BIOLOGICAL NUTRIENT REMOVAL USING SEQUENCING BATCH BIOREACTOR

MUHAMAD YASIN BIN ISMAIL

UNIVERSITI MALAYSIA PAHANG

UNIVERSITI MALAYSIA PAHANG

PSZ 19:16 (Pind. 1/97)

BORANG PENGESAHAN STATUS TESIS♦

JUDUL : BIOLOGICAL NUTRIENT REMOVAL USING SEQUENCING BATCH BIOREACTOR

SESI PENGAJIAN : 2008/2009

Saya **MUHAMAD YASIN BIN ISMAIL**

(HURUF BESAR)

mengaku membenarkan tesis (PSM/~~Sarjana~~/~~Doktor Falsafah~~)* ini disimpan di Perpustakaan Universiti Malaysia Pahang dengan syarat-syarat kegunaan seperti berikut :

1. Tesis adalah hakmilik Universiti Malaysia Pahang.
2. Perpustakaan Universiti Malaysia Pahang dibenarkan membuat salinan untuk tujuan pengajian sahaja.
3. Perpustakaan dibenarkan membuat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
4. **Sila tandakan (√)

☐	SULIT	(Mengandungi maklumat yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di dalam AKTA RAHSIA RASMI 1972)
☐	TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana penyelidikan dijalankan)
√	TIDAK TERHAD	

Disahkan oleh

(TANDATANGAN PENULIS) (TANDATANGAN PENYELIA)

Alamat Tetap **TL 19 Pt Hj Abd Wahab**
Kayu Ara Pasong, 82010
Pontian, Johor.

Pn. Norazwina Bte Zainol
Nama Penyelia

Tarikh : **2 Mei 2009** Tarikh: **2 Mei 2009**

CATATAN :
* Potong yang tidak berkenaan.
** Jika tesis ini **SULIT** atau **TERHAD**, sila lampirkan surat daripada pihak berkuasa/organisasiberkenaan dengan menyatakan sekali sebab dan tempoh tesis ini perlu dikelaskan sebagai **SULIT** atau **TERHAD**.
♦ Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana secara penyelidikan, atau disertasi bagi pengajian secara kerja kursus dan penyelidikan, atau Lapuran Projek Sarjana Muda (PSM).

"I hereby declare that I have read this thesis and in my opinion this thesis has fulfilled the qualities and requirements for the award of the degree of Bachelor of Chemical Engineering"

Signature : ……

Name of Supervisor : Pn. Norazwina Bte Zainol

Date : ……

BIOLOGICAL NUTRIENT REMOVAL USING SEQUENCING BATCH BIOREACTOR

MUHAMAD YASIN BIN 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 2009

DECLARATION

I declare that this thesis entitled *"Biological nutrient removal using sequencing batch bioreactor"* is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

Signature : ……

Name : Muhamad Yasin bin Ismail

Date : 2 May 2009

DEDICATION

Special Dedication to

Pn Naizum Zaidun, my beloved mother, you are everything to me,
En Ismail Nordin, my beloved father, you are my inspiration,

My family members, for your love and support,
My friends, for your care and support.

ACKNOWLEDGEMENT

In the name of Allah, the most Gracious and Merciful.

Praise to God for His help and guidance that I am finally able to complete this final year project as one of the requirement to complete my study.

First and foremost, I would like to extend my deepest gratitude to all parties directly involved in this project. A special thank to my supervisor, Pn. Norazwina bte Zainol for her willingness in overseeing the progress of my research work from its initial phases till the completion of this project. I do believe that all her advices and comments are for the benefit of producing the best research work.

Secondly, I would like to extend my words of appreciation to all my lecturers in the Faculty of Chemical Engineering and Natural Resources (FKKSA), for their support and motivation during this project development.

To all my close friends and all my course mates, thank you for believing in me and helping me to go through the difficult time. The experiences and knowledge I gained throughout the process of completing this final project would prove invaluable to better equip me for the challenges which lie ahead.

Last but definitely not least to my parents, I can never thank you enough for your love and support throughout my studies in University Malaysia Pahang (UMP).

ABSTRACT

In order to control the eutrophication problems, the removal of phosphorus has been increasingly important. The biological phosphorus removal using sequencing batch reactor (SBR) was investigated using simulated municipal wastewater. Experiments were carried out in eight sequencing batch reactors (SBRs) at hydraulic retention time (HRT) of 5 d. The experiment is operated at loading rates (LR) of 5.0, 4.5, 4.0, 3.5 and 3.0 mg/L.d. The mixed cultured growth is increased with the increasing of LR according to the suspended solids readings. The graph of the suspended solid concentration, the phosphorus removal and the chemical oxygen demand (COD) removal is almost same which is increasing by time and loading rate. The higher the loading rate, the higher the value of suspended solid, phosphorus removal and COD removal. LR 5 mg/L.d shows the highest suspended solid (SS) concentration with average value of 846.24 mg/L. The highest value of phosphorus removal is at loading rate 5.0 mg/L.d with average removal of 57.38%. The COD removal is also highest at loading rate 5.0 mg/L.d with average removal of 64.33%. The removal efficiency was influenced by the biofilm growth according to the suspended solid readings. The highest SS reading give the highest removal efficiency for both phosphorus and COD. According to the Design Expert plotted, the highest phosphorus removal can be achieved at LR 5.0 mg/L with 56.89% removal. Same result in Design Expert for COD removal which give the highest removal of 61.69% at LR 5.0 mg/L.d. As for the SS concentration, the Design Expert determine that the optimum value of SS is at LR 5.0 mg/L.d with the value of 840.92 mg/L.

ABSTRAK

Bagi mengawal masalah eutrofikasi, penyingkiran fosforus telah menjadi perkara yg semakin penting. Penyingkiran fosforus secara biologi menggunakan reactor sesekumpul berjujukan telah dilakukan menggunakan air sisa simulasi. Eksperimen telah dijalankan menggunakan 8 reaktor sesekumpul berjujukan dengan masa menahan hidraulik selama 5 hari. Eksperimen telah dijalankan pada kadar beban 5.0, 4.5, 4.0, 3.5, dan 3.0 mg/L.h. Pertumbuhan kultur campuran meningkat dengan peningkatan kadar beban berdasarkan bacaan pepejal terampai. Graf kepekatan pepejal terampai, penyingkiran fosforus dan penyingkiran kemahuan oksigen kimia hampir serupa iaitu meningkat berdasarkan masa dan kadar beban. Lebih tinggi kadar beban, lebih tinggi nilai pepejal terampai, penyingkiran fosforus dan penyingkiran kemahuan oksigen kimia. Kadar beban 5.0 mg/L.h menunjukkan kepekatan pepejal terampai yg paling tinggi dengan nilai purata sebanyak 846.24 mg/L. Nilai penyingkiran fosforus yg tertinggi ialah pada kadar beban 5.0 mg/L.h dengan nilai purata sebanyak 57.38%. Kadar penyingkiran kemahuan oksigen kimia yg tertinggi juga berada pada kadar beban 5.0 mg/L.h dengan nilai purata sebanyak 64.33%. Kadar keefektifan penyingkiran adalah didorong oleh pertumbuhan biofilem berdasarkan kepada bacaan pepejal terampai. Nilai pepejal terampai yang tertinggi memberikan kadar keefektifan penyingkiran yang tertinggi kepada fosforus dan kemahuan oksigen kimia. Berdasarkan hasil yg diplot menggunakan *Design Expert*, nilai penyingkiran fosforus yang tertinggi boleh dicapai pada kadar beban 5.0 mg/L.h dengan penyingkiran sebanyak 56.89%. keputusan yg sama di dalam *Design Expert* bagi penyingkiran kemahuan oksigen kimia yg mana memberikan nilai penyingkiran tertinggi sebanyak 61.69% pada kadar beban 5.0 mg/L.h. Begitu juga dengan kepekatan pepejal terampai, *Design Expert* menentukan nilai optimum bagi pepejal terampai ialah pada kadar beban 5.0 mg/L.h dengan nilai sebanyak 840.92 mg/L.

TABLE OF CONTENTS

LIST OF TABLE

LIST OF FIGURES

LIST OF ABBREVIATION

ACGIH	-	American Conference of Governmental Industrial Hygienists
A2O	-	Anaerobic-Anoxic-Oxic
BNR	-	Biological Nutrient Removal
BOD	-	Biological Oxygen Demand
COD	-	Chemical Oxygen Demand
CISTR	-	Continuous Ideally Stirred-Tank Reactor
CSTR	-	Continuous Stirred-Tank Reactor
DNOs	-	Denitrifying Organisms
EBPR	-	Enhanced Biological Phosphorus Removal
IR	-	Infrared
LR	-	Loading Rate
NIOSH	-	National Institute for Occupational Safety and Health
OSHA	-	Occupational Safety and Health Administration
PAHs	-	Polycyclic Aromatic Hydrocarbons
PAOs	-	Phosphate Accumulating Organisms
PO	-	Phosphorus
PO_4^{3-}	-	Phosphate
SBR	-	Sequencing Batch Reactor
SS	-	Suspended Solids
XMSM	-	Xanthomonas Maltophilia Selective Medium

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background

Water that is been used every day by humans comes from multiple sources such as streams, rivers and lakes. This makes the rivers an increasingly scarce resource. Water scarcity is in fact a result of excessive exploitation of water bodies which in turns may result in depletion of those resources. Human tend to have clean water but at the same time contribute to the increased rate of pollution in water. Many lakes, rivers, and streams are becoming increasingly polluted by industrial and agricultural activities, and most frequently by domestic wastes that are being discharge without proper treatment. The need of conducting a water quality treatment is significantly important for the evaluation of physical, chemical and biological nature of water in order to verify whether the observed water quality is suitable for the intended uses.

One of domestic wastes that known to be toxic and carcinogenic to loving organism is phosphorus. There are several physical, chemical and biological parameters to be taken into account as an indication of phosphorus pollution level. The excess content of phosphorus in receiving waters leads to extensive algae growth (eutrophication). Eutrophication is one of the main problems encountered in monitoring the environmental water sources in the industrial countries. It caused by the excess phosphorus concentration in the effluents from municipal or industrial plants discharged in the environment (Lenntech, 1998). The phenomenon of

eutrophication usually decreases the water quality and as a result it may increase significantly the cost of water treatment at treatment plants for surface water. The load of phosphorus discharged to receiving waters comes from various groups of sources of which the main sources are agricultural use of fertilizers, domestic and industrial wastewater, and atmospheric deposition. Eutrophication of waterways through delivery of phosphorus from farmland is an increasing problem in many countries (Haygarth and Jarvis, 1999). 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 14000 people daily. (Pink and West, 2006).

There are several methods that can be used in order to remove the phosphorus from wastewater such as trickling filter, biofilm process, activated sludges process and enhanced biological phosphorus removal. All of this methods have their own capabilities, advantages and disadvantages.

1.2 Problem Statement

Modernization and industrialization, added by the increase in the world population has greatly affected our river system. Industrial processes coupled by various domestic activities often produced discharged that are harmful and toxic to the environment. In a typical wastewater treatment, optimum dosing of the appropriate chemicals such as phosphorus may result in water being untreated and in the same time enhance pollution in water thus contribute to the poor surface water quality. When phosphorus occurs in nature this can be a serious danger to our health. Phosphorus is extremely poisonous and in many cases exposure to it will be fatal. Breathing phosphorus for short periods may cause coughing and irritation of the throat and lungs. Breathing phosphorus for long periods may cause a condition known as "phossy jaw" which involves poor wound healing of the mouth and breakdown of the jaw bone. Eating or drinking small amounts of phosphorus may cause liver, heart, or kidney damage, vomiting, stomach cramps, drowsiness, or death (Bowen *et al*. 1971; Eldad and Simon 1991).

This research is proposed because this method is quite economical because we are using microorganism that we get from drain. It is very easy to get microorganism from drain and to growth it into large scale. The usage of sequencing batch reactor with no aeration enables this system to operate with the minimal maintenance and because of using microflora, it is easy to maintenance due to its biological regeneration, thus this system is sustainable, effective and economical.

1.3 Objective

The main objectives of this research are

- To study the effects of different loading rate on the phosphorus waste water treatment.
- To study the effects of phosphorus to the growth of microflora.
- To study the amount of suspended solid that will exist during the experiment.

1.4 Research scope

The scope of study in this research is to treat phosphorus in waste water using appropriate nutrients from mixed culture in drain. This is done by monitoring the mixed culture growth using suspended solid test. The experiments are without aeration but with ambient oxygen. Each reactor has working volume 5 liter. The efficiency of treatment for different phosphorus concentration was evaluated in terms of water quality parameters (COD) and the removal of initial phosphorus concentration. Besides that, the effects of phosphorus concentration on biomass growth in terms of suspended solid that exists throughout experiments will also been studied.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Phosphorus is the chemical element that has the symbol P and atomic number 15. The name comes from the Greek word φώς (meaning "light") and φόρος (meaning "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, hence its Greek derivation, Φωσφόρος meaning "light-bearer" (Latin Lucifer), the planet Venus as "Morning Star". Phosphorus was first isolated in 1669 by Hennig Brand, a German physician and alchemist, by boiling, filtering and otherwise processing as many as 60 buckets of urine (Wikipedia, 2008).

Phosphorus was recognized as a chemical element at the emergence of the atomic theory that gradually occurred in the late part of the 18th century and the early 19th century, and was formulated by John Dalton. Phosphorus was first made commercially, for the match industry, in the 19th century, by distilling off phosphorus vapor from precipitated phosphates heated in a retort. The precipitated phosphates were made from ground-up bones that had been de-greased and treated with strong acids. This process became obsolete in the late 1890s when the electric arc furnace was adapted to reduce phosphate rock (Wikipedia, 2008).

2.2 Phosphorus

2.2.1 Properties of Phosphorus

Phosphorous is a multivalent nonmetal of the nitrogen group**.** Phosphorus has three main allotropes which is white, red and black. White phosphorus is poisonous and can spontaneously ignite when it comes in contact with air. Red phosphorus is formed by heating white phosphorus to 250°C (482°F) or by exposing white phosphorus to sunlight. Red phosphorus is not poisonous and is not as dangerous as white phosphorus, although frictional heating is enough to change it back to white phosphorus. Black phosphorus is also formed by heating white phosphorus, but a mercury catalyst and a seed crystal of black phosphorus are required. The chemical properties of phosphorus are given in Table 2.1. The physical properties of phosphorus are given in Table 2.2 (Busch and Marianna, 1999).

Table 2.1: Chemical Properties of Phosphorus

Atomic radius	93 pm
Atomic number	15
Atomic mass	30.973762(2) g·mol^{-1}
Oxidation states	± 3, 4, 5
Van der waals radius	1,04 Å

Table 2.2: Physical Properties of Phosphorus

Name, Symbol,	Phosphorus, P,
Chemical series	Nonmetals
Appearances	Waxy white, black, red, colorless, yellow
Density (near r.t.)	(white) 1.823 g·cm^{-3} (red) 2.34 g·cm^{-3}

	(black) 2.69 $g \cdot cm^{-3}$
Melting point	(white) 317.3 K (44.2 °C, 111.6 °F)
Boiling point	550 K (277 °C, 531 °F)
Heat of fusion	(white) 0.66 $kJ \cdot mol^{-1}$
Heat of vaporization	12.4 $kJ \cdot mol^{-1}$
Specific heat capacity (25 °C)	(25 °C) (white) 23.824 $J \cdot mol^{-1} \cdot K^{-1}$

2.2.2 Disadvantages of Phosphorus and effects to the environment

There are three allotropic forms of elemental phosphorus which are white, red, and black phosphorus. At room temperature, pure white phosphorus is a tetrahedral crystal with a molecular formula of P_4. In the pure form, white phosphorus is an ivory-colored, waxy solid. Phosphorus in its pure form has a white color. White phosphorus is the most dangerous form of phosphorus that is known to us. When white phosphorus occurs in nature this can be a serious danger to our health. White phosphorus is extremely poisonous and in many cases exposure to it will be fatal. In most cases people that died of white phosphorus exposure had been accidentally swallowing rat poison. Before people die from white phosphorus exposure they often experience nausea, stomach cramps and drowsiness. White phosphorus can cause skin burns. Skin contact with burning white phosphorus may burn skin or cause liver, heart, and kidney damage. While burning, white phosphorus may cause damage to the liver, the heart or the kidneys (Eldad and Simon, 1991).

Breathing white phosphorus for short periods may cause coughing and irritation of the throat and lungs. Breathing white phosphorus for long periods may cause a condition known as "phossy jaw" which involves poor wound healing of the mouth and breakdown of the jaw bone. Eating or drinking small amounts of white

phosphorus may cause liver, heart, or kidney damage, vomiting, stomach cramps, drowsiness, or death (Bowen *et al*. 1971; Eldad and Simon, 1991).

This phosphorus will also affect the environment. In water with low oxygen, white phosphorus may react with water to form a compound called phosphine. Phosphine is a highly toxic gas and quickly moves from water to air. Phosphine in air is changed to less harmful chemicals in less than a day. In water, white phosphorus builds up slightly in the bodies of fish (Garry *et al*., 1989).

2.2.3 Level of discharge for Phosphorus

Surface waters contain certain level of phosphorus in various compounds which is very important for living organisms. When the input of phosphorus to waters is higher than it can be assimilated by a population of living organism, then the problem occurs. Many countries set 1mg/L and 2mg/L as a limit for total phosphorus concentrations in discharges of wastewater treatment plants. The National Institute for Occupational Safety and Health (NIOSH), the Occupational Safety and Health Administration (OSHA), and the American Conference of Governmental Industrial Hygienists (ACGIH) have all set the inhalation exposure limit for white phosphorus in the workplace during an 8-hour workday at 0.1 milligram of white phosphorus per cubic meter of air (0.1 mg/m^3). For Malaysia, the level measurement to discharge phosphorus is 0.2 mg/L which has been set by the Department of Environment in national water quality standard in Malaysia.

2.3 Phosphorus Removal Method

2.3.1 Activated Sludge Process

A common method for the treatment of municipal and plant sewage wastes is the activated sludge process in which large numbers of microorganisms are present, and in fact necessary to treat the sewage. Activated Sludge Process is a biological treatment process in which a mixture of sewage and activated sludge is agitated and aerated. The activated sludge is subsequently separated from the treated sewage by settlement and may be re-used. Such systems generally comprise a rapid flocculation of the materials in the biomass followed by their entrapment and subsequent settling and the reduction of the sludge liquor Biological Oxygen Demand (B.O.D.) (Fremont and Henry A., 1981). One condition have been defined as essential for excess biological phosphorus uptake and storage which is exposing the activated sludge bacteria to influent wastewater in an anaerobic contacting zone, followed by an aerobic or anoxic zone (Barnard, 1976; Stensel, 1991).

The dynamics of microbial community structure of activated sludges in a small-scale domestic wastewater treatment process were examined using a novel approach of quinone profiles. The composition and content of quinones in the activated sludges were analyzed monthly over a period of one year. Activated sludge is a process in sewage treatment in which air or oxygen is forced into sewage liquor to develop a biological floc which reduces the organic content of the sewage. In all activated sludge plants, once the sewage has received sufficient treatment, excess mixed liquor is discharged into settling tanks and the supernatant is run off to undergo further treatment before discharge. Part of the settled material, the sludge, is returned to the head of the aeration system to re-seed the new sewage entering the tank. The remaining sludge is further treated prior to disposal. Activated sludge is the biomass produced in raw or settled wastewater (primary effluent) by the growth of organisms in aeration tanks in the presence of dissolved oxygen. The term activated comes from the fact that the particles are teeming with bacteria, and protozoa. Activated sludge is different from primary sludge in that the sludge

contains many living organisms which can feed on the incoming wastewater (Transgalactic Ltd., 2005).

The term anaerobic digestion can be define as a naturally occurring decomposition of organic material by numerous strains of bacteria working together in an oxygen free environment. The primary products formed during the decomposition of the organic material are sugars, organic acids, alcohols, methane, carbon dioxide, and hydrogen. Nutrients are preserved during the anaerobic digestion process. Nitrogen actually becomes more usable by plants during the anaerobic digestion process. Phosphorus and potassium along with other nutrients and undigested fiber can be returned to the soil and act as excellent soil amendments (Farm Bureau Seminar, February 28, 2007). In an anaerobic system, there is an absence of gaseous oxygen. In an anaerobic digester, gaseous oxygen is prevented from entering the system through physical containment in sealed tanks. Anaerobes access oxygen from sources other than the surrounding air. The oxygen source for these microorganisms can be the organic material itself or alternatively may be supplied by inorganic oxides from within the input material. When the oxygen source in an anaerobic system is derived from the organic material itself, then the 'intermediate' end products are primarily alcohols, aldehydes, and organic acids plus carbon dioxide. In the presence of specialized methanogens, the intermediates are converted to the 'final' end products of methane, carbon dioxide with trace levels of hydrogen sulfide. In an anaerobic system the majority of the chemical energy contained within the starting material is released by methanogenic bacteria as methane. The phosphorus removal performance of this system was attractive. Phosphate release under anaerobic conditions and phosphate uptake under aerobic conditions was significant. This phenomenon was consistent with the conventional enhanced biological phosphorus removal (EBPR) processes reported by Mino *et al*. in 1998.

The anaerobic–anoxic–oxic (A2O) activated sludge process has been currently modified from the conventional activated sludge process to simultaneously remove nutrient such as phosphorus in wastewater which will otherwise be the critical nutrient for eutrophication of closed water body. The anaerobic and anoxic

zones of this biological nutrient removal (BNR) process selectively favor the floc-forming microorganisms as example the phosphate accumulating organisms (PAOs), denitrifying organisms (DNOs), and discourage the possible filamentous microorganisms; therefore, the anaerobic and anoxic zones are usually termed as anaerobic and anoxic selectors, respectively (As example of activated sludges process, each aeration basin received identical wastewater which was the effluent from a high rate trickling filter. One of the aeration basins was dosed with aluminum sulfate for the purpose of phosphorus removal. The other aeration basin (control) was operated in the conventional manner without alum addition. Plate counts performed on combined chemical-biological sludge and control activated sludge revealed that a higher number of viable micro-organisms was contained in the chemical-biological sludge, but the magnitude of difference between the two sludges was significant depending on the culture medium employed. Results suggest the aluminum flocs formed in the chemical-biological treatment enmesh dispersed wastewater micro-organisms, some of which are qualitatively unlike those indigenous to natural activated sludge. The combined chemical-biological sludge contained significantly higher numbers of lipolytic, gelatinolytic, and thiosulfate oxidizing micro-organisms and, possibly, fewer nitrite oxidizing micro-organisms than did control activated sludge. Alum did not appear to affect flagellated protozoa in mixed liquor; however, amoeboid and ciliated protozoa were found less frequently in alum dosed than in control mixed liquor. The settled effluent from the combined chemical-biological aeration basin generally contained fewer total coliforms, fecal coliforms, and fecal streptococci than did counterpart control effluents (Judith *et al*., 2003).

2.3.2 Trickling Filter

Trickling filters are one of the oldest types of biological filters. They are still the most common type of biological filter for several reasons. They are reliable, simple, effective, rugged and inexpensive when built properly. There are much fancier, complicated and sexy biological filters available for aquaculture but fancy, complicated and sexy typically mean more money as in Figure 2.1 and Figure 2.2. In

the late 1800 the first trickling filters were built for sewage treatment. These filters were filled with rock or coal. Incredibly, some trickling filters are still built with rock or gravel today. Unfortunately, the high capital cost and maintenance costs associated with this type of filter lead some people to think that trickling filters are an outdated and inefficient type of biological filter (Smith, 2003).

Trickling filters are rugged and easy to operate. They can be very simple to build. Trickling filters are completely scalable and they can be built to handle water flows from 4 to 4 million GPM. They have the ability to treat a wide variety of nutrient levels. Properly designed systems can handle solids very well. One of the big advantages of a trickling filter is that the water can leave with more oxygen than it entered. Because trickling filters have a large - air water interface, they also act as strippers to remove CO2, H2S, N2 or other undesirable volatile gases. Very few other types of biofilters perform all these functions. There are only two minor disadvantages to trickling filters. One is the energy cost required to pump the water to the top of the filter. However, trickling filters are typically more energy efficient than bead filters. The other disadvantage to trickling filters is their size. They are larger and take more space than some other types of biofilters (Smith, 2003).

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 (Beychok, 2007).

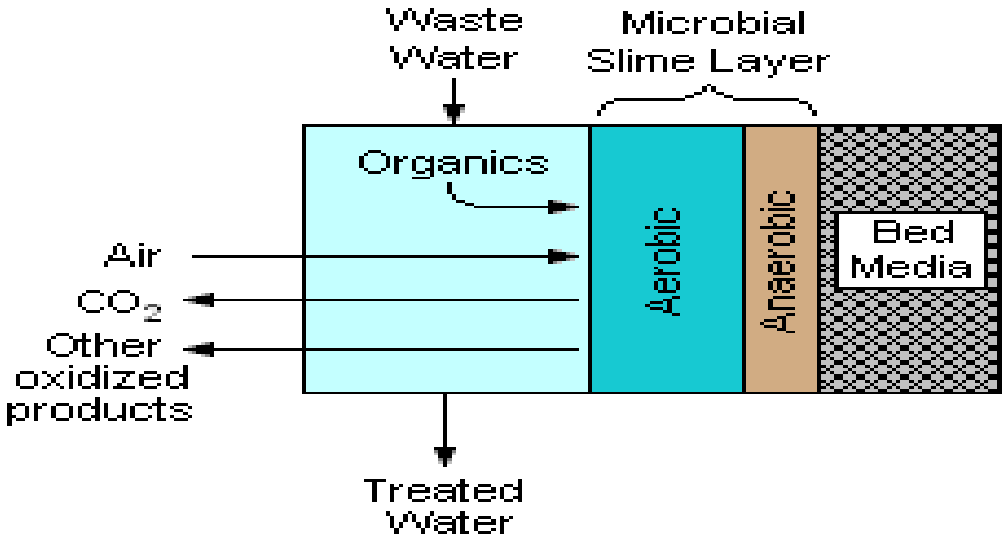


Figure 2.1: Filter Process

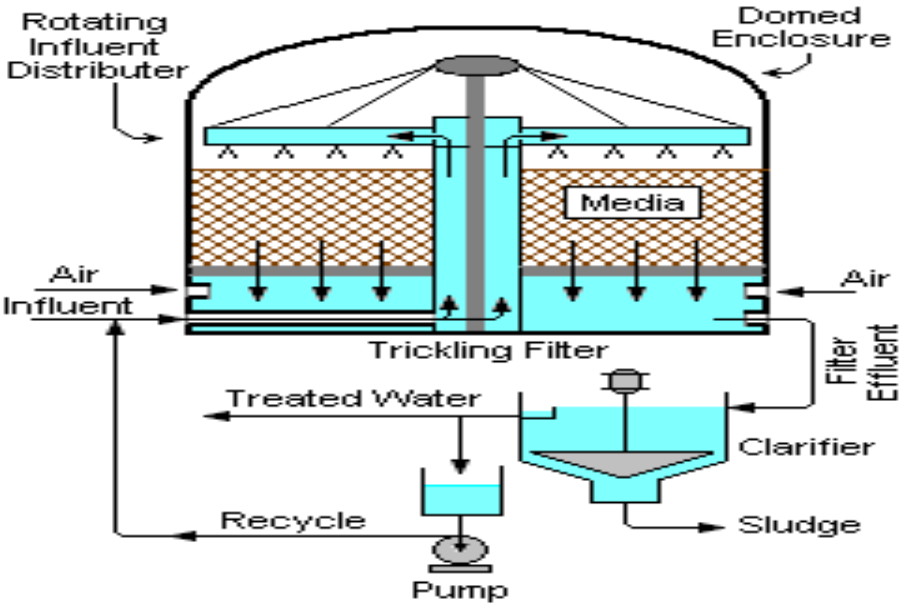


Figure 2.2: Trickling Filter

2.3.3 Biofilm Process

A biofilm is a complex aggregation of microorganisms growing on a solid substrate. Biofilms are characterized by structural heterogeneity, genetic diversity, complex community interactions, and an extracellular matrix of polymeric substances. Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. These first colonists adhere to the surface initially through weak, reversible van der Waals forces. If the colonists are not immediately separated from the surface, they can anchor themselves more permanently using cell adhesion molecules such as pili. The first colonists facilitate the arrival of other cells by providing more diverse adhesion sites and beginning to build the matrix that holds the biofilm together. Only some species are able to attach to a surface on their own. Others are often able to anchor themselves to the matrix or directly to earlier colonists. Once colonization has begun, the biofilm grows through a combination of cell division and recruitment (Transgalactic Ltd, 2005).

Biofilms are usually found on solid substrates submerged in or exposed to some aqueous solution. Biofilms consist of many species of bacteria and archaea living within a matrix of excreted polymeric compounds. This matrix protects the cells within it and facilitates communication among them through chemical and physical signals. Some biofilms have been found to contain water channels that help distribute nutrients and signalling molecules. This matrix is strong enough that in some cases, biofilms can become fossilized. Bacteria living in a biofilm can have significantly different properties from free-floating bacteria, as the dense and protected environment of the film allows them to cooperate and interact in various ways. One benefit of this environment is increased resistance to detergents and antibiotics, as the dense extracellular matrix and the outer layer of cells protect the interior of the community. Biofilms can also be harnessed for constructive purposes. For example, many sewage treatment plants include a treatment stage in which waste water passes over biofilms grown on filters, which extract and digest harmful organic compounds (Transgalactic Ltd, 2005).

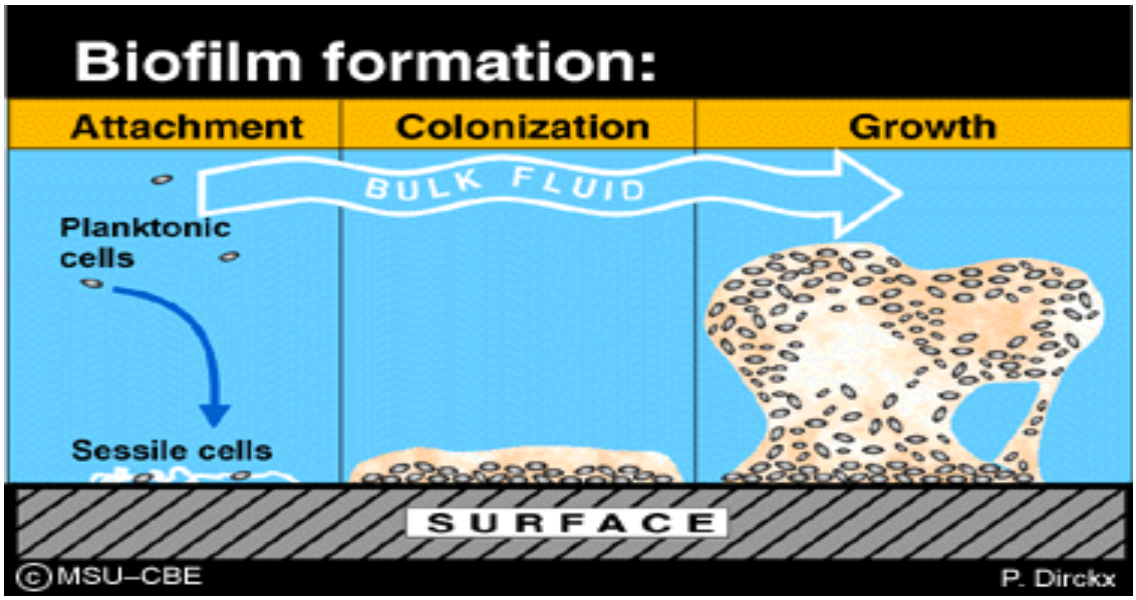


Figure 2.3: Biofilm formation

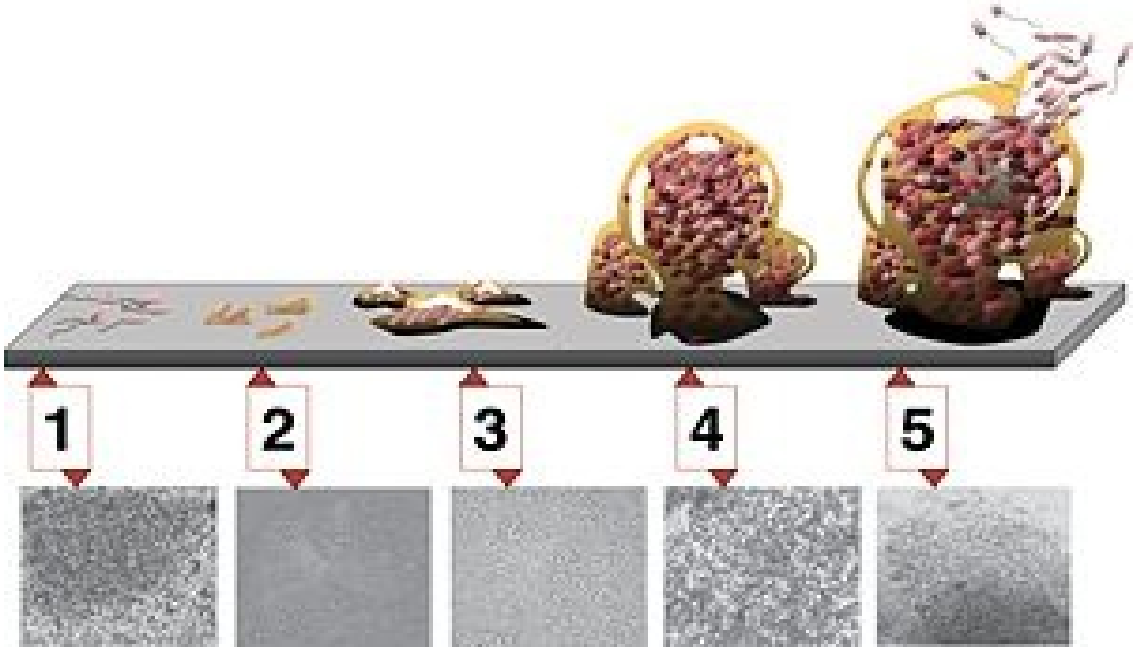


Figure 2.4: Biofilm formation

The biofilm process includes 5 main stages which is initial attachment, irreversible attachment, maturation I, maturation II and dispersion like shown in Figure 2.3. Formation of a biofilm begins with the attachment of free-floating microorganisms to a surface. These first colonists adhere to the surface initially through weak, reversible van der Waals forces like in Figure 2.4. If the colonists are not immediately separated from the surface, they can anchor themselves more

permanently using cell adhesion structures such as pili. The first colonists facilitate the arrival of other cells by providing more diverse adhesion sites and beginning to build the matrix that holds the biofilm together. Some species are not able to attach to a surface on their own but are often able to anchor themselves to the matrix or directly to earlier colonists. It is during this colonization that the cells are able to communicate via quorum sensing. Once colonization has begun, the biofilm grows through a combination of cell division and recruitment. The final stage of biofilm formation is known as development, and is the stage in which the biofilm is established and may only change in shape and size. This development of biofilm allows for the cells to become more antibiotic resistant (Wikipedia, 2008).

2.3.4 Selection of Biofilm as Removal Method

The biofilm process has been chosen as the removal process because of several reasons. The first one is the costing. The biofilm process is very economic compared to the other process because of the system itself and the retention time. Biofilm will decreases the filtration time from months to days. The second reason is the oxygen supply. In my experiment, I'm dealing with aerobic situation but without aeration. Only using the ambient air. The biofilm process is very good in the aerobic condition. The last reason is the system itself. In biofilm, the system is more simple and easy enough to handle and maintenance. So, it will be easier doing biofilm if comparing with activated sludge process or trickling filter.

2.4 Mixed Culture

2.4.1 Mixed Culture from River

Bacillus spp was on type bacteria found in river. There are six main types of Bacillus spp but the most famous was Bacillus cereus. Although Bacillus spp used in

degradation of petroleum pollutant but it have bad effect in our health (okerentugba *et al*., 2003). Bacillus anthracis can causes anthrax.Bacillus subtilis, Bacillus licheniforms, Bacillus amyloliquefaciens and Bacillus pumilis all cause food borne illness. There are symptoms causes by Bacillus spp which were nausea, vomiting, stomach pains, diarrhea, headache and flushing (Lancaster City Council, 2005).

Aspergillus was one of fungi found in river. It also used in degradation of petroleum pollutant (okerentugba *et al*., 2003). Aspergillus was a genus of around 200 molds found throughout much of nature worldwide. Aspergillus was first catalogued in 1729 by the Italian priest and biologist Pier Antonio Micheli. Aspergillus species were highly aerobic and were found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a substrate, as a result of the high oxygen tension. Aspergillosis was the group of diseases caused by Aspergillus. The most common subtype among paranasal sinus infections associated with aspergillosis is aspergillus fumigatus. The symptoms include fever, cough, chest pain or breathlessness, which also occur in many other illnesses so diagnosis can be difficult. Usually, only patients with already weakened immune systems or who suffer other lung conditions are susceptible. (Wikipedia, 2008).

The other bacterium found useful in degradation of petroleum pollutant was Micrococcus spp. In the experiment, result show that the pH of Micrococcus was 8. (Okerentugba *et al*., 2003). It categorized in gram-positive cocci. Most strains are saprophytic and non-pathogenic found in soil, water, dust, frequently found on the skin of man and other animals. Hazard of infection is low and there weren't any report about the infection by this bacterium (Health Canada, 2001).

2.4.2 Mixed Culture from Soil

Bacteria in soil occur singly and in aggregates. To estimate the number of bacteria in a gram of soil, the soil must be both diluted and mixed thoroughly so that the aggregates are broken up such that a suspension of single cells is achieved.

Pseudomonas species is new discovered bacterium in soil. Some of it species used in degradation of wastewater especially in degradation of cyanides, thiocyanate and toluene. Some studies have done on Pseudomonas species. Optimum growth rates and maximum population yields of the four strains in distilled water were obtained at 37 C, although high population levels (106-107/ml) were reached and maintained over extended incubation periods at temperatures from 18 C to 42 C. Two strains were able to grow in distilled water at temperatures ranging from 12 C to 48 C and to survive 48 hand 21 days at 50 C and 10 C, respectively (Carson *et al*., 1972).

The growth and biodegradation rates of the mixed cultures did not increase over those of the individual strain. The mixed culture isolated from soil in oil refinery sites could offer advantages for several reasons. The first one is most of them are adapted to this contaminated environment to allowing the inoculums to survive, and the second one is as they are able to extend through the soil by propagation, bacteria can access xenobiotics. Therefore, the present study aimed to investigate the biodegradation potential of the enriched mixed bacterial cultures obtained from different soils, which were contaminated with petroleum hydrocarbons in oil refinery fields, and to test any relationship between polycyclic aromatic hydrocarbons (PAHs) contamination in soil and phenanthrene biodegradation (Kim *et al*., 2004).

2.4.3 Mixed Culture from Drain

Pseudomonas aeruginosa is a major cause of nosocomial infections that are difficult to treat, partly because of a relatively high intrinsic resistance of the bacterium to clinically useful antibiotics and partly due to the formation of antibiotic-tolerant biofilms. The bacterial cell used in biosorption studies was P. aeruginosa and it was identified according to . The tolerance to heavy metals was determined by the agar dilution method and it was found that it was highly resistant to nickel (300 ppm) and lead (500 ppm) on tris minimal agar medium. The organic functional groups and their corresponding wave numbers were identified in the lyophilized cells

of P. aeruginosa ASU 6a biomass. A fast look on Figure 2.5(a) below can recognize the presence of amine R–NH_2 (amino acids, proteins, glycoproteins, etc.), carboxylic acids (fatty acids, lipopolysaccharides, etc.), sulfonates and phosphates. . The IR spectra of Ni- and Pb-loaded biomass are shown in Figure 2.5(b) and Figure 2.5(c). A trial to compare the native biomass with that found in the case of metal-loaded one can reveal that the bands that appeared at 1075 and 1034 cm^{-1}may be due to the interaction of adsorbed metals with phosphate groups. It was also assumed that P. aeruginosa ASU 6a contains S_1 (COOH), S_2 (NH_2) and S_3 PO_4^{3-} acidic sites capable of binding protons (Shoreit *et al*.**,** 2008).

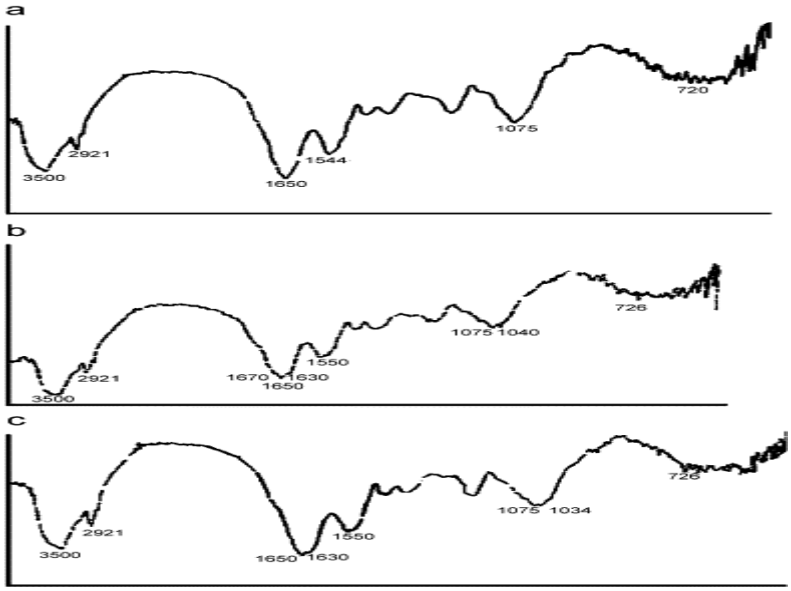


Figure 2.5: IR Spectra for P.Aeruginosa

The second bacterium in drain is Stenotrophomonas Maltophilia. Stenotrophomonas maltophilia is an opportunistic pathogen ubiquitous in nature. Little is known about acquisition of the organism in the community, and hospital sources of infection or colonisation are rarely identified. Historically, there have been problems with media for the culture of S. maltophilia from the environment, being expensive and laborious to prepare, and lacking in sensitivity or specificity. These issues may have hampered efforts to identify environmental sources of the organism (Kerr *et al*., 1996) developed VIA medium for the selective culture of S.Maltophilia from clinical and environmental samples. It demonstrated superiority over Xanthomonas maltophilia selective medium (XMSM) (Juhnke and Jardin,

1989) with regards to ease and cost of preparation, specificity, and sensitivity, when evaluated using faecal samples. The medium incorporated vancomycin, imipenem and amphotericin B as selective agents, and a mannitol/bromothymol blue indicator system. Unfortunately, imipenem is now largely unobtainable. A modification of the medium, incorporating meropenem instead of imipenem was evaluated in an environmental survey of Sir Charles Gairdner Hospital, a large Western Australian teaching hospital, and surrounding locations in the Perth metropolitan area. All but two non-S. maltophilia isolates (95%) were oxidase positive; oxidase negative, non-S. maltophilia isolates were unable to grow on MacConkey type 3 (MCC). S. maltophilia can therefore be easily distinguished from contaminating bacteria **(**Riley *et al*., 2008).

2.4.4 Selection Mixed Culture of Drain

For this study, mixed culture from the drain is selected to be used in removal of phosphorus. This is supported by fact that the bacteria contain in drain such as Pseudomonas Aeruginosa has the ability to catch phosphorus while the removal process was done (Gersberg and Alien, 1985; Suresh *et al*., 1985; Buchan, 1981; Lotter, 1985). So, the removal can be done successfully. The second factor is because the mixed culture from drain is easy to get and easy to handle.

2.5 Reactor

2.5.1 Batch Reactor

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 batch reactor is used for small-scale operation,for testing new process that have not been fully developed, for manufacture of expensive product, and for process that are difficult to convert to contionous operation (Fogler, 2006).

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 1 litre to more than 15,000 litres. 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 (Wikipedia, 2008).

The usual agitator arrangement is a centrally mounted driveshaft with an overhead drive unit as shown in Figure 2.6. Impeller blades are mounted on the shaft. A wide variety of blade designs are used and typically the blades cover about two thirds of the diameter of the reactor. Where viscous products are handled, anchor shaped paddles are often used which have a close clearance between the blade and the vessel walls. Most batch reactors also use baffles. These are stationary blades which break up flow caused by the rotating agitator. These may be fixed to the vessel cover or mounted on the side walls. Despite significant improvements in agitator blade and baffle design, mixing in large batch reactors is ultimately constrained by the amount of energy that can be applied. On large vessels, mixing energies of more than 5 Watts per litre can put an unacceptable burden on the cooling system. High agitator loads can also create shaft stability problems. Where mixing is a critical parameter, the batch reactor is not the ideal solution. Much higher mixing rates can be achieved by using smaller flowing systems with high speed agitators, ultrasonic mixing or static mixers (Wikipedia, 2008).

Figure 2.6: Batch Reactor with Single Cooling Jacket

2.5.2 Continuous Stirred-Tank Reactor(CSTR)

The continuous stirred-tank reactor (CSTR), also known as 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 which is 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 assume 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 (Wikipedia, 2008).

Integral mass balance on number of moles N_i of species i in a reactor of volume V.

[accumulation] = [in] - [out] + [generation]

1. $$\frac{dN_i}{dt} = F_i o - F_i + V \nu_i r_{i\,[1]}$$

where F_{io} is the molar flow rate inlet of species i, F_i the molar flow rate outlet, and ν_i stoichiometric coefficient. The reaction rate, r, is generally dependent on the reactant concentation and the rate constant (k). The rate constant can be figured by using the Arrhenius temperature dependence. Generally, as the temperature increases so does the rate at which the reaction occurs. Residence time, τ, is the average amount of time a discrete quantity of reagent spends inside the tank (Wikipedia, 2008).

These types of reactors employ a stirred tank, to which reactants are continuously added and products continuously withdrawn. 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:

- 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.
- The reaction proceeds at the reaction rate associated with the final (output) concentration.
- 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.
- It can be seen that an infinite number of infinitely small CSTRs operating in series would be equivalent to a PFR.

2.5.3 Sequencing Batch Reactor (SBR)

Sequencing batch reactors are conventionally utilized for waste water treatment to provide a predetermined sequence of treatment conditions. In this regard, waste water may typically be introduced into a sequencing batch reactor treatment system and subjected to extensive mixing and aeration for a predetermined period of time to provide biological oxidation or consumption of waste water components. The mixing and aeration may subsequently be stopped and the waste water maintained in a quiescent state to permit waste water solids, including microbiological treatment organisms, to settle in the reactor. A clarified portion of the treated waste water may be removed from the upper portion of the reactor, which in turn may be conducted to subsequent treatment and discharge steps. Additional waste water which is to be treated may then be introduced into the sequencing batch reactor, and the cycle repeated. Although sequencing batch reactor systems have significant potential as efficient, flexible and economical waste water treatment systems, conventional systems for removing the clarified waste water in such systems have various disadvantages and have imposed significant limitations on the utility of sequencing batch reactor systems. For example, conventional decanting apparatus has been utilized which is positioned above the water level of the reactor. Such decanting apparatus is subject to freezing in cold weather, and may be adversely affected in its operation by scum accumulation on the surface of