The initial simulated wastewater was analyzed. Then, experiments were conducted separately in reactor with different operation loading rate (0.4, 1.3, 2.2, 3.1, and 4.0 mg/l/day). The amount of NH_4-N in each tank was 5 liters. The efficiency of treatment for different NH_4-N concentrations was evaluated in terms of water quality parameters (COD) and the changes in concentration. Besides that, the suspended solids parameter is used to measure the quality of the effluent.

CHAPTER 2

LITERATURE REVIEW

2.1 Ammonium-nitrogen

One member from the group of nitrogen-containing compounds that act as important nutrients for aquatic plants and algae is ammonium ion, NH_4^+. However, if ammonium nitrogen levels in surface waters are too high, they can be toxic to some aquatic organisms. If the levels are only moderately high, plant and algal growth will usually increase due to the abundance of nitrogen available as a nutrient (Bitto and Morel, 1998). This will have a ripple effect on other attributes of water quality, such as increasing biochemical oxygen demand and lowering dissolved oxygen levels. Dissolved oxygen levels can also be lowered when ammonium nitrogen is high due to the increased amount of nitrification occurring nutrient (Bitto and Morel, 1998).

Bacteria can convert the nitrogen in decaying plant and animal matter and waste products in the soil or water to ammonium in a process called ammonification (Bitton, 2005). Other sources of organic matter for ammonification include industrial waste, agricultural runoff, and sewage treatment effluent. Some trees and grasses are able to absorb ammonium ions directly, but most require their conversion to nitrate. Animals require nitrogen as well. They obtain the nitrogen they need by eating plants or by eating other animals, which in turn have eaten plants.

Ammonium-nitrogen levels are usually quite low in moving surface waters. This is because there is little decaying organic matter collecting on the bottom. If there is a high level of ammonium nitrogen in a moving stream, it may be an indication of pollution of some kind entering the water. Ponds and swamps usually have a higher ammonium nitrogen level than fast-flowing water. While levels of ammonium nitrogen in drinking water should not exceed 0.5 mg/L, streams or ponds near heavily fertilized fields may have higher concentrations of this ion. Fertilizers containing ammonium sulfate, $(NH_4)^2SO_4$, or ammonium nitrate, NH_4NO_3, may result in runoff from fields containing a high level of ammonium ions (Bitton, 2005).

2.2 Eutrophication

Eutrophication occurs when there is such an abundance of nutrients available that there is a significant increase in plant and algal growth. As these organisms die, they will accumulate on the bottom and decompose, releasing more nutrients and compounding the problem. In some cases, this process of eutrophication can become so advanced that the body of water may become a marsh, and eventually fill in completely. If too little ammonium nitrogen is present, it may be the limiting factor in the amount of plant and algal growth. Ammonium nitrogen can quickly be converted into nitrites or nitrates; therefore, a low level of ammonium-nitrogen does not necessarily indicate a low level of nitrogen in general (Bitton, 2005).

In aquatic environments, enhanced growth of choking aquatic vegetation or phytoplankton (that is, an algal bloom) disrupts normal functioning of the ecosystem, causing a variety of problems. Human society is impacted as well: eutrophication decreases the resource value of rivers, lakes, and estuaries such that recreation, fishing, hunting, and aesthetic enjoyment are hindered. Health-related problems can occur where eutrophic conditions interfere with drinking water treatment (Bartram *et al*., 1999).

Although traditionally thought of as enrichment of aquatic systems by addition of fertilizers into lakes, bays, or other semi-enclosed waters (even slow-moving rivers), terrestrial ecosystems are subject to similarly adverse impacts (Rodhe, 1969). Increased content of nitrates in soil frequently leads to undesirable changes in vegetation composition and many plant species are endangered as a result of eutrophication in terrestric ecosystems.

2.3 Nitrification

Microbial activity is responsible for the two steps of nitrification. Nitrosomonas (obligate autotrophic bacteria) convert ammonium to nitrite (Bitton, 2005). Nitrification inhibitors, such as nitrapyrin or dicyandiamide interfere with the function of these bacteria, blocking ammonium conversion to leachable nitrate. The second step of nitrification occurs through Nitrobacter species, which convert nitrite to nitrate. This step rapidly follows ammonium conversion to nitrite, and consequently nitrite concentrations are normally low in soils (Forcht and Verstraete, 1977).

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+$$

$$2NO_2^- + O_2 \rightarrow 2NO_{3^-}$$

Mineralization and nitrification are influenced by environmental factors that affect biological activity such as temperature, moisture, aeration and pH (Forcht and Verstraete, 1977). Nitrification, for example, occurs very slowly at cold temperatures and ceases once the temperature declines below freezing (Figure 2.1). The rate increases with increasing temperature until the point at which bacterial viability is reduced, (around 95^o F to 100^o F) and then nitrification begins to decline with increasing temperature. Moisture is necessary for microbial function in both the mineralization and nitrification processes. Excessive moisture limits oxygen availability, reducing mineralization and nitrification rates, which, perhaps, lead to anaerobic conditions in the soil. Rates of mineralization and nitrification proceed most rapidly at pH levels near 7, and decline as soils become either excessively acid or alkaline.

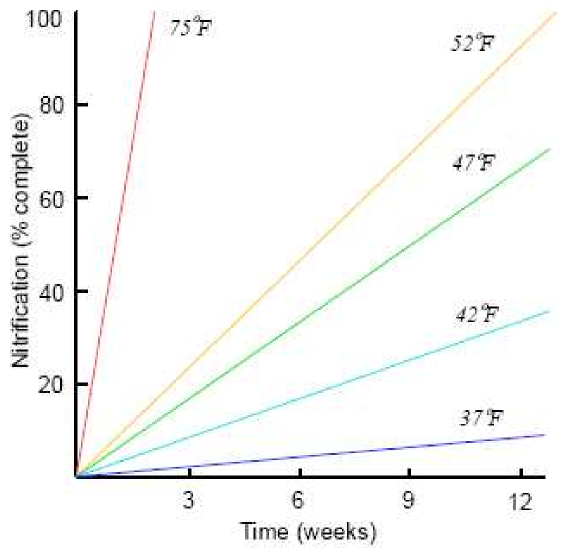

Figure 2.1: Reductions in nitrification based on temperature.

2.4 Factors Controlling Nitrification

Nitrification is the conversion of ammonium to nitrate by microbial action. This process is carried out by two categories of microorganisms, in two stages: conversion of ammonia to nitrite and conversion of nitrite to nitrate. There is several factors control the process which is:

1 Oxygen level – Dissolved oxygen (DO) concentration remains one of the most important factors controlling nitrification. The half-saturation constant for oxygen (K_0) is 1.3mg/L (Metcalf and Eddy, 1991). For nitrification to proceed the oxygen should be well disturbed in the aeration tank of an activated sludge system and its level should not be less than 2mg/L.

$$NH_3 + 2O_2 \rightarrow NO_3^- + H^+ + H_2O$$

To oxidize 1mg of ammonia, 4.6mg of O_2 are needed. Pure culture studies have demonstrated the possible growth of Nitrobacter in the absence of dissolved oxygen, with NO_3 used as electron acceptor and organic substances as the source of carbon. Furthermore, nitrifiers may behave as microaerophiles in aquatic environments such as the sediment – water interface. The nitrate produced helps support denitrification in subsurface sediments (Bitton, 2005).

2 Temperature – The growth rate of nitrifiers is affected by temperature in the range 8-30^{o}C. The optimum temperature has been reported to be in the range 25-30^{o}C (Bitton, 2005).

3 pH – Biological treatment of wastewater occurs generally at neutral pH. In general, the optimum pH for bacterial growth is around 7. However, the optimum pH for Nitrosomonas and Nitrobacter lies between 7.5 to 8.5 (EPA, 1975). Nitrification ceases at or below pH 6.0. Alkalinity is destroyed as a result of ammonia oxidation by nitrifier. Theoretically, nitrification destroys alkalinity as $CaCO_3$, in amounts of 7.14mg/1mg of NH_4^+ - N oxidized. Therefore, there should be sufficient alkalinity in wastewater to balance the acidity produced by nitrification. The pH drop that results from nitrification can be minimized by aerating the wastewater to remove CO_2. Lime is sometimes added to increase wastewater alkalinity.

4 BOD_5/TKN ratio – The fraction of nitrifying organisms decreases as the BOD_5/TKN ratio increase. In combined carbon oxidation – nitrification processes this ratio is greater than 5, whereas in separate stage nitrification processes, the ration is lower than 3 (Metcalf and Eddy, 1991)

5 Toxic inhibition – Nitrifiers are subject to product and substrate inhibition and are also quite sensitive to several toxic compounds found in wastewater. It appears that many of those compounds are more toxic to Nitrosomonas than to Nitrobacter. Organic matter in wastewater is not directly toxic to nitrifiers. Apparent inhibition by organic matter may be due to O_2 depletion by heterotrophs (Bitto and Morel, 1998). The most toxic compounds to nitrifiers are cyanide, thiourea, phenol, anilines and heavy metal.

2.5 Denitrification

Denitrification—the conversion of nitrate to various gaseous forms of nitrogen which can be lost to the atmosphere (nitric oxide, nitrous oxide, dinitrogen)—occurs under oxygen-limiting conditions when anaerobic bacteria use nitrate in respiration in the presence of a carbon source such as organic matter. Low areas of fields that are subject to ponded water for sustained periods during the irrigation season often exhibit nitrogen deficiencies related to denitrification losses.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$$

Denitrification losses from saturated soil will vary with temperature and the amount of carbon (organic matter) available (Sias *et al.*, 1980). Table 2.1 illustrates the effect that time and temperature can have on potential nitrogen losses from denitrification.

Table 2.1: Denitrification rates from saturated soil*

Time	Temperature	N loss
Days	Degrees F	Percent
5	55 – 60	10
10	55 – 60	25
3	75 - 80	60

*Denitrification loss will be less with soils having less than 1% organic matter

2.6 Disadvantages of ammonium-nitrogen

Wastewater treatment plants may discharge effluents with high ammonium or nitrate concentrations into receiving aquatic environments. This may lead to several environmental problems, which are summarized as follows:

2.6.1 Toxicity

Un-ionized ammonia is toxic to fish. At neutral pH, 99% of the ammonia occurs as NH_4^+, whereas NH_3 concentration increases at pH greater than 9. Therefore, ammonia toxicity is particularly important after discharge of alkaline wastewaters or rapid algal photosynthesis, which leads to high pH (Bitton, 2005).

2.6.2 Oxygen depletion in receiving waters

Ammonia may results in oxygen demand in receiving waters (recall 1 mg ammonia exerts an oxygen demand of 4.6mg O_2). The oxygen demand exerted by nitrifiers is called nitrogenous oxygen demand (NOD). Oxygen depletion adversely affects aquatic life (Bitton, 2005).

2.6.3 Eutrophication of surface waters

Discharge of nitrogen into receiving water may stimulate algal and aquatic plant growth. These, in turn, exert a high oxygen demand at night time, which adversely affects fish and other aquatic life and has a negative impact on the beneficial use of water resources for drinking or recreation. Nitrogen and phosphorus often are limiting nutrients in aquatic environments. Algal assay procedures help determine which of these two nutrients is the limiting one (Bitton, 2005).

2.6.4 Corrosion

Ammonia at concentration exceeding 1 mg/L may cause corrosion of copper pipes (Bitton, 2005).

2.6.5 Public health aspects of nitrogen discharge

Nitrate may be the cause of methemoglobinemia in infants and certain susceptible segments of the adult population and it can lead to the formation of carcinogenic compound. Methemoglobinemia is due to the conversion of nitrate to nitrite by nitrate-reducing bacteria in the gastrointestinal tract. Hemoglobin is converted to brown pigment, methemoglobin, after oxidation by nitrite of Fe^{2+} in hemoglobin to Fe^{3+} (Bitton, 2005).

Since methemoglobin is incapable of binding molecular oxygen, the ultimate result is suffocation. Babies are more susceptible to methemoglobinemia because the higher pH in their stomach allows a higher reduction of nitrate to nitrite by nitrate-reducing bacteria. Vitamin C offers a protective effect and helps maintain lower levels of methemoglobin. An enzyme, methemoglobin reductase, keeps methemoglobin at 1-2% of the total hemoglobin in healthy adults (Bitton, 2005).

2.7 Process description of sequence batch reactor

The term sequence batch reactor stems from the sequence of steps that the reactor goes through as it receives wastewater, treats it and discharges it, since all steps are accomplished in a single tank/reactor. The typical sequence is illustrated in Figure 2. The cycle starts with the fill period in which the wastewater enters the bioreactor. The length of the fill period is chosen by the designer and depends upon a variety factors, including the nature of the facility and the treatment objectives. The main effect of the fill period is to determine the hydraulic characteristics of the bioreactor. If the fill period is short, the process will be characterized by a high instantaneous process loading factor, thereby making the system analogous to a continuous system with a tanks-in-series configuration. In that case, the biomass will be exposed initially to high concentration of organic matter and other wastewater constituents, but the concentrations will drop over time. Conversely, if the fill period is long, the instantaneous process loading factor will be small and the system will be similar to a completely mixed continuous flow system in its performance. This means that the biomass will experience only low and relatively constant concentration of the wastewater constituents (Bitton, 2005).

The fill period is followed by the react period in which the biomass is allowed to act upon the wastewater constituents. Actually, reactions for example biomass growth and substrate utilization also occur during the fill period, so the fill period should really be though of as ‘fill plus react’ with react continuing after fill has ended. Since a certain total react period will be required to achieve the process objectives, if the fill period is short, the separate react period will be long, whereas if the fill period is long, the separate react period will be short to nonexistent. The two periods are usually separately specified because of the impact that each has on the performance of the system (Bitton, 2005).

After the react period has been completed, all mixing and aeration are stopped and the biomass is allowed to settle. Just as in continuous processes, this accomplishes two things. It produces a clear effluent suitable for discharge and it retains biomass for solid retention time control. Solid wastage may be accomplished at the end of the settle period, simulating the conventional wastage strategy the for continuous flow system (Bitton, 2005).

After sufficient settling has occurred, treated effluent may be removed by decantation during the draw period. The amount of liquid and biomass retained in the bioreactor constitutes the biomass recycle for the next cycle. Finally, an idle period is generally allowed in each cycle to provide flexibility. This is particularly important for a system with several sequence batch reactor because it allows their operation to be synchronized for maximum effectiveness. The beginning of the new fill period terminates the idle period and initiates a new cycle (Bitton, 2005).

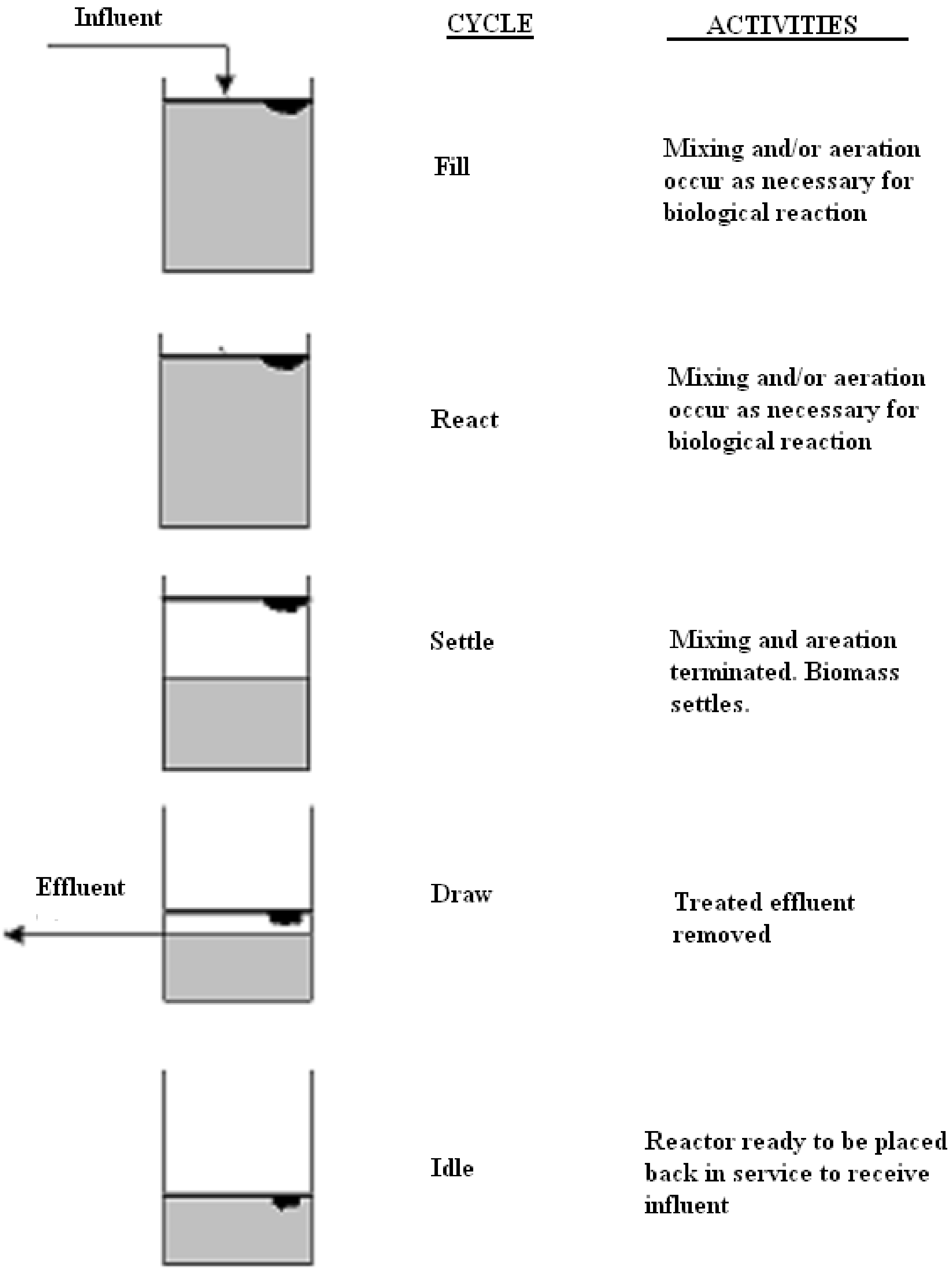

Figure 2.2: The sequence of events in a sequencing batch reactor

2.8 Microorganism and its nutrient

Microorganisms are major important in industrial wastewater treatment, agricultural and aquaculture. They reside in the sediment and other substrates, and in the water of aquaculture facilities, as well as in and on the cultured species. Microorganisms may have positive or negative effects on the outcome of aquaculture operations. Positive microbial activities include elimination of toxic materials such as ammonia, nitrite, and hydrogen sulfide, degradation of uneaten feed, and nutrition of aquatic animals such as shrimp, fish; production of aqua-farmer. These and other functions make microorganisms' key players in the health and sustainability of aquaculture. Yet, microorganisms are among the least known and understood elements in aquaculture. Like other areas in aquaculture, microorganisms require management and manipulation.

The world of microorganisms is made of bacteria, fungi, algae, protozoa, and viruses. They are group together only because of their small size, and not by their function (Organic Aquaculture). The microorganisms such as fungus, bacteria and algae are believed can adsorb the heavy metal or inorganic compounds that exist in drain. One of the microorganisms that synthesize the nutrients and obtain the energy from inorganic raw materials is autotrophic (Michael and John, 2006). The major nutrients for growth required by autotrophic microorganisms are nitrogen and phosphorus. These nutrients are dilute in the aquatic medium relative to major cations and anions; autotrophic microorganisms therefore must be able to assimilate these and other required solutes effectively at low concentrations. Microorganisms as well as multicellular organisms are also sensitive to toxicants at low concentrations and can release organic constituents to the medium that have specific functions (e.g., vitamins).

Typically, algae are autotrophic (derive cell carbon from inorganic carbon dioxide), photosynthetic (derive energy for cell synthesis from light), and contain chlorophyll. They are also chemotrophic in terms of nighttime respiration, e.g., metabolism of molecular oxygen (O_2). Algae utilize photosynthesis (solar energy) to convert simple inorganic nutrients into more complex organic molecules. Ammonium is then oxidized (combined with oxygen) by specialized bacteria to form nitrites (NO_2^-)

and nitrates (NO_3^-). Conversion to nitrate occurs more rapidly at higher water temperatures. Nitrate is usually the most prevalent form of nitrogen in lakes. Both NO_3^- and NH_4^+ can be used by most aquatic plants and algae (http://www.umass.edu/tei/mwwp/factsheets.html).

2.9 Microbial Growth

There are a number of factors that affect the survival and growth of microorganisms. The parameters that are inherent to the environment include the following (Bitton, 2005):

- nutrient content
- moisture content
- pH
- available oxygen

Nutrient Requirements: While the nutrient requirements are quite organism specific, the microorganisms of importance require the following:

- water
- energy source
- carbon/nitrogen source
- vitamins
- minerals

Moisture Content: All microorganisms require water but the amount necessary for growth varies between species. Each microorganism has a maximum, optimum, and minimum amount of water for growth and survival.

pH: Most microorganisms have approximately a neutral pH optimum (pH 6-7.5). Yeasts are able to grow in a more acid environment compared to bacteria. Moulds can grow over a wide pH range but prefer only slightly acid conditions.

Available Oxygen: Microorganisms can be classified according to their oxygen requirements necessary for growth and survival:

- Obligate Aerobes: oxygen required
- Facultative: grow in the presence or absence of oxygen
- Microaerophilic: grow best at very low levels of oxygen
- Aerotolerant Anaerobes: oxygen not required for growth but not harmful if present
- Obligate Anaerobes: grow only in complete absence of oxygen; if present it can be lethal.

CHAPTER 3

METHODOLOGY

3.1 Set-up of sequence batch reactor for ammonium-nitrogen treatment.

The equipment that use in this set-up is four reactor which contains one acclimatization reactor which is 10L and three treatment reactors 5L, gauze wire and smooth stone (the diameter of it approximately 1-2cm). The function of the acclimatization reactor is as the reactor for acclimatizing the microflora. The sample of wastewater that contains the microflora was taken from the canteen drain. This microflora was acclimatized in the main reactor by supplying the glucose as the nutrient. The smooth stone was place in the reactors as the medium. Another three reactors were operated with different loading rate. Table 3.1 shows the various value of loading rate that have been used in this experiment. The value of hydraulic retention time (HRT) that was used in this experiment is 5 days. The wastewater was simulated by using ammonium chloride (NH_4-Cl). The concentration of simulated wastewater can be calculated by using equation 3.1. For initial set up all the reactors were filled with 1.5L of microflora. The duration for each loading rate is two weeks. The experiments were conducted under natural environmental condition with open space.

$$Organic\ Loading\ Rate\ (OLR) = \frac{Concentration}{Hydraulic\ retention\ time\ (HRT)} \qquad 3.1$$

Table 3.1: Value of loading rate

No.	Loading rate (mg/day)	Concentration (mg/l)
1	2.20	11.00
2	2.20	11.00
3	1.30	6.50
4	4.00	20.00
5	0.40	2.00
6	4.00	20.00
7	0.40	2.00
8	3.10	15.50

3.2 Preparation of simulated wastewater

In this experiment, ammonium-chloride (NH_4Cl) was used as the chemical to produce ammonium ions (NH_4^+). The concentration of stock solution that has been used is 1000mg/l of NH_4Cl. This stock solution will dilute to get low standard solution and it can use for almost five weeks.

3.3 Experimental analysis

In this study, the experimental analysis involved three parameters which are analysis of ammonium-nitrogen (NH_4-N), Chemical oxygen demand (COD) test and measurement of suspended solids.

3.3.1 Analysis of ammonium-nitrogen (NH_4-N)

The wastewater was sampled in each reactor by using grab sampling. Sampling was done twice per week (for difference concentration and loading rate). The parameters for NH_4-N analysis were listed as follow:

a) Chemical oxygen demand (COD)

COD test was conducted according to the standard method by using COD reactor HACH model (DBR200 Reactor) and spectrophotometer HACH DR 24000 model as shown in figure 3.1

(a) (b)

Figure 3.1: Hach equipment. (a) COD reactor HACH model (DBR200 Reactor). (b) spectrophotometer HACH DR 24000 model.

b) Ammonium-nitrogen (NH_4-N)

NH_4-N analysis was conducted according to the standard Salicylate method by using spectrophotometer HACH DR 2400 model.

3.3.2 Measurement of suspended solids

The simple method was used to measure the suspended solid by using spectrophotometer HACH DR 2400 model.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Introduction

In order to give a clear understanding of the study work, the results and the respective discussion are grouped and arranged accordingly in the following manner: the first part of this chapter presents on the growth of the microflora in the reactor by analyze the biomass (suspended solid). Next, the discussion focused on the reduction of the ammonium-nitrogen concentration and reduction of chemical oxygen demand (COD) in different loading rate. The expected result from the experiment is ammonium-nitrogen can be degrading from the wastewater by the microflora in drain. The degradation can be determined if there are reduction in concentration of ammonium-nitrogen between the influent and effluent. It is believed that the microorganisms such as fungus, bacteria and algae can adsorb the heavy metal or inorganic compounds that exist in drain. Autotrophic is a one of the microorganisms that can synthesize the nutrients (such nitrogen and phosphorus) and obtain the energy from inorganic raw materials. This microorganism can use their ability to degrade the ammonium-nitrogen and indirectly it applies biological treatment (www.bio-medicine.org/biology).

4.2 The growth of microflora

The growth of microflora was determined by analyze the suspended solid (biomass) in the reactor. In this experiment the suspended solid was measured in concentration value, mg/l. The increasing value of suspended solid per week shows that the microflora is growth. Table 4.1 and figure 4.1 indicate that the growth of microflora in the main reactor.

Table 4.1: Value of suspended solid in the main reactor

week	biomass (mg/l)
1	45
2	33
3	107
4	68
5	61
6	80
7	55
8	690
9	591.335
10	579
11	634
12	700
13	660.67
14	774.67
15	648.33
16	593.33
17	575

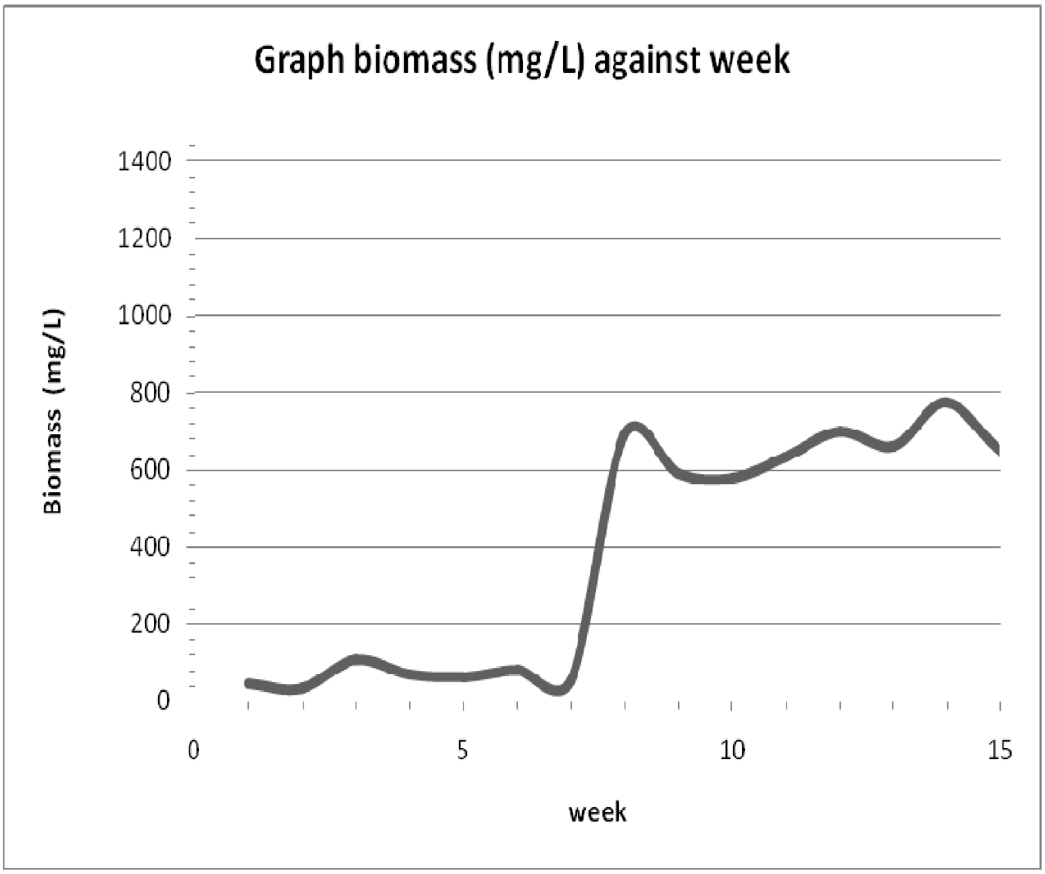

Figure 4.1 Graph biomass against week for main reactor

From figure 4.1, the graph shows that the microflora is growth in the main reactor. At week eight the concentration of biomass achieve the highest peak. The growth cycle of microflora including lag phase, exponential phase, stationary phase and death phase. According to Michael and John (2006) the growth of microflora is influenced by environmental conditions (temperature, compositon of the culture medium) as well as genetic characteristics of the microflora itself. For the growth of the microflora in the experiment reactor, it was illustrated by the graph 4.2 to 4.4.

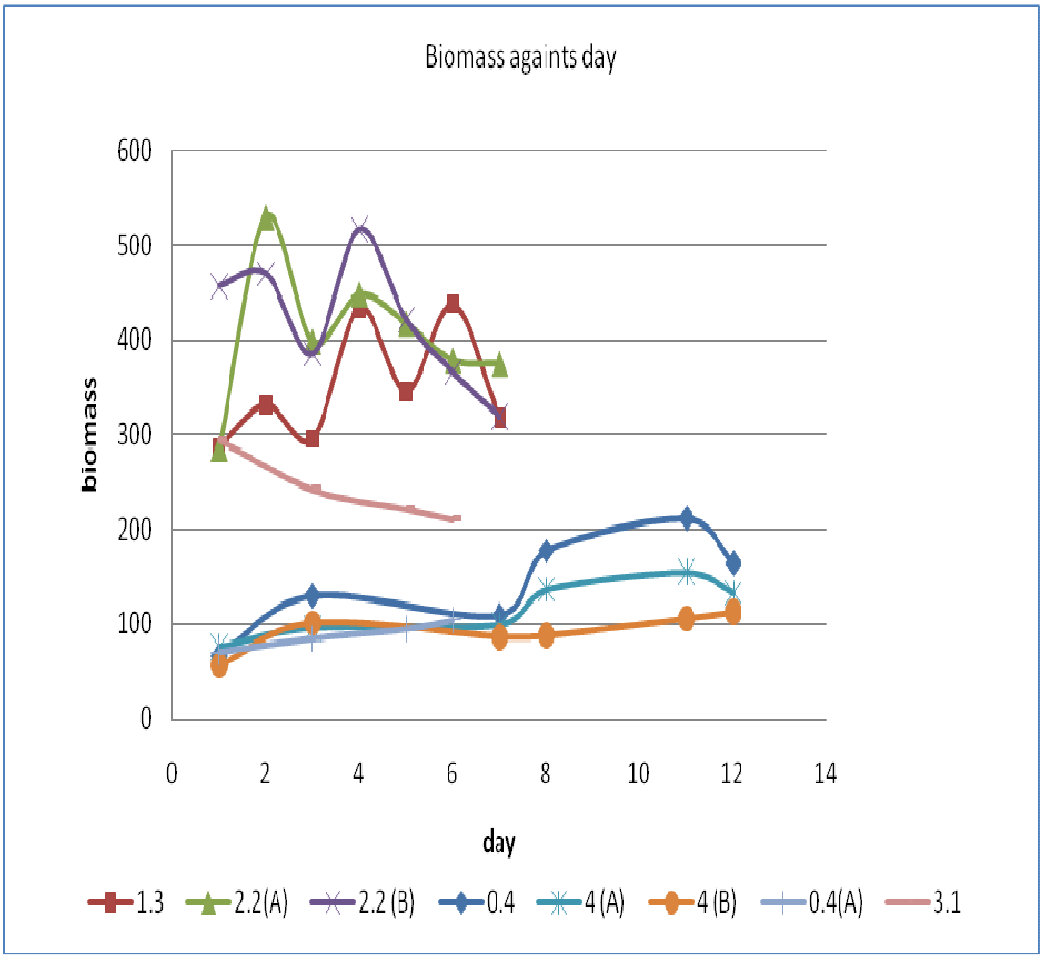

Figure 4.2 Graph biomass against day

From the Figure 4.2 it shows that the growth of microflora is rapidly increase in the experiment reactor that have organic loading rate (OLR) 1.3 mg/l/day and 2.2 mg/l/day. For the experiment reactors that have OLR 0.4 mg/l/day, 3.1 mg/l/day and 4.0 mg/l/day the growth of microflora is too slow. Base on the design expert result, for maximum percentage COD reduction and maximum percentage concentration reduction the optimum value OLR that need is 3.59 mg/l/day with 38% COD reduction, 24.9% concentration reduction and 208.49 mg/l suspended solid. These results may be affected by a variety of factors such as inhibiting substances that exist in the simulated waste water stock solution, temperature around the experiment reactor and also solids retention times and operating loading rate itself. According to Makaya et al., (2007) a variety of substances could be present in the wastewater may affect the growth of bacterial species.

This inhibiting substance will inhibit from achieving high ammonium-nitrogen conversion to nitrate and nitrite. Besides that, the surrounding temperature is also effect the biochemical reactions of the effluent treatment system. Changes in the temperature can also be brought by weather condition. As temperature effects nitrification it also has a direct relationship with the growth of microflora. For solids retention times and operating loading rate, its can affect both nitrification and denitrification. However, depending upon how an effluent treatment system is designed.

4.3 The reduction of the ammonium-nitrogen concentration

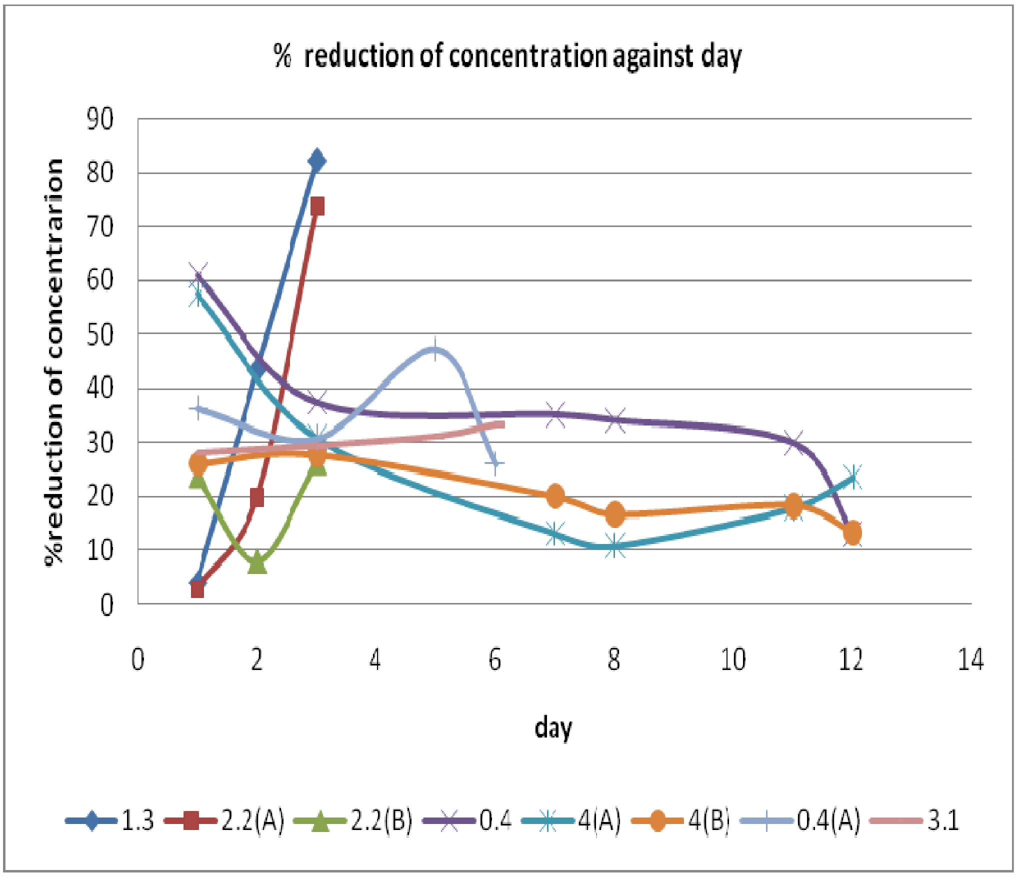

Figure 4.3 Graph percentage reduction of concentration against day

Figure 4.3 describe that the reduction of ammonium-nitrogen concentration at each OLR. From this result it shows that the reduction of ammonium-nitrogen concentration for OLR 1.3 mg/l.day and 2.2 mg/l.day have increase until it achived 82% and 74% reduction. At both OLR (1.3 and 2.2 mg/l.day) it achieve higher percentage of concentration reduction compared to the others operating loading rate (0.4, 3.1 and 4.0 mg/l/day). This result is parallel with the growth of microflora as discuss in section 4.2. Meanwhile from design expert result the value of OLR for the maximum percentage concentration reduction can be achieved at 0.4 mg/l/day with 36.71% concentration reduction and 124.13 mg/l suspended solid. The degradation of ammonium-nitrogen indicates that the microflora is degrade the wastewater and lead to its growth. According to Zheng *et al* (2004) microflora could be divided into two different groups which are slow-growth and fast growth pattern. One group of ammonia oxidation bacteria (fast-growth pattern) has higher affinity for substrate ammonia (lower half-saturation coefficient for ammonia) and thus can grow under the condition of low substrate concentration with the higher specific growth rate. Oppositely, another has lower affinity for substrate and can only grow under the condition of high substrate concentration. In conventional wastewater treatment process, ammonia concentration generally would be controlled at the lower level to satisfy discharge standard.

4.4 The reduction of chemical oxygen demand (COD)

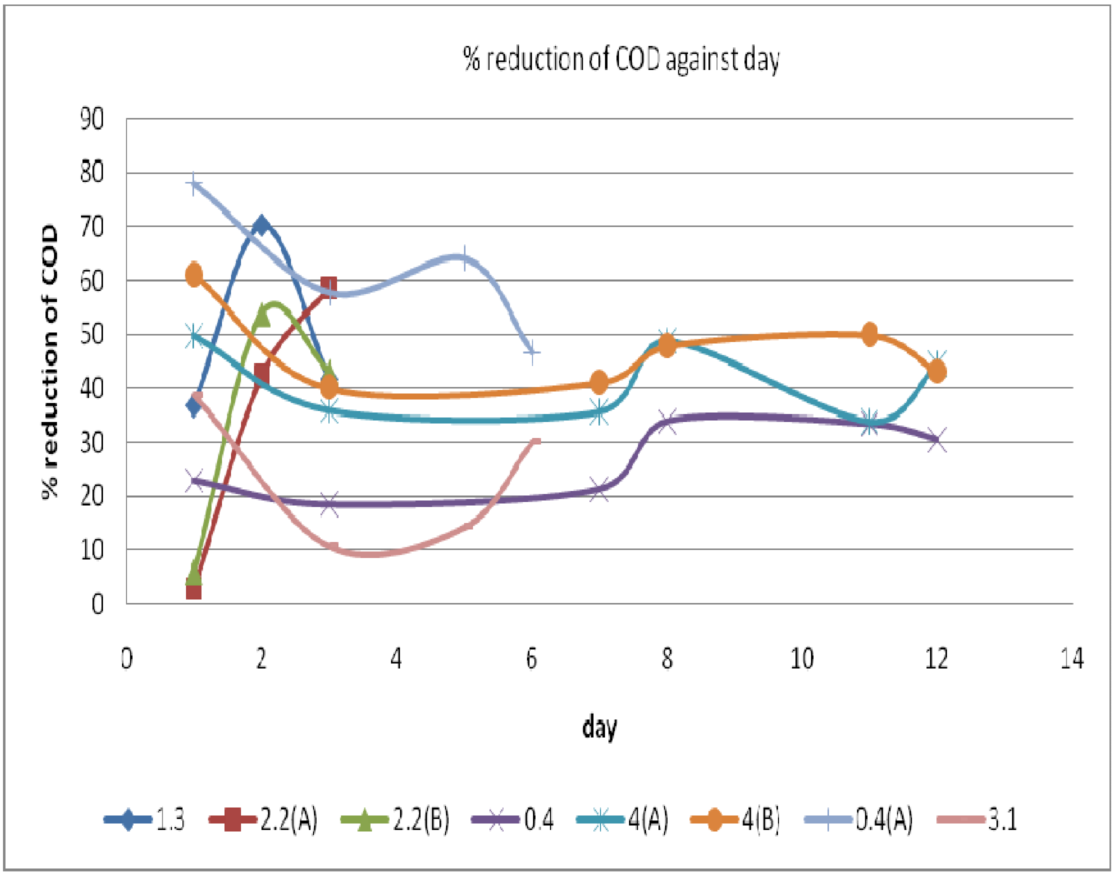

Figure 4.4 Percentage reduction of COD against day

In Figure 4.4 the percentage reduction of COD are represented. From this result it shows that most of the trend during initial duration at each OLR, the COD reduction is decreased. For certain range of time the COD reduction is increase. However at OLR 1.3 and 2.2 mg/l/day the initial COD reduction is rapidly increased compared to the other trend of OLR. The maximum percentage of COD reduction for operating loading rate 0.4,1.3, 2.2, 3.1 and 4.0 mg/l/day are 78%, 70%, 58%, 39% and 61% respectively. Base on design expert the maximum percentage COD reduction is 0.4 mg/l/day with 47.05% COD reduction and 124.13 mg/l suspended solid. This result may be due to the efficiency during analyzed the sample. COD should be analyzed on a routine basis in reactor designed to remove nitrogen (Gujer et al., 1995). In addition, if a plant is designed to remove phosphorus (total phosphorus and orthophosphate) and nitrogen (ammonium, nitrate nitrogen, and nitrite) COD needs to be monitored more frequently in each reactor of the treatment process (Henze et al., 2002).

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusion

Generally, the study was carried out to determine the possibility of using microflora in drain to degrade the wastewater that contains ammonium-nitrogen. The system that used in this study is known as sequencing batch reactor (SBR). Each reactor was operated at different operating loading rate for two weeks. Within two weeks the suspended solid, concentration and COD was analyzed. Based on the design of expert results, it can be conclude that the optimum value for organic loading rate to get maximum percentage COD reduction is 0.4 mg/l/day with 47.05% COD reduction and 124.13 mg/l suspended solid. Meanwhile the value of organic loading rate to get the maximum percentage concentration reduction is also 0.4 mg/l/day with 36.71% concentration reduction and 124.13 mg/l suspended solid. For maximum percentage COD reduction and maximum percentage concentration reduction the optimum value organic loading rate that need is 3.59 mg/l/day with 38% COD reduction, 24.9% concentration reduction and 208.49 mg/l suspended solid. As a conclusion this result is accompanied with previous study. It is shows that, there are obvious advantages of eliminating ammonium-nitrogen from wastewater using biological treatment. First, it does not generate secondary pollution by generation of ammonia (NH_3) and second the biomass can be harvested and used as a slow-release fertilizer or soil conditioner (Jing *et al.*, 2006). Besides the treatment is environmentally approach it is also give low cost of operating. By using this method hopefully it can decrease the serious pollution problems that usually happen in developed region.

5.2 Recommendation

In order to get better result it is need some precaution and suggestion before/during implement the experiment. First suggestion is, the duration for experiment should be done longer than two weeks. This is because at the early duration the micro flora is tried to adapt with the new condition. So, sometimes the reading is not consistence. After the condition of micro flora is stable, we can take the reading for analysis sample. This long duration time is also can give clear profile for micro flora growth. Besides that another precaution that should be taken is, we must place the wastewater stock solution inside the refrigerator. This action can prevent from any reaction happen inside the wastewater stock solution. The others suggestion is we must apply aseptic technique during we take the sample micro flora in drain. This action will prevent the micro flora from contaminate with the other microorganism that contains in the bottle sample that we used. Besides, more extensive studies could be conducted for future research in order to understand more clearly the biological treatment by using micro flora. Below are some recommendations for future research:

(i) Use the micro flora in the other drain (such as lab drain) to treat the wastewater and compare its efficiency of removal the wastewater with micro flora in canteen drain;

(ii) Identify and characterize the micro flora that was used to treat the wastewater;

(iii) Use the single culture of microflora to treat the waste water.

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APPENDIX

A.1: Value of suspended solid for experiment reactor

day	1.3	2.2(A)	2.2 (B)
1	287.6667	285	457.33
3	332	529.33	470.33
4	296	398	385
5	434.67	448	517.67
10	346.3	417	421.6667
11	438.33	378.33	365.67
13	318.33	374.67	318.33
day	0.4	4 (A)	4 (B)
1	67.333	75.33	56.667
3	130	95.5	102
7	109.67	99	87.33
8	177.33	136	88.33
11	211.67	155	106.67
12	164.33	133	113.67
day	0.4(A)	3.1	
1	70.33	294.33	
3	84.67	242.33	
5	94.67	221.67	
6	104	210.67	

A.2: Value of percentage reduction of the ammonium-nitrogen concentration

	1.3	2.2(A)	2.2(B)	0.4	4(A)	4(B)
day	% reduction	% reduction	% reduction	% reduction	% reduction	% reduction
1	-	-	-	61.11	57.69	26.09
2	-	-	-	37.50	30.86	27.78
4	-	-	-	35.29	13.04	20.00
8	4.12	2.84	23.84	34.21	10.71	16.67
9	43.42	19.77	7.95	30.00	17.81	18.37
12	82.19	73.68	26.19	13.04	23.53	13.33

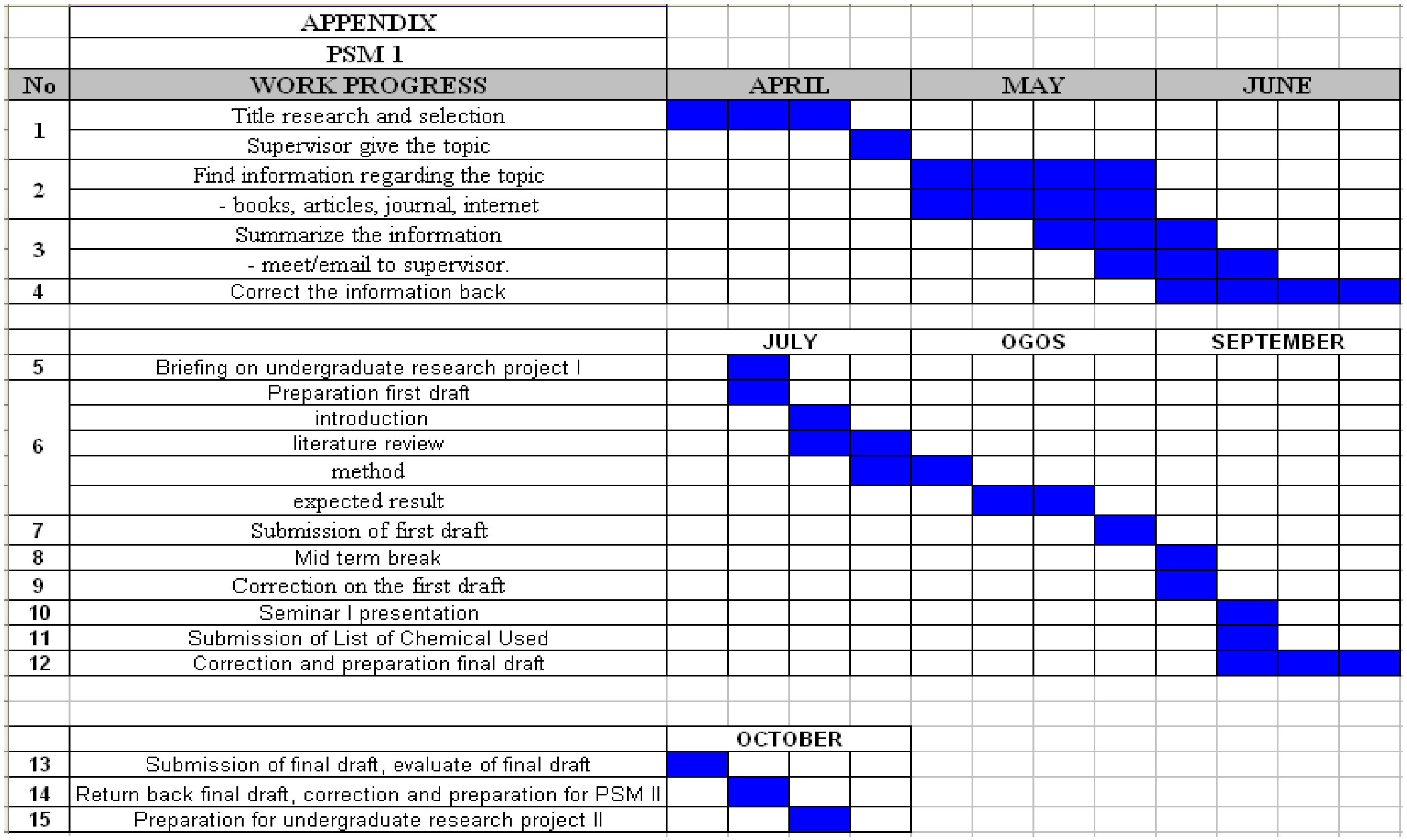
	0.4(A)	3.1
day	% reduction	% reduction
1	36.36	28.21
3	30.77	29.55
5	47.37	31.25
6	26.09	33.33

A.3: Value of percentage reduction of chemical oxygen demand (COD)

	1.3	2.2(A)	2.2(B)	0.4	4(A)	4(B)
day	% reduction	% reduction	% reduction	% reduction	% reduction	% reduction
1	-	-	-	22.82	49.85	61.06
2	-	-	-	18.36	35.83	40.05
4	-	-	-	21.23	35.57	41.09
8	36.97	2.88	5.87	33.83	48.89	47.83
9	70.40	42.37	53.85	33.33	33.73	49.89
12	41.89	58.74	43.02	30.42	44.57	43.00

	0.4(A)	3.1
day	% reduction	% reduction
1	77.941	38.75
3	57.746	10.71
5	64.096	14.33
6	46.715	29.98

A.4: Gantt chart for Undergraduate Research Project 1



A.5: Gantt chart for Undergraduate Research Project 2

	PSM 2	WEEK													
No	**WORK PROGRESS**	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	Take the sample														
2	Growth the microflora														
3	Experiment and data collection														
4	Discussion														
5	Final report														
6	Presentation														

A.6: Result from Design of Expert

Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
loading rate	**is in range**	**0.4**	**4**	**1**	**1**	**3**
COD	**maximize**	**23.444**	**61.6248**	**1**	**1**	**3**
concentration	**is in range**	**19.3271**	**43.2438**	**1**	**1**	**3**
SS	**is in range**	**88.4175**	**419.428**	**1**	**1**	**3**

Solutions

Number	loading rate	COD	concentration	SS	Desirability	
1	0.40	47.052	36.7127	124.128	0.618	Selected
2	4.00	41.3266	23.0728	89.5356	0.468	

Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
loading rate	**is in range**	**0.4**	**4**	**1**	**1**	**3**
COD	**is in range**	**23.444**	**61.6248**	**1**	**1**	**3**
concentration	**maximize**	**19.3271**	**43.2438**	**1**	**1**	**3**
SS	**is in range**	**88.4175**	**419.428**	**1**	**1**	**3**

Solutions

Number	loading rate	COD	concentration	SS	Desirability	
1	0.40	47.052	36.7127	124.128	0.727	Selected

Constraints

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
loading rate	**maximize**	**0.4**	**4**	**1**	**1**	**3**
COD	**maximize**	**23.444**	**61.6248**	**1**	**1**	**3**
concentration	**maximize**	**19.3271**	**43.2438**	**1**	**1**	**3**
SS	**is in range**	**88.4175**	**419.428**	**1**	**1**	**3**

Solutions

Number	loading rate	COD	concentration	SS	Desirability	
1	3.59	38.0062	24.8976	208.49	0.429	Selected

A.7: Method for Ammonium-nitrogen test