NUTRIENT REMOVAL USING BIOFILM REACTOR WITH SUPPORT MEDIA

SHAHRUL AMRI BIN SHAFIE @ SAFIE

UNIVERSITI MALAYSIA PAHANG

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Alamat Tetap **Lot 2048 TBK Kg Chekok** **Pn. Norazwina Binti Zainol**

Panji, 16100 Kota Bharu Nama Penyelia

Kelantan Darul Naim

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NUTRIENT REMOVAL USING BIOFILM REACTOR WITH SUPPORT MEDIA

SHAHRUL AMRI BIN SHAFIE @ SAFIE

Thesis submitted to the Faculty of Chemical and Natural Resources Engineering in
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Faculty of Chemical and Natural Resources Engineering
Universiti Malaysia Pahang

MAY 2009

I declare that this thesis entitled "*Nutrient Removal Using Biofilm Reactor with Support Media"* is the result of my own research except as cited in the 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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Special Dedication of This Grateful Feeling to My…

Beloved father and mother;
Mr. Shafie @ Safie Bin Che Mohd Salleh and Mrs. Fauziah Binti Ibrahim

Loving brothers and sisters;
Nariah, Nor Aieda, Siti Normazura and Sallehudin

Supportive friends

For Their Love, Support and Best Wishes.

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ABSTRACT

The phosphorus removal becomes very important nowadays in order to reduced eutrophication. The aim of this study is to find the suitable loading rate for the highest phosphorus removal efficiency using biofilm reactor. Experiments are carried out in eight sequencing batch reactors (SBRs) at hydraulic retention time (HRT) of 5 d. This experiment is operated at loading rates of 5.0, 4.5, 4.0, 3.5 and 3.0 mg/L.d. The biofilm growth is increased with the increasing of loading rate according to the suspended solids readings. Loading rate 5 mg/L.d shows the highest suspended solid concentration which is 1585 mg/L at average reading. The Phosphorus removal efficiency was increasing according to the increasing of loading rate while the chemical oxygen demand (COD) removal is decreased with the increasing of loading rates. The loading rate 5.0 mg/L.d shows the highest average removal efficiency ranged from 66% to 87%. The COD removal is highest at loading rate 3.5 mg/L.d with 83% of average removal. The removal efficiency was influenced by the biofilm growth according to the suspended solid readings. The highest suspended solids reading give the highest removal efficiency. According to the Design Expert plotted, the highest predicted phosphorus removal can be achieved at loading rate 5.0 mg/L.d with 72.53% of phosphorus removal, 76.14% COD removal and 1142.85 mg/L of suspended solid (SS) concentration. The highest predicted COD removal can be achieved at loading rate (LR) 3.0 mg/L.d with 77% removal. The expected phosphorus removal is 66% and the expected suspended solid (SS) concentration is 984 mg/L. As the conclusion, the mixed culture from soil is capable of degrading phosphorus at which the effective loading rate is 5.0 mg/L.d.

ABSTRAK

Penyingkiran fosforus daripada air sisa menjadi sangat penting pada masa ini dalam mengurangkan masalah eutropikasi. Matlamat kajian ini adalah untuk mencari kadar beban yang sesuai untuk mendapatkan peratusan penyingkiran fosforus yang paling tinggi menggunakan reaktor biofilem. Eksperimen ini di jalankan di dalam lapan reaktor sesekumpul berjujukan pada masa penahanan hidraulik 5 h. Eksperimen ini dijalankan pada kadar beban 5.0, 4.5, 4.0, 3.5 dan 3.0 mg/L.h. Bacaan kepekatan pepejal terampai yang diperolehi menunjukkan peningkatan dalam pertumbuhan biofilem seiring dengan peningkatan kadar beban. Kadar beban 5 mg/L.h menunjukan bacaan purata kepekatan pepejal terampai yang paling tinggi iaitu 1585 mg/L. Kenaikan kadar beban menunjukkan peningkatan kadar penyingkiran fosforus tetapi mengurangkan kadar penyingkiran permintaan oksigen kimia. Kadar beban 5.0 mg/L.h menunjukan purata peratusan penyingkiran yang tertingi iaitu di antara 66% kepada 87%. Peratusan penyingkiran dipengaruhi oleh pertumbuhan biofilem. Bacaan pepejal terampai yang paling tinggi menunjukkan peratusan penyingkiran yang paling tinggi. Berdasarkan plot dari *Design Expert*, penyingkiran fosforus yang paling tinggi diramalkan akan dapat dicapai pada kadar beban 5.0 mg/L.h dengan 72.53% penyingkiran fosforus dan 76.14% penyingkiran permintaan oksigen kimia pada kepekatan pepejal terampai 1142.85 mg/L. Penyingkiran permintaan oksigen kimia yang paling tinggi pula diramalkan akan dapat diperolehi pada kadar beban 3 mg/L.h dengan peratusan penyingkirannya sebanyak 77%. Peratusan penyingkiran fosforus yang diramalkan pada kadar beban ini ialah 66% pada kepekatan pepejal terampai 984 mg/L. Sebagai kesimpulan, kultur campuran daripada tanah berupaya mengurai fosforus di mana kadar beban yang efektif ialah 5.0 mg/L.h.

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LIST OF ABBREVIATIONS

AIDS	-	Acquired immune deficiency syndrome
A/O	-	Anaerobic and oxic
Bio-P	-	Biological phosphorus
BOD	-	Biological oxygen demand
CISTR	-	Continuous Ideally Stirred-Tank Reactor
CSTR	-	Continuous Stirred-tank Reactor
COD	-	Chemical oxygen demand
DIN	-	Dissolved inorganic nitrogen
DIP	-	Dissolved inorganic phosphorus
DNA	-	Deoxyribonucleic acid
DO	-	Dissolved oxygen
EBPR	-	Enhanced biological phosphorus removal
EHS	-	Environmental Health and Safety
HRT	-	Hydraulic retention time
LR	-	Loading rate
N	-	Nitrogen
P	-	Phosphorus
RAS	-	Return activated sludge
rRNA	-	Ribosomal ribonucleic acid
SBR	-	Sequencing batch reactor
SS	-	Suspended solids
TEM	-	Transmission electron microscopy
WCMC	-	Weill Cornell Medical College

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CHAPTER 1

INTRODUCTION

1.1 Background

Water pollutants come from point and non-point sources. Their effects on aquatic systems largely depend on whether polluted waters are standing (lakes and ponds) or flowing (rivers). Standing systems are generally more susceptible because of slow turnover. The major water pollutants are organic nutrients, inorganic nutrients, infectious agents, toxic organics, sediment and heat. Organic nutrients come from feedlots, municipal sewage treatment plants, and industry. They promote growth of natural populations of aquatic bacteria. Bacterial decomposition of organic materials results in declines in dissolved oxygen, with dire effects on other oxygen-requiring organisms.

Two inorganic plant nutrients of major concern are nitrogen and phosphorus. They come primarily from septic tanks, barnyards, heavily fertilized crops, and sewage treatment plants, and cause excessive plant growth that clogs navigable waterways. Bacterial decay of plants in the fall result in a drop in dissolved oxygen, which may suffocate fish and other organisms.

As well known, nowadays eutrophication is one of the main problems nowadays encountered in the monitoring of the environmental water sources in the industrialized countries. It caused by the excess phosphorus concentration in the effluents from municipal or industrial plants discharged in the environment (Lenntech, 1998). Eutrophication of waterways through delivery of phosphorus (P)

and nitrogen (N) from farmland is an increasing problem in many countries (Haygarth and Jarvis, 1999). Eutrophication is the fertilisation of surface water by nutrients that were previously scarce. Water pollution is a major problem in the global context. It has been suggested that it is the leading worldwide cause of deaths and diseases and that it accounts for the deaths of more than 14,000 people daily (Pink and West, 2006).

This research is done to treat phosphorus from wastewater by using microorganism from soil. This study is undertaken to evaluate the effectiveness of microorganism in soil to remove phosphorus. Laboratory scale study will be conducted to determine the effects of different loading rate in the degradation of phosphorus, reduction of chemical oxygen demand (COD) and also the effects to the mixed culture growth in terms of suspended solid that exist during the experiment.

1.2 Problem Statement

Nowadays, world has become aware about the seriousness of one of the major effect of the waste discharged by the industrial activities, that is the quantity and diversity of hazardous waste. This harmful and toxic discharge will affect our aquatic system. So, it is important to control the waste contain to assure that our ecosystem are prevented from damage.

In typical wastewater treatment process, optimum dosing of the appropriate chemicals, such as phosphorus is added into the water. Phosphorus will contribute to the poor surface water quality when discharged it to the river. The excess phosphorus concentration in the effluents from municipal or industrial plants discharged in the environment will cause eutrophication. The increasing of phosphor concentrations in surface waters raises the growth of phosphate-dependent organisms, such as algae (Phytoplankton). These organisms use great amounts of oxygen and prevent sunlight from entering the water. This makes the water fairly unliveable for other organisms (Lenntech, 1998).

This research is proposed because the method that will be use is quite economical since it only uses the microorganism from soil which is easy to get from our surrounding. These microorganisms are assumed can treat the phosphorus in the wastewater.

1.3 Objective of the Study

The main objectives of this research are:

1. To study the degradation of phosphorus by microorganism in soil.
2. To study the effect of different loading rate on phosphorus removal.
3. To study the effect of phosphorus concentration to the growth of mixed culture.

At the end of the study, this objective will help in understanding the used of mixed culture in phosphorus removal process which is the main concern of this study.

1.4 Scope of the Study

The scope of this study is to acclimatize the bacteria in order to treat phosphorus from wastewater and to monitoring the mixed culture growth using suspended solid test. The wastewater that contains of phosphorus in the phosphate form (PO_4^{3-}) was simulated with the appropriate nutrients for mixed culture. The experiments were conducted in biofilm reactor with different loading rate. The efficiency of the treatment for different phosphate concentration is evaluated in terms of water quality parameters; chemical oxygen demand (COD) and the removal of the initial concentration. Besides, the effect of the phosphate concentration on microorganism growth in terms of suspended solids concentration.

CHAPTER 2

LITERATURE REVIEW

2.1 Phosphorus

Phosphorus is the chemical element that has the symbol P and atomic number 15. The name comes from the Greek meaning “light” and “bearer”. A multivalent nonmetal of the nitrogen group, phosphorus is commonly found in inorganic phosphate rocks. Due to its high reactivity, phosphorus is never found as a free element in nature on Earth. One form of phosphorus (white phosphorus) emits a faint glow upon exposure to oxygen, and hence in Greek derivation, meanings "light-bearer" (Latin *Lucifer*), the planet Venus as "Morning Star". Phosphorus is a component of DNA and RNA and an essential element for all living cells. The most important commercial use of phosphorus-based chemicals is the production of fertilizers. Phosphorus compounds are also widely used in explosives, nerve agents, friction matches, fireworks, pesticides, toothpaste, and detergents. Phosphorous is a multivalent nonmetal of the nitrogen group. It is found in nature in several allotropic forms, and is an essential element for the life of organisms. There are several forms of phosphorous, called white, red and black phosphorous. Eventhough their colours are more likely to be slightly different. White phosphorous is the one manufactured industrial; it glows in the dark, is spontaneously flammable when exposed to air and is deadly poisoning. Red phosphorous can vary in colours from orange to purple, due to slight variations in its chemical structure. The third form, black phosphorous, is made under high pressure, looks like graphite and, like graphite, has the ability to conduct electricity (Lenntech, 1998).

2.1.1 Phosphorus Sources

In the natural world, phosphorous is never encountered in its pure form, but only as phosphates, which consists of a phosphorous atom bonded to four oxygen atoms. This can exists as the negatively charged phosphate ion (PO_4^{3-}), which is how it occurs in minerals, or as organophosphates in which there are organic molecules attached to one, two or three of the oxygen atoms (Lenntech, 1998).

Municipal wastewaters may contains from 5 to 20 mg/L of total phosphorous of which 1-5 mg/L is organic and the rest in inorganic. The individual contribution tend to increase, because phosphorous is one of the main constituent of synthetic detergents. The individual phosphorous contribution varies between 0.65 and 4.80 g/inhabitant per day with an average of about 2.18 g. The usual forms of phosphorus found in aqueous solutions include orthophosphate, polyphosphate and organic phosphate (Lenntech, 1998).

Phosphate rock, which is partially made of apatite (an impure tri-calcium phosphate mineral) is an important commercial source of this element. About 50 per cent of the global phosphorus reserves are in the Arab nations. Large deposits of apatite are located in China, Russia, Morocco, Florida, Idaho, Tennessee, Utah, and elsewhere (Albright and Wilson, 1956).

2.1.2 Disadvantage of Phosphorus

As well known, nowadays eutrophication is one of the main problems nowadays encountered in the monitoring of the environmental water sources in the industrialized countries. It caused by the excess phosphorus concentration in the effluents from municipal or industrial plants discharged in the environment (Lenntech, 1998). Eutrophication of waterways through delivery of phosphorus (P) and nitrogen (N) from farmland is an increasing problem in many countries (Haygarth and Jarvis, 1999).

In white phosphorus form, it enters the environment through discharge of wastewater, white phosphorus ends up in surface waters near the factories that use it. White phosphorus is not likely to spread, because it reacts with oxygen fairly quickly. When phosphorus ends up in air through exhausts it will usually react with oxygen right away to be converted into less harmful particles. However, when phosphorus particles are in air they may have a protective coating that prevents chemical reactions. In water, white phosphorus is not reacting with other particles that quickly and as a result it will accumulate in the bodies of aquatic organisms. In soil, phosphorus will remain for several days before it is converted into less harmful substances. But, phosphorus may remain for a thousand years in deep soils, the bottom of rivers and lakes (Lenntech, 1998).

Phosphorus exist in phosphate form in natural world, phosphates have many effects upon organisms. The effects are mainly consequences of emissions of large quantities of phosphate into the environment due to mining and cultivating. During water purification phosphates are often not removed properly, so that they can spread over large distances when found in surface waters. The increasing phosphor concentrations in surface waters raise the growth of phosphate-dependent organisms, such as algae (Phytoplankton). These organisms use great amounts of oxygen and prevent sunlight from entering the water. This makes the water fairly unlivable for other organisms (Lenntech, 1998). The key role of this substance is particularly evident when one realizes that 1g of PO^4-P enables the development of 100g Phytoplankton. For the aerobic breakdown of these biomes, further 150g oxygen is required. In other words, the presence of 1g of PO^4-P in waterways induces a secondary overhead of 150g biochemical oxygen demand (BOD). This phenomenon is commonly known as eutrophication (Uhlmann, 1982).

Forsberg and Ryding, (1980) give the trophic status of waterways with respect to the total phosphorus concentration as; Concentration < 15µg PO^4-P/l are oligitrophic, concentrations of 15-25µg PO^4-P/l is mesotrophic and concentrations of >25µg PO^4-P/l is eutrophic. From these concentration figures, the importance of phosphate removal from wastewater becomes clear.

2.1.3 Level Measurement to Discharge Phosphorus

Several types of wastes (chemical, biological, radioactive, universal, and recyclable) are generated by a variety of laboratory, maintenance, and cleaning operations at the Weill Cornell Medical College (WCMC). Wastes must be properly managed by personnel in their work areas prior to collection for disposal. These Waste Disposal Procedures have been established as part of the WCMC Environmental Health and Safety (EHS) Program Manual to provide generators guidance in the proper management of chemical, biological, radioactive, universal, and recyclable wastes (EHS).

Many countries set 1 mg/L and 2 mg/L as the limit for total phosphorus concentration in discharge of wastewater treatment plants. One of the reasons for this low limit is that P concentrations below 0.5 mg/L have been shown to be the limiting value for Algae growth (Dryden and Stern, 1968).

For Asian country such as Malaysia, the level measurement to discharge phosphorus is 0.2 mg/L; this is based from Department of Environment in national water quality standard in Malaysia.

2.2 Mixed Culture

2.2.1 Microorganism in Sediment

The deep sea and its sediments represent the largest permanently cold environment on Earth. We incubated cultures at low temperatures (2°–10°C) in order to increase the number of psychrophilic microorganisms and to explore their potential enzyme activities in situ. We isolated and characterized organisms that are phylogenetically related to *Photobacterium, Halomonas, Shewanella*, and *Vibrio* species. All isolates are closely related (by >98% 16S rRNA similarity) to previously

isolated deep-sea strains, consistent with their being from the core sample rather than contaminants. These genera are also commonly found in deep sediment studies, especially those in the Pacific (Wang *et al.,* 2004). The trend in extracellular degradative enzyme production agrees with previously published results of deep-sea sediment isolates and corresponds to available nutrient sources, suggesting a possible adaptation to this environment or competitive advantage within this ecosystem (Wang *et al.,* 2004). The one *Vibrio* sp. isolate from the sediment column (0.67 mbsf) adds to the database of other sediment-dwelling microbes isolated from deeper than 0.5 mbsf (Bale *et al.,* 1997). Further, the characterization of these isolates increases the numbers of described species for these genera and provides information on their production of cold-active enzymes of possible industrial interest.

The presence of a number of facultative anaerobic microorganisms in our cultivations suggests that the capability for anaerobic growth may allow cell survival after burial by the accumulation of the sediment column over time. However, because facultative organisms grow more rapidly aerobically, especially at low temperatures, we used aerobic cultivation to examine this population. In such an aerobic enrichment culture, it was surprising to find crenarchaeal signatures. *Crenarchaea* have been shown to be members of the seafloor community and have been detected deeper in sediment columns (Vetriani *et al.,* 1998; Bidle *et al.,* 1999). However, to our knowledge, they have not yet been reported as members of a cultivated community from marine sediment. Here we report the existence of a marine benthic *Crenarchaeon* in a bacteria-dominated enrichment culture at 10°C for more than a week. The conditions needed to prolong the existence of these *Crenarchaea* in liquid culture are being investigated.

The importance of the bacterial population with respect to enhanced biological phosphorus removal (EBPR) has been noted in a number of papers (Carucci *et al.,* 1995). Kavanaugh, (1991) found that bacteria belonging to *Aeromonas*/*Vibrio,* Coliforms, *Pseudomonas* and *Acinetobacter* were present in a continuous flow EBPR system, which was also operated to achieve biological nitrogen removal. In the same study, *Acinetobacter* which is widely reported to be responsible from phosphorus uptake only accounted for 5% of the population. This

finding was supported that it was difficult to find *Acinetobacter* in well-operated fill and draw activated sludge systems (Hascoet, 1985). In a study carried out by Okada *et al*. (1992) however, *Acinetobacter* and *Pseudomonas* were the predominant species and high phosphorus removal efficiencies were obtained. *Pseudomonas* is present in fish and sediments from aquaculture in Australia (Olasumbo**,** 2007**)**.

2.2.2 Microorganism in Soil

The soil represents a favorable habitat for microorganisms and is inhabited by a wide range of microorganisms, including bacteria, fungi, algae, viruses and protozoa. Microorganisms are found in large numbers in soil usually between one and ten million microorganisms are present per gram of soil with bacteria and fungi being the most prevalent. However, the availability of nutrients is often limiting for microbial growth in soil and most soil microorganisms may not be physiologically active in the soil at a given time.

Soil microorganisms are very important as almost every chemical transformation taking place in soil involves active contributions from soil microorganisms. In particular, they play an active role in soil fertility as a result of their involvement in the cycle of nutrients like carbon and nitrogen, which are required for plant growth. For example, soil microorganisms are responsible for the decomposition of the organic matter entering the soil (e.g. plant litter) and therefore in the recycling of nutrients in soil. Certain soil microorganisms such as mycorrhizal fungi can also increase the availability of mineral nutrients (e.g. phosphorus) to plants. Other soil microorganisms can increase the amount of nutrients present in the soil. For instance, nitrogen-fixing bacteria can transform nitrogen gas present in the soil atmosphere into soluble nitrogenous compounds that plant roots can utilize for growth. These microorganisms, which improve the fertility status of the soil and contribute to plant growth, have been termed 'bio-fertilizers' and are receiving increased attention for use as microbial inoculants in agriculture. Similarly, other soil microorganisms have been found to produce compounds (such as vitamins and plant hormones) that can improve plant health and contribute to higher crop yield. These

microorganisms (called 'phytostimulators') are currently studied for possible use as microbial inoculants to improve crop yield (Impact, 1998).

2.2.3 Microorganism in Geyser

The ribbons of color that stream from hot springs are usually formed by a variety of bacteria. The green and orange mats that you see here live in water of varying temperatures; in essence, the colors serve as temperature indicators. Hot springs *cyanobacteria* are wonders of life at high temperatures. Some live in waters as hot as 167° F (76° C). At this temperature they are usually yellow, but become darker - orange, rust or brown - as the water cools. Between 113 °F and 131°F (45°C and 55° C), other species may appear which will modify the colors even more. Certain varieties are scientific curiosities because they are extremely specific for their environment. They may be found around the world living only in hot spring waters. Yellow or pink strands of bacteria sometimes appear in water as hot as 196° F (92° C), just below the boiling point (water boils at 199°F (93° C) at this elevation). Chemical deposits of sulphur, iron oxides, arsenic sulfide, and other substances add vivid colors to the hot springs in a few areas of the park, but not generally in the Midway and Fountain Paint Pots Basins. At Norris Geyser Basin the Echinus Geyser and Emerald Spring release acidic water. Hardy microscopic plants, like lime-green *Cyanidium* algae, thrive in these warm acid waters. *Orangish cyanobacteria* may be found in many runoff streams in Porcelain Basin. From a distance these bacteria look like rusty iron-rich mineral deposits. Amazingly, living organisms thrive even in the extreme environments of Norris' acid hot springs. These bacteria are on the cutting edge of research in the fields of medicine and criminal investigation, yielding new tools in such complex areas as AIDS research and DNA "fingerprinting" (Karen, 1995).

The acidophilic and thermophilic unicellular red alga, Cyanidium caldarium (Tilden) Geitler, is widely distributed in acidic hot springs. Observation by transmission electron microscopy (TEM) showed that algae grown in Allen's medium contained electron-dense bodies with diameters from 100 to 200 nm.

Electron dispersive x-ray analysis indicated that the electron-dense bodies contained high levels of iron, phosphorous, and oxygen; P/Fe ratios were from 1.3 to 2.0 (Mori *et al.,* 2003).

Preconditions for the development of diazotrophic cyanobacteria have been described many times for marine and freshwater ecosystems (Berman *et al.,* 1998). One of the most important preconditions is a low N to:P ratio of inorganic the availability of dissolved inorganic phosphorus (DIP) in water when dissolved inorganic nitrogen (DIN) is already exhausted (Kononen *et al.,* 1996). This precondition exists in the Baltic Sea. In comparison with the Redfield ratio, the low N: P ratio of about 8:1 in the winter surface layer generated by vertical convection is the result of denitrification and DIP release in the anoxic deep water (Hille *et al.,* 2005).

2.2.4 Microorganism Selection

Soil microorganisms are very important as almost every chemical transformation taking place in soil involves active contributions from soil microorganisms. Certain soil microorganisms such as mycorrhizal fungi can also increase the availability of mineral nutrients (e.g. phosphorus) to plants. Thus, microorganism from soil is chosen for this research because it seems to be effective way to treat phosphorus. Microorganism from soil also easy to get because can be taken from our surrounding.

2.3 Phosphorus Removal Process

2.3.1 Biofilm Process

The biofilm process, which has been extensively researched and has been applied to practical wastewater treatment, is effective to nitrogen and biochemical oxygen demand (BOD) removal (Aesoy and Odegaard, 1988). In a mature biofilm process, even though the biomass detached from the biofilm becomes suspended, the concentration of the immobilized biomass in the biofilm is much higher than that of the suspended biomass in the liquid phase, indicating that the biomass of the biofilm mainly governs the efficiency of the wastewater treatment (Rittmann and McCarty, 2002). Moreover, with an increase of biofilm thickness, coexistence of aerobic and anaerobic regions in the biofilm can appear which allows the simultaneous removal of nitrogen and BOD to occur. Biofilm reactors have been used in many wastewater treatment processes, and the engineering basis for designing and operating the biofilm processes has well been established (Rittmann and McCarty, 2002). However, there has been little research of phosphorus removal and simultaneous removal of nitrogen and phosphorus by biofilm. In bacterial cells, phosphorus accounts for 2% of the total dry weight (Sherrard and Schroeder, 1972) Some studies of nutrient removal by the continuous-flow biofllm process have shown that it is only effective for nitrogen removal (Wang *et al.*, 1992). Less than 20% at most of phosphorus in sewage can be removed because the A (anaerobic) and O (oxic) conditions necessitated by phosphorus removal microbes cannot be fully provided (Wang *et al.*, 1992). Basically, a sequencing batch reactor (SBR) process combines an aeration tank and a sedimentation tank and the A/O conditions are easily achieved. The operation can be feasibly conducted in the order of inflow, reaction, sedimentation and discharge of the treated water and/or the excess biomass.

Li *et al.* (2003) from School of Civil Engineering and Architecture, Beijing University of Technology, Beijing have studied the process for biofilm formed on fibrous carriers for nitrogen and BOD removal from wastewater and have successfully applied this process to real wastewater treatment in China. The basic

configuration of the biofilm process is an aeration tank wherein the fibrous carriers are distributed in a determined pattern. The ideal operating condition for achieving efficient biofilm performance is to supply enough dissolved oxygen (DO) while avoiding the negative effect of the shear stress caused by aeration to form a biofilm thick enough to be active in wastewater treatment. Maintaining the DO concentration in the liquid phase at about 5 mg/L enables the biofilm process to work well. Compared with other biofilm reactors such as rotating contactors and trickling filters, our biofilm process for wastewater treatment has several advantages. It has a low cost because it uses cheaper and more easily-processed carriers, it has a high biomass concentration due to its large specific surface area; the A/O conditions are controllable, the detached sludge is capable of sedimentation and the process is easy to operate and maintain. In order to remove phosphorus by biofilm, a sequencing batch reactor consisting of submerged biofilm on fibrous carriers was developed by the authors. In this biofilm SBR system, microorganisms are allowed to experience anaerobic and aerobic status alternatively as the aeration is controlled, and the surplus sludge detached from the biofilm which contains the phosphorus in wastewater can be discharged from the system periodically.

2.3.2 Activated Sludge

The activated sludge process is a continuous or semi-continuous (fill and draw) aerobic method for biological wastewater treatment, including carbonaceous oxidation and nitrification. This process is based on the aeration of wastewater with flocculating biological growth, followed by separation of treated wastewater from this growth. Part of this growth is then wasted, and the remainder is returned to the system. Usually, the separation of the growth from the treated wastewater is performed by settling (gravity separation) but it may also be done by flotation and other methods.

The activated sludge process presently represents the most widespread technology for wastewater purification. Activated sludge plants can be found in different climate conditions from the tropics to the polar regions, from sea level

(wastewater treatment plants in ships) to extreme elevations (mountainous hotels). The scale of activated sludge plants ranges from package plants for one family to huge plants serving big metropolises. Wastewater treatment plants equipped with the activated sludge process are able to fulfill the most stringent effluent criteria.

The invention of the activated sludge process is connected with the efforts of British and American engineers at the end of the last century to intensify biological purification in fixed-film systems. The experiments with wastewater aeration did not provide the expected results until a recycle of suspension formed during the aeration period are introduced (Ardern and Lockett, 1914). The suspension, known as activated sludge was in fact an active biomass responsible for the improvement of treatment efficiency and process intensity (Dohse and Heywood, 1998).

According to Activated Sludge, Manual of Practice 9 (Water Environment Association, 1987), the activated-sludge process contains five essential interrelated equipment components. The first is an aeration tank or tanks in which air or oxygen is introduced into the system to create an aerobic environment that meets the needs of the biological community and that keeps the activated sludge properly mixed. At least seven modifications in the shape and number of tanks exist to produce variations in the pattern of flow. Second, an aeration source is required to ensure that adequate oxygen is fed into the tank(s) and that the appropriate mixing takes place. This source may be provided by pure oxygen, compressed air or mechanical aeration. Just as there are modifications in the shape and number of aeration tanks that can be used in the activated-sludge process, different equipment systems exist to deliver air or oxygen into aeration tanks. Third, in the activated-sludge process, aeration tanks are followed by secondary clarifiers. In secondary clarifiers, activated-sludge solids separate from the surrounding wastewater by the process of flocculation (the formation of large particle aggregates, or flocs, by the adherence of floc-forming organisms to filamentous organisms) and gravity sedimentation, in which flocs settle toward the bottom of the clarifier in a quiescent environment. This separation leads ideally to the formation of a secondary effluent (wastewater having a low level of activated-sludge solids in suspension) in the upper portion of the clarifier and a thickened sludge comprised of flocs, termed return activated sludge, or RAS, in the

bottom portion of the clarifier. Next, return activated sludge must be collected from the secondary clarifiers and pumped back to the aeration tank(s) before dissolved oxygen is depleted. In this way, the biological community needed to metabolize influent organic or inorganic matter in the wastewater stream is replenished. Finally, activated sludge containing an overabundance of microorganisms must be removed, or wasted (waste activated sludge, or WAS), from the system. This is accomplished with the use of pumps and is done in part to control the food to microorganism ratio in the aeration tanks.

2.3.3 Trickling Filters

A trickling filter consists of a fixed bed of rocks, gravel, slag, polyurethane foam, sphagnum peat moss, or plastic media over which sewage or other wastewater flows downward and causes a layer or film of microbial slime to grow, covering the bed of media. Aerobic conditions are maintained by splashing, diffusion, and either by forced air flowing through the bed or natural convection of air if the filter medium is porous. The process mechanism, or how the removal of waste from the water happens, involves both absorption and adsorption of organic compounds within the sewage or other wastewater by the layer of microbial slime. Diffusion of the wastewater over the media furnishes dissolved air, the oxygen which the slime layer requires for the biochemical oxidation of the organic compounds and releases carbon dioxide gas, water and other oxidized end products. As the slime layer thickens, it becomes more difficult for air to penetrate the layer and an inner anaerobic layer is probably formed. This slime layer continues to build until it eventually sloughs off, breaking off longer growth into the treated effluent as a sludge that requires subsequent removal and disposal. Typically, a trickling filter is followed by a clarifier or sedimentation tank for the separation and removal of the sloughing. Other filters utilizing higher-density media such as sand, foam and peat moss do not produce a sludge that must be removed, but require forced air blowers and backwashing or an enclosed anaerobic environment

The terms trickle filter, trickling biofilter, biofilter, biological filter and biological trickling filter are often used to refer to a trickling filter. These systems have also been described as roughing filters, intermittent filters, packed media bed filters, alternative septic systems, percolating filters, attached growth processes, and fixed film processes. The treatment of sewage or other wastewater with trickling filters is among the oldest and most well characterized treatment technologies. Wastewaters from a variety of industrial processes have been treated in trickling filters. Such industrial wastewater trickling filters consist of two types: (1) Large tanks or concrete enclosures filled with plastic packing or other media. (2)Vertical towers filled with plastic packing or other media.

The availability of inexpensive plastic tower packings has led to their use as trickling filter beds in tall towers, some as high as 20 meters (Beychok, 1967). As early as the 1960s, such towers were in use at the Great Northern Oil's Pine Bend Refinery in Minnesota, the Cities Service Oil Company Trafalgar Refinery in Oakville, Ontario and at a kraft paper mill (Bryan and Moeller, 1960).

The treated water effluent from industrial wastewater trickling filters is very often subsequently processed in a clarifier-settler to remove the sludge that sloughs off the microbial slime layer attached to the trickling filter media. Currently, some of the latest trickle filter technology involves aerated biofilters which are essentially trickle filters consisting of plastic media in vessels using blowers to inject air at the bottom of the vessels, with either downflow or upflow of the wastewater (Sperling, 2007).

2.3.4 Process Selection

Biofilm process is chosen as the method in phosphorus removal for this research. It has a low cost because it uses cheaper and more easily-processed carriers, it has a high biomass concentration due to its large specific surface area, the anaerobic or oxic conditions are controllable, the detached sludge is capable of sedimentation and the process is easy to operate and maintain.

2.4 Types of Reactor

2.4.1 Sequencing Batch Reactor

Sequencing batch reactors (SBR) or sequential batch reactors are industrial processing tanks for the treatment of wastewater. SBR reactors treat waste water such as sewage or output from anaerobic digesters or mechanical biological treatment facilities in batches. Oxygen is bubbled through the waste water to reduce biochemical oxygen demand (BOD) and chemical oxygen demand (COD) to make suitable for discharge into sewers or for use on land.

In recent years, sequencing batch reactor (SBR) has been employed as an efficient technology for wastewater treatment, especially for domestic wastewaters, because of its simple configuration (all necessary processes are taking place time-sequenced in a single basin) and high efficiency in BOD and suspended solids removal. SBRs could achieve nutrient removal using alternation of anoxic and aerobic periods (Rim *et al.,* 1997). The SBR has received considerable attention since (Irvine and Davis, 1971) described its operation and studies of SBR process were originally conducted at the University of Notre Dame, Indiana (Irvine and Busch, 1979).

Sequencing batch reactors operate by a cycle of periods consisting of fill, react, settle, decant, and idle. The duration, oxygen concentration, and mixing in these periods could be altered according to the needs of the particular treatment plant. Appropriate aeration and decanting is essential for the correct operations of these plants. The aerator should make the oxygen readily available to the microorganisms. The decanter should avoid the intake of floating matter from the tank. The many advantages offered by the SBR process justify the recent increase in the implementation of this process in industrial and municipal wastewater treatment (Norcross and Chambers, 1993).

2.4.2 Batch Reactor

A batch reactor is used in chemical processes for small scale operation, for testing new processes that have not been fully developed, for the manufacture of expensive products, and for processes that are difficult to convert into continuous operations. The main advantage of a batch reactor is high conversion, which can be obtained by leaving the reactant in the reactor for long periods of time, but it also has the disadvantages of high labor costs per batch and the difficulty of large scale production. In a batch reactor, all the reactants are loaded at once. The concentration then varies with time, but at any one time it is uniform throughout. Agitation serves to mix separate feeds initially and to enhance heat transfer. Batch reactors are popular in practice because of their flexibility with respect to reaction time and to the kinds and quantities of reactions that can be performed. Characteristics of a batch reactor are that the total mass of each batch is fixed, each batch is a closed system, and the reaction (residence) time for all elements of fluid is the same.

The Batch reactor is the generic term for a type of vessel widely used in the process industries. Its name is something of a misnomer since vessels of this type are used for a variety of process operations such as solids dissolution, product mixing, chemical reactions, batch distillation, crystallization, liquid/liquid extraction and polymerization. In some cases, they are not referred to as reactors but have a name which reflects the role they perform (such as crystallizer, or bio reactor).

A typical batch reactor consists of a tank with an agitator and integral heating/cooling system. These vessels may vary in size from less than 1L to more than 15,000L. They are usually fabricated in steel, stainless steel, glass lined steel, glass or exotic alloy. Liquids and solids are usually charged via connections in the top cover of the reactor. Vapors and gases also discharge through connections in the top. Liquids are usually discharged out of the bottom.

The advantages of the batch reactor lie with its versatility. A single vessel can carry out a sequence of different operations without the need to break containment. This is particularly useful when processing, toxic or highly potent compounds (Levenspiel, 1993).

2.4.3 Continuous Stirred-Tank Reactor (CSTR)

The continuous stirred-tank reactor (CSTR), also known as vat- or backmix reactor is a common ideal reactor type in chemical engineering. A CSTR often refers to a model is used to estimate the key unit operation variables when using a continuous agitated-tank reactor to reach a specified output. The mathematical model works for all fluids: liquids, gases, and slurries.

The behavior of a CSTR is often approximated or modeled by that of a Continuous Ideally Stirred-Tank Reactor (CISTR). All calculations performed with CISTRs assisted with the assumption of the reactor are well mixed or perfect mixing. If the residence time is 5-10 times the mixing time, this approximation is valid for engineering purposes. The CISTR model is often used to simplify engineering calculations and can be used to describe research reactors. In practice it can only be approached, in particular in industrial size reactors (Schmidt, 1998).

In a CSTR, one or more fluid reagents are introduced into a tank reactor equipped with an impeller while the reactor effluent is removed. The impeller stirs the reagents to ensure proper mixing. Simply dividing the volume of the tank by the

average volumetric flow rate through the tank gives the residence time, or the average amount of time a discrete quantity of reagent spends inside the tank. Using chemical kinetics, the reaction's expected percent completion can be calculated. Some important aspects of the CSTR are: (1) At steady-state, the flow rate in must equal the mass flow rate out, otherwise the tank will overflow or go empty (transient state). While the reactor is in a transient state the model equation must be derived from the differential mass and energy balances. (2) The reaction proceeds at the reaction rate associated with the final (output) concentration. (3) Often, it is economically beneficial to operate several CSTRs in series. This allows, for example, the first CSTR to operate at a higher reagent concentration and therefore a higher reaction rate. In these cases, the sizes of the reactors may be varied in order to minimize the total capital investment required to implement the process. (4) It can be seen that an infinite number of infinitely small CSTRs operating in series would be equivalent to a PFR (Levenspiel, 1993).

2.4.4 Reactor Selection

For this research, sequencing batch reactor (SBR) has been selected because it is simple configuration and high efficiency in BOD and suspended solids removal. SBRs could achieve nutrient removal by using alternation of anoxic and aerobic periods. Sequencing batch reactor technology is well known for its simplicity and low cost. It has been widely used for municipal and industrial wastewater treatment applications to meet specific effluent requirements.

CHAPTER 3

METHODOLGY

3.1 Acclimatization Reactor

For this research, two types of reactors with different volume will be used. One for treatment reactor and another one are for acclimatization reactor. The treatment reactor volume is 5L and the volume acclimatization reactor is 10L. For acclimatization reactor, 2kg fresh soils are taken from the waste disposal area. Eight 1L conical flasks are filled with 250g soil that has been taken. Then, 10L distilled water is sterilized at 121°C and then cooled to reach room temperature. After that, each conical flask that containing 250g soil are filled with 500mL distilled water that has been sterilized. All 8 conical flasks are shakes at 150rpm for 1 hour. Then, 10L reactor is filled with rocks to act as a support media. Then, 4L mixture from all conical flasks that has been shakes is filled in the 10L reactor and assures that the precipitate is not filled together with the mixture into the reactor. Then, the 10L reactor is filled with 6L distilled water that has been sterilized.

For feed purpose, 1L solutions from the acclimatization reactor are discarded to give the HRT equal to 5 day. Then, 10mL solution that has been discarding is taken for suspended solid test and the remaining solution is let for a moment until the precipitate settled down. After it settled down, about 700mL solutions are discarded and assured the precipitate is not discarded together. Then, 20mL glucose stock solutions are mixed with remaining solution above. Distilled water that has been sterilized is filled into the solution until it reached 1L to give the concentration of the glucose is 200mg/L. Then, this solution is filled back into the acclimatization reactor.

3.2 Treatment Reactor

In providing the treatment rector, 1L mixture from the acclimatization reactor are discarded and then filled into 5L reactor. Water that has been sterilized is then filled into the reactor until reached 5L point. The rocks that have diameter about 1.5 cm to 2 cm are placed in the reactor as a support media to the mixed culture. Plastic net is used to prevent unneeded things from entering the reactor. The treatment reactor is shows in Figure 3.1 and Figure 3.2.

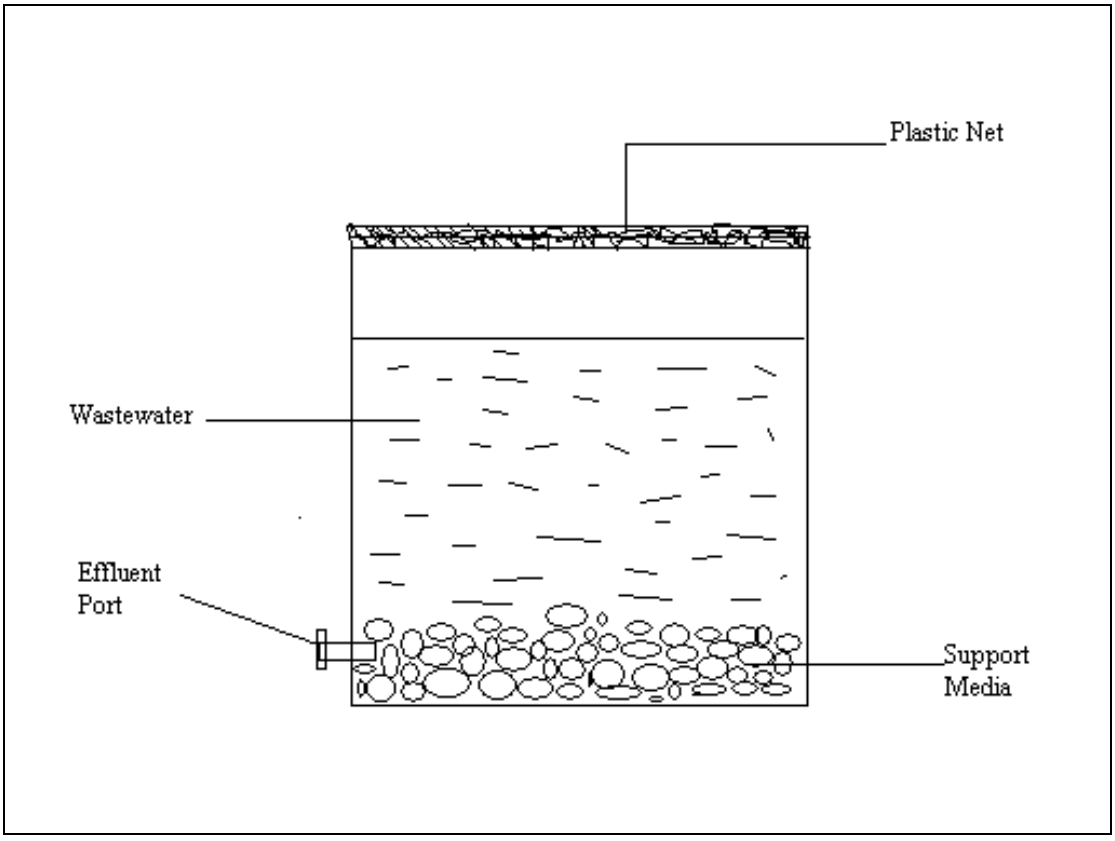

Figure 3.1: Schematic treatment reactor

Figure 3.2: Treatment reactor

3.3 Operational Condition

Table 3.1 shows the operational parameters used in the experiment. This experiment is run at loading rate 5.0, 4.5, 4.0, 3.5 and 3.0 mg/L.d. Eight treatment rectors are used in the experiment. The concentration of the phosphorus fed into each reactor was different according to the loading rate. The reactors are fed everyday with the phosphate solution during the treatment process.

For every two days during treatment period, the sample from the each reactor was taken to test the COD and phosphorus concentration while the suspended solids concentration is tested for every single day during treatment process. COD, suspended solid and concentration of phosphorus was analyzed during this experiments using HACH Spectrophotometer.

Table 3.1: Operational parameters

Loading Rate(mg/L.d)	Concentration(mg/L)	HRT(d)	Flow rate(L/d)
5.00	25.0	5	1
4.50	22.5	5	1
4.00	20.0	5	1
3.00	15.0	5	1
4.00	20.0	5	1
3.50	17.5	5	1
5.00	25.0	5	1
3.00	15.0	5	1

3.4 Chemical Composition

For this research, phosphate powder is used to produce phosphorus solution. The concentration of phosphate ion (PO_4^{3-}) used are based on the loading rate that has been decided. Glucose is used to feed the mixed culture in the acclimatization reactor. It is for microorganism growth.

3.4.1 Glucose and Phosphate Stock Solution

For making glucose stock solution, 10g glucose powders are weighed. Then, the 10g glucose is mixed into 1L distilled water to give the concentration of 10g/L. For the phosphate stock solution, 0.33g phosphate powders are weighed and then the powder is diluted with 3 L distilled water to give the concentration of 1000mg phosphate /L. The mixture is sterilized at 121°C and then cooled until it reached room temperature. Then, the stock solution is stored in the chiller.

3.5 Analytical Method

COD, suspended solid and concentration of phosphorus were analyzed during this experiment using HACH Spectrophotometer DR 2400 and DR 2800.

For suspended solids test, two sample cells are used. 25mL sample is added to the first sample cell and then the second sample cell is filled with distilled water to act as blank. This sample cell is then tested using HACH Spectrophotometer DR 2400.

COD high range (HR) vial are used for the COD tested. Two vials is used for each test, one vial for the sample test and another one is for blank. 2mL sample is added to the first vial and 2mL deionized water is added to the second vial. Then, the vials are heated in the COD reactor at 150 °C for 2 hours. After that, the vials is inverted for several time and then cooled to the room temperature. The vials are then tested using HACH Spectrophotometer.

Reactive phosphorus, orthophosphate are used to test the phosphorus concentration. 5mL sample is added to the vials and then tested using the barcode program in the HACH Spectrophotometer DR 2800.

Figure 3.3: HACH Spectrophotometer DR 2800.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Suspended Solid Concentration for Acclimatization Reactor

Suspended solids (SS) refer to small solid particles which remain in suspension in water as a colloid or due to the motion of the water. It is used as one indicator of water quality. It is sometimes abbreviated Suspended solids (SS), but is not to be confused with settleable solids, also abbreviated Suspended solids (SS), which contribute to the blocking of sewer pipes. Suspended solids are important as pollutants and pathogens are carried on the surface of particles. The smaller the particle size, the greater the surface area per unit mass of particle, and so the greater the pollutant load that is likely to be carried.

From the plotted graph in Figure 4.1, the suspended solid reading was decreased from 13220 mg/L to 3060 mg/L from day 3 to day 9. It is because the mixed culture is not fed at the first week in order to let the mixed culture to well adapt to the new environment. The microorganism must first become acclimated to their surrounding and the food provided. After a week, the mixed culture is fed with the glucose solution at day 7, the suspended solid reading increasing from 3060 mg/L to 5190 mg/L on day 9 to day 21. The lack of micronutrients, responsible for the decrease of the removal performance had also a negative impact on biofilm growth. Indeed, the part of carbon converted to biomass decreased (Cresson *et al.*, 2006).

After day 21, the Suspended solids (SS) reading were decreased and increased drastically. According to the suspended solid concentration graph in Figure 4.1, overall suspended solids concentration readings are decreased from day 1 to day 84. It is because, increasing in biofilm growth will contributed to the slime formation. The slime will give a negative impact to the biofilm growth because it can limited the nutrient and oxygen supply for microorganism growth. (Thormann *et al.*, 2005) stated that at sloughing events, in general, are proportional to biofilm thickness and could be attributed to oxygen or nutrient limitation.

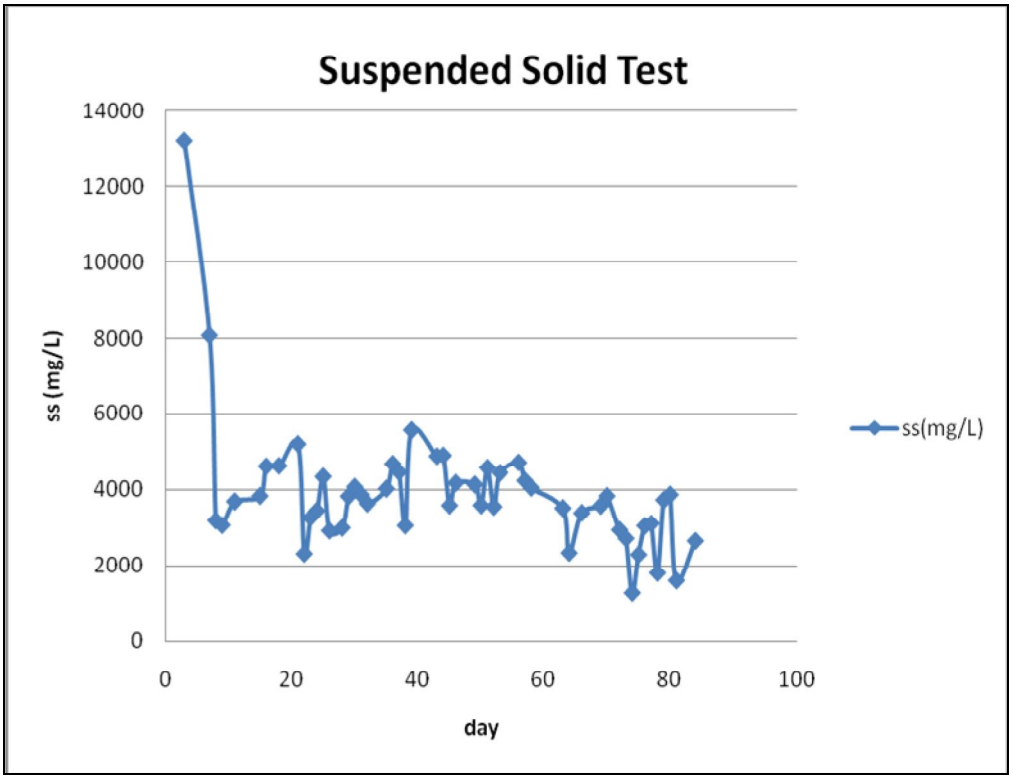

Figure 4.1: Suspended solid concentration for acclimatization reactor

4.2 Effect of Loading Rate on Suspended Solid Concentration

From the Figure 4.2, it shows that the increasing of loading rate (LR) will contributed to the increasing of the biofilm growth. The suspended solids (SS) reading increased with the increasing of the loading rate. Loading rate 5.0 mg/L.d shows the highest suspended solids concentration readings compared to other loading

rate. The concentration of phosphate solution fed into the treatment reactor increased with the increasing of loading rate. It proved that the biofilm growth is increased with the increasing of nutrient concentration. Like other living creatures, bacteria require certain nutrients for growth and reproduction. Limiting these nutrients will limit bacteria growth, but nutrient levels in high-purity systems are unequivocally sufficient to permit microbial growth and reproduction to a troublesome extent (Husted, 1994).

The results from the experiment are plotted in Figure 4.2. It shows that the microorganism growth rate decreased with the decreasing in the loading rate. Only the loading rate 5.0 mg/L.d show an increasing in the suspended solids readings. Loading rate 3.0 (A) mg/L.d shows an increasing in the suspended solids readings. But, the increasing rate at loading rate 3.0 (A) mg/L.d is quite low compared to the loading rate 5.0 mg/L.d. It shows that the fed phosphorus concentration of 25 mg/L at loading rate 5.0 mg/L.d gives the highest biofilm growth. The phosphorus concentration below 25 mg/L is not enough contributed to the biofilm growth. This statement can be reviewed in the Table 1 in the Appendix A1.

Figure 4.2 also reveals that there is a different of the suspended solid readings between loading rates (A) and loading rates (B). Logically, there is no big different of the suspended solids readings because the mixed cultures is taken from the same acclimatization reactor. The difference is because of the error in the lab handling which can contribute to the errors in suspended solids readings.

Based on the result from the Design Expert in Figure 4.3, the predicted suspended solids concentration increased with the increasing of loading rate. It shows that, the biofilm growth increased when the fed phosphorus concentration increased. The result from the experiment is the same as the result plotted using Design Expert. The suspended solids reading are depend on the nutrient availability.

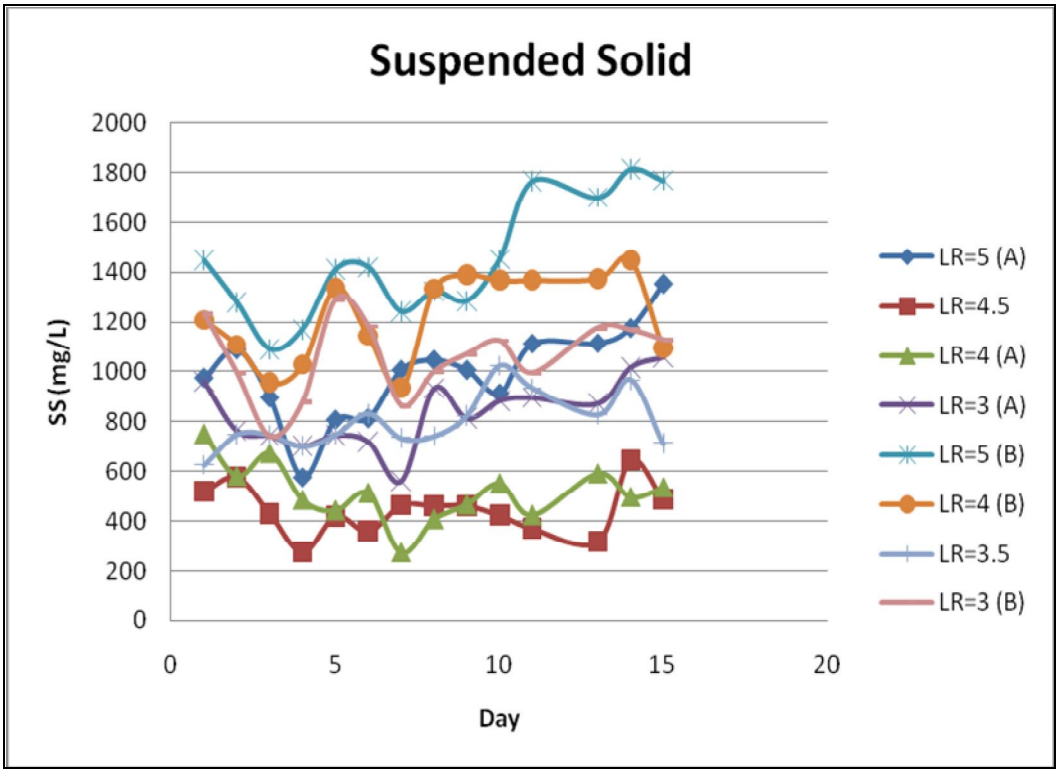

Figure 4.2 : Suspended solids concentration for treatment reactor

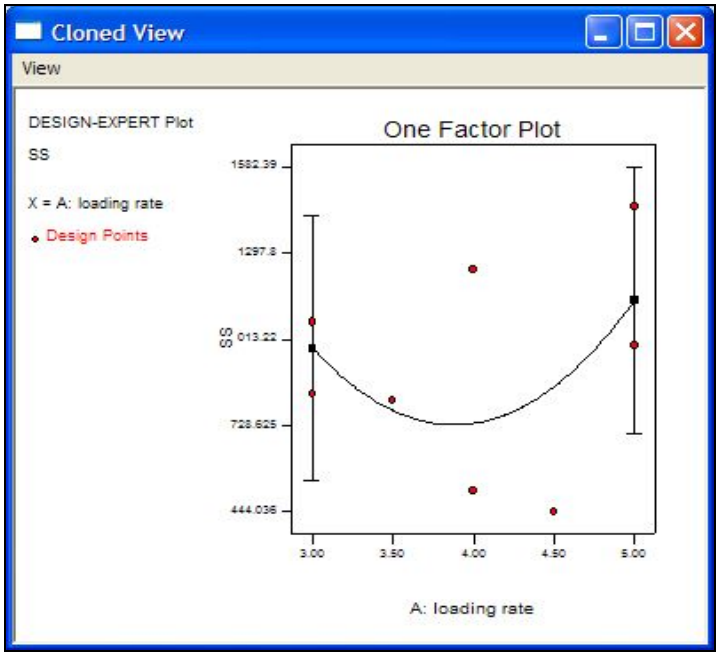

Figure 4.3: Suspended solid plotted from Design Expert.

4.3 Effect of Different Loading Rate on Phosphorus Removal

The effect of different loading rate on phosphorus removal is best described in Figure 4.4, the loading rate 5.0 mg/L.d gives the highest percent of phophorus removal. Loading rate 4.0 and 4.5 mg/L.d shows the lowest percent of phosphorus removal. It is because of the biofilm at this loading rate 4.0 and 4.5 mg/L.d is lowest compared to other loading rate. It is only give 38% and 43% of average removal. It shows that the phosphorus removal efficiencies are depends on the biofilm growth. The highest suspended solids reading will give the highest percent of the phosphorus removal. It is because of Biological phosphorus (Bio-P) is necessary for microorganism growth and metabolism, thus another possibility for phosphorus removal in the study was that phosphorus removal was the result of normal growth and metabolism by microorganism (Wang *et al*., 2007). It takes the point that microorganism need the phosphorus to growth. Phosphorus removal are increased when the biofilm growth increased.

Figure 4.5 illustrate the predicted phosphorus removal efficiency using Design Expert. The phosphorus removal increased with increasing of the loading rate. This experiment shows the same result as the Design Expert plotted. The biofilm growth increased will attribute to the high removal percentage. (Suresh *et al*., 1985) stated that not only one species but also several of bacteria which existed in activated sludge could remove phosphorus. It explained that the microorganisms consumed the phosphorus for their growth and metabolism.

In comparison to the previous studies, the result of the removal efficiency is lowest compared to the previous study. This comparison can be reviewed in the Table 4.1. This is because of the loading rate that was used in this experiment is small and not suitable to give the high biofilm growth rate in order to treat phosphorus.

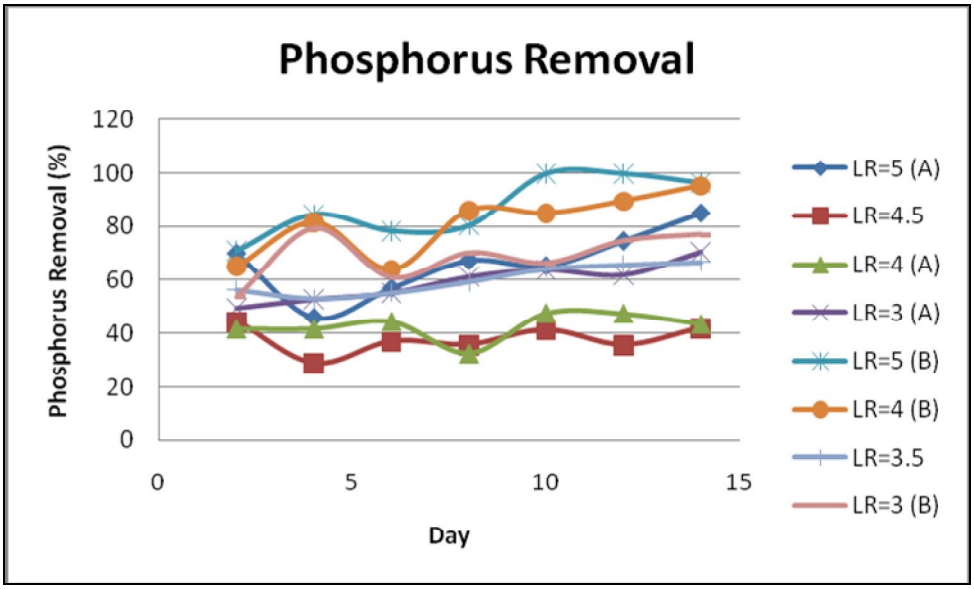

Figure 4.4: Percentage of the phosphorus removal.

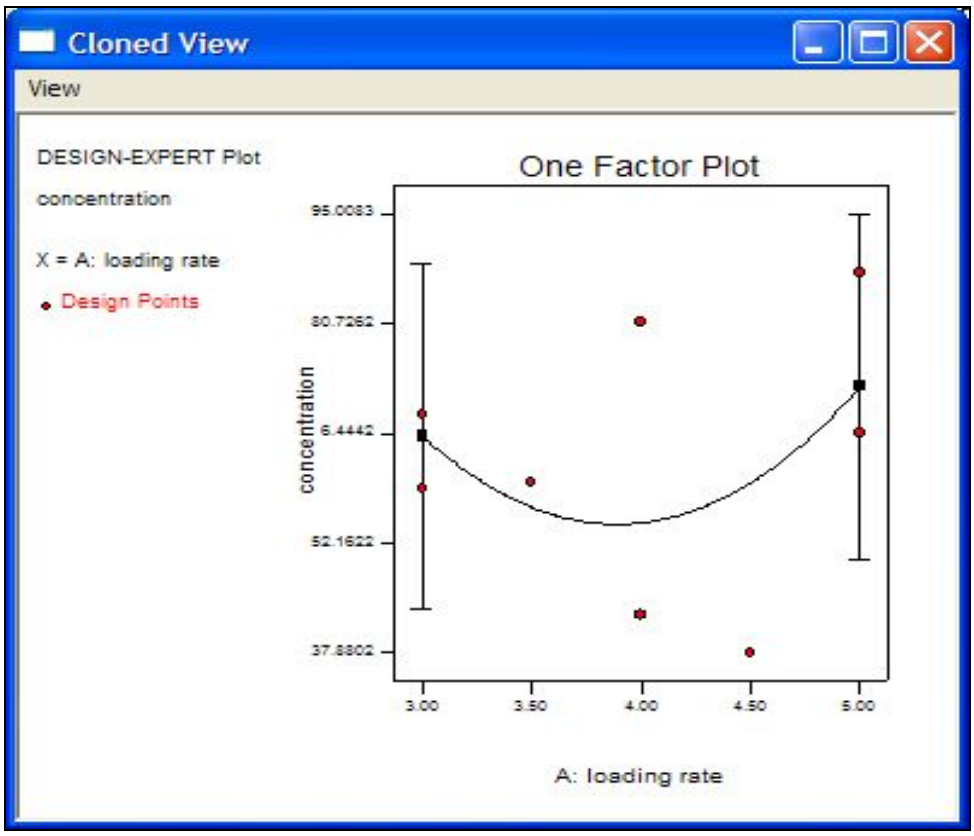

Figure 4.5: Phosphorus removal from Design Expert.

Table 4.1: Comparison of phosphorus removal.

STUDIES	% REMOVAL	METHOD
This Study	87%	-sequencing batch reactors -mixed culture from soil
Zhang *et al.,* 2008	96.7%	-membrane bioreactors -sludge microorganisms
Wang *et al.,* 2007	98.59%	-sequencing batch reactors -sludge microorganism
Li *et al.,* 2003	90%	-sequencing batch reactors -sludge microorganism

4.4 Effect of Different Loading Rate on COD Removal

The chemical oxygen demand (COD) test is commonly used to indirectly measure the amount of organic compounds in water. Most applications of COD determine the amount of organic pollutants found in surface water for examples lakes and rivers, that makes COD a useful measurement of water quality. It is expressed in milligrams per liter (mg/L), which indicates the mass of oxygen consumed per liter of solution. Older references may express the units as parts per million (ppm) (Parkin *et al*., 2003).

Theoritically, increasing in the phosphorus removal will attributed to the increased of the COD removal. In this study, LR 3.5 mg/L.d shows the highest average percentage of COD removal compared to other loading rate. LR 5.0 mg/L.d only give 75% to 78% of average removal. It is opposite from the previous research that shows the COD removal is increased with the increasing of loading rate (LR). It is because of the higher detachment rate. At the highest phosphate level, the higher detachment rate could be attributed to increased precipitation of calcium phosphate which would decrease calcium crosslinking with extracellular polysaccharides and thus weaken the biofilm matrix (Turakhia *et al*., 1989). It will result in the higher organics compound in the reactor. The phosphorus removal and increasing

precipitation of calcium phosphate occurred at the same time but in different rate. It can contribute to the less COD removal (see Figure 4.6).

COD removal plotted from Design Expert, Figure 4.7 shows that the removal percentage decreased with the increasing of loading rate. It is the same as the result from the experiment.

From this experiment, the highest COD removal is only 83%. It is quite low when compared to other studies. With supporting evidence in Table 4.2 and Figure 4.6, this hypothesis is best explain due to the increasing in calcium phosphate precipitate with the increasing of the biofilm growth.

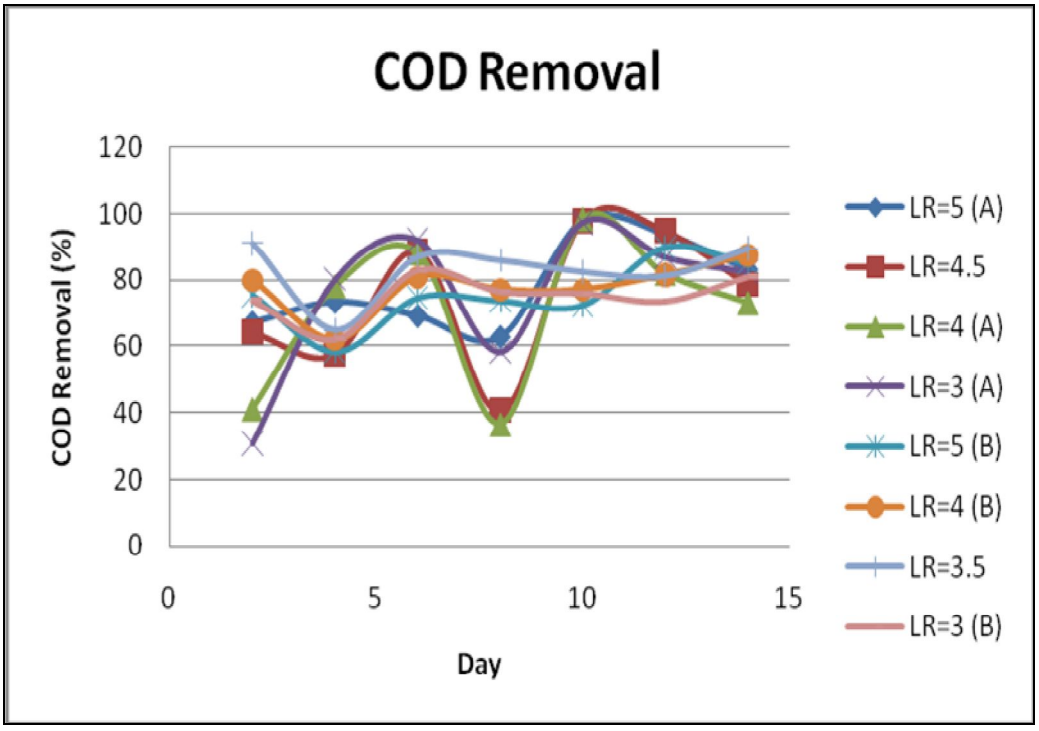

Figure 4.6: Percentage of the COD removal.

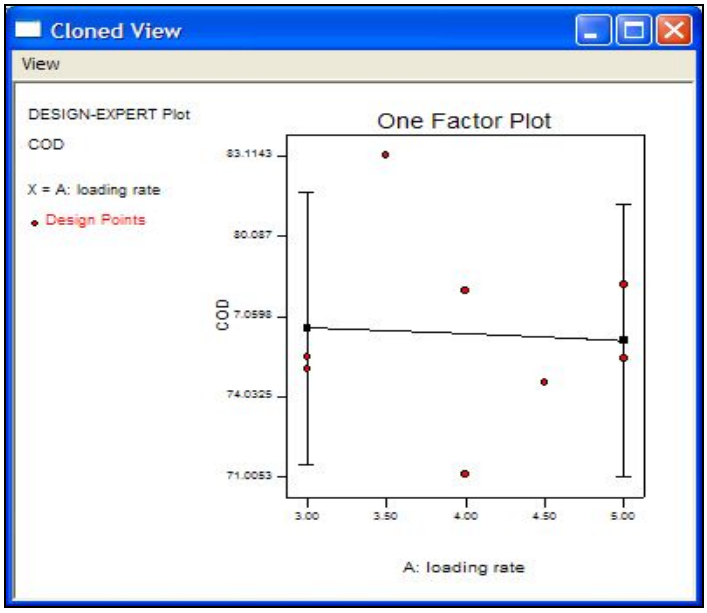

Figure 4.7: COD removal from Design Expert.

Table 4.2: Comparison of COD removal.

STUDIES	% REMOVAL	METHOD
This Study	83%	-sequencing batch reactors -mixed culture from soil
Zhang *et al.*, 2008	90%	-membrane bioreactors -sludge microorganisms
Kulikowska *et al.*, 2006	76.7%	-sequencing batch reactors -leachate microorganisms
Devi and Dahiya, 2006	95.87%	-sequencing batch reactors -sludge microorganisms

4.5 Optimization of Phosphorus and COD removal

The Design Expert result in Figure 4.8 shows that the best predicted phosphorus removal is at LR 5.0 mg/L.d with 72.53% phosphorus removal, 76.14% COD removal and 1142.85 mg/L of suspended solid (SS) concentration. The highest COD removal can be achieved at loading rate 3.0 mg/L.d with the COD removal of 77%. The expected phosphorus removal at this loading rate is 66% at 984 mg/L of suspended solid concentration.

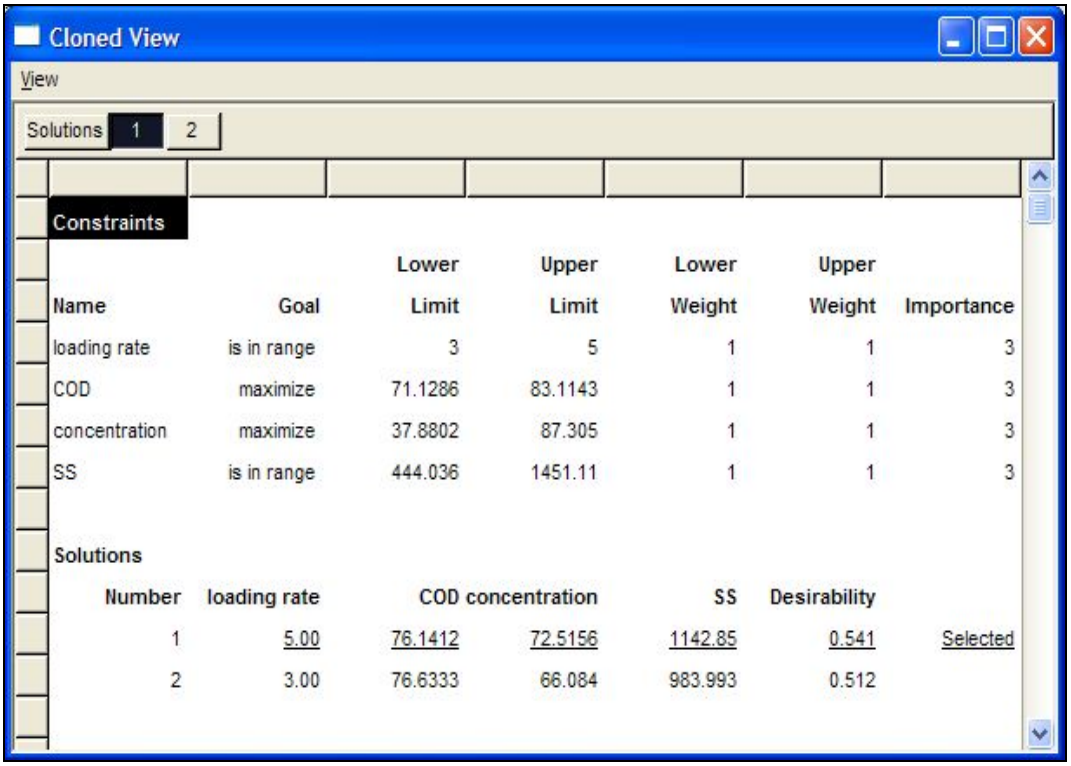

Figure 4.8: Optimization data

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The phosphorus removal by using the mixed culture from soil is a quite promising wastewater treatment method. From this study, it can be conclude that the mixed culture from soil can degrade the phosphorus at average removal percentage of 66% to 87%. The loading rates 5.0, 4.5, 4.0, 3.5 and 3.0 mg/L.d give the different effect on removal efficiency and biofilm growth. Loading rate 5 mg/L.d shows the highest suspended solid readings and highest phosphorus removal efficiency. But, the COD removal efficiency is decreased with the increasing of loading rate. The varying of the phosphorus concentration ranged from 15 to 25 mg/L gives an effect to the biofilm growth. The biofilm growth is increased with the increasing of the phosphorus concentration. The suspended solids readings are highest at loading rate 5 mg/L.d compared to other loading rate. Result from the Design Expert shows that the best predicted phosphorus removal is at LR 5.0 mg/L.d. The phosphorus removal of 72.53% and COD removal of 76.14% can be achieved at LR 5.0 mg/L.d with 1142.85 mg/L of suspended solid (SS) concentration. The highest COD removal can be achieved at loading rate 3.0 mg/L.d with the COD removal of 77%. The expected phosphorus removal at this loading rate is 66% and the expected suspended solid concentration is 984 mg/L. Hence, this research can be applied to control the phosphorus concentration in urban and industrial wastewater to assure that our ecosystem is prevented from eutrophication.

5.2 Recommendation

5.2.1 Increasing the Loading Rate

From this study, it shows that the removal efficiency increased with the increasing of loading rates. For the future study, it is necessary to used high loading rate. Regarding the result of this experiment, it shows that the highest growth rate is obtained at loading rate 5.0 mg/L.d. The loading rate below than 5.0 mg/L.d shows the negative impact to the biofilm growth. The biofilm growth will affect the phosphorus removal efficiency because the biofilm consumed the phosphorus to growth. It is necessary to used loading rate higher than 5.0 mg/L.d for the future study. The high removal efficiency can be achieved with the increasing of the loading rate (Bernet *et al*., 2006).

5.2.2 Using Suitable Micronutrient

From the result, the suspended solid readings are decreasing from the starting day to the end of the acclimatization period. Chosen of the suitable nutrient will contribute to the biofilm growth. For the future study, the suitable micronutrient must be chosen to give the best result in biofilm growth. The micronutrients other than glucose are needed to be finding in order to give highest biofilm growth rate. The highest growth rate will contributed to a highest removal performance.

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APPENDIX A1

TABLE 1: suspended solid concentration

	Suspended Solid Concentration							
Day	LR=5(A)	LR=4.5	LR=4(A)	LR=3(A)	LR=5(B)	LR=4(B)	LR=3.5	LR=3(B)
1	976.7	520	751.7	960	1448.3	1208.3	626.7	1233.3
2	1090	576.5	581.5	765	1280	1108	750	995
3	898.5	430	673.5	745	1090	956.7	748.3	741.7
4	578.5	276.5	485	703.5	1171.7	1028.3	701.7	880
5	810	420	443.5	746.5	1411.7	1338.3	748.3	1291.7
6	816.5	360	513.5	721.5	1421.7	1145	833.3	1183.3
7	1008.5	463.5	275	561.5	1243.3	933.3	735	865
8	1048	461.7	408.3	933.3	1325	1335	743.3	1000
9	1008	461.7	466.7	815	1286.7	1390	825	1070
10	915	423.3	551.7	885	1453.3	1368.3	1023.3	1125
11	1113.3	371.7	425	896.7	1766.7	1368.3	935	995
13	1115	318.3	591.7	873.3	1700	1375	828.3	1176.7
14	1176.7	645	496.7	1016.7	1815	1450	968.3	1168.3
15	1355	488.3	535	1058.3	1768.3	1093.3	713.3	1128.3

TABLE 2: phosphorus removal

	Phosphorus Concentration (%)							
Day	LR=5(A)	LR=4.5	LR=4(A)	LR=3(A)	LR=5(B)	LR=4.0(B)	LR=3.5	LR=3(B)
2	70.0126	44.4543	41.7681	49.4156	71.0494	65.1065	56.3157	53.7651
4	45.7427	28.9223	41.7756	52.7509	84.6367	81.5265	52.9621	79.2431
6	56.8671	36.9946	44.4649	55.1433	78.4459	64.0879	55.152	61.01
8	67.4042	35.8211	32.6925	61.1951	80.4573	85.9798	59.4212	70.2102
10	65.3672	41.2611	47.6423	63.846	100	84.9165	64.3632	66.4712
12	74.6552	35.7193	47.4903	61.9415	100	89.4958	65.8413	74.6557
14	85.0584	41.9885	43.5728	70.4305	96.546	95.2471	66.8253	77.0073

TABLE 3: chemical oxygen demand (COD) removal

	COD Concentration							
Day	LR=5(A)	LR=4.5	LR=4(A)	LR=3(A)	LR=5.0(B)	LR=4.0(B)	LR=3.5	LR=3(B)
2	67.2	64.1	41.2	30.7	75.3	79.9	91	73.6
4	73.4	57.6	78.2	80.3	58.3	62.1	64.8	62.1
6	69.2	88.7	88.3	92.1	74.4	80.9	87	83
8	62.9	40.6	36.6	58.3	73.5	77.1	85.9	76.4
10	97.9	97.8	98.5	97.5	72.2	77.1	82.4	75.9
12	93.9	94.7	82	87.1	89.7	81.6	81.2	73.5
14	83.3	78.6	73.1	82.6	85	87.6	89.5	81

APPENDIX B1

1. Touch **Hach Programs**. Select program **Barcode Program.**
2. Add 5mL sample to the reactive phosphorus, orthophosphate vial.
3. Clean the outside of the vials with a damp towel followed by a dry one to remove fingerprints or other marks.
4. Place the vial in the adapter and the reading will appear.

APPENDIX B2

1. Homogenize 100mL of sample for 30 seconds in a blender.
2. For the 200-15,000 mg/L range or to improve accuracy and reproducibility of the other ranges, pour the homogenized sample into a 250-mL beaker and gently stir with a magnetic stir plate.
3. Turn on the COD Reactor. Preheat to 150^{o}C. Place the safety shield in front of the reactor.
4. Remove the caps from two COD Digestion Reagent Vials.
5. Hold one vial at a 45-degree angle. Use a clean volumetric pipet to add 2.00 mL of sample to the vial. This is the prepared sample.
6. Hold a second vial at a 45-degree angle. Use a clean volumetric pipet to add 2.00 mL deionized water to the vial. This is the blank.
7. Cap the vials tightly. Rinse them with deionized water and wipe with a clean paper towel.
8. Hold the vials by the cap over the sink. Invert gently several times to mix. Place the vials in the preheated COD Reactor. The sample vials will become very hot during mixing.
9. Heat the vials for two hours.
10. Turn the reactor off. Wait about 20 minutes for the vials to cool to 120^{o}C or less.
11. Invert each vial several times while still warm. Place the vials into rack and cool to room temperature.

12. Touch **Hach Programs**. Select program **430 Cod LR** or **435 COD HR**. Touch **start**.
13. Clean the outside of the vials with a damp towel followed by a dry one to remove fingerprints or other marks.
14. Install the 16-mm adapter. Place the blank into the adapter.
15. Touch **Zero**.
16. When the timer beeps, place the sample vial into the adapter. Touch **read**.
17. If using High Range Plus COD Digestion Reagent Vials, multiply the result by 10.

APPENDIX B3

1. Touch Hach Programs. Select program 630 Suspended Solids. Touch Start.
2. Blend 500mL of sample in a blender at high speed for exactly two minutes.
3. Pour the blended sample into a 600-mL beaker.
4. Stir the sample and immediately pour 25 mL of the blended sample into a sample cell (the prepared sample).
5. Fill a second sample cell with 25 mL tap water or deionized water (the blank).
6. Place the blank into the cell holder.
7. Touch zero. The display will show: 0 mg/L Susp.Solids
8. Swirl the prepared sample to remove any gas bubbles and uniformly suspend any residue.
9. Place the prepared sample into the cell holder.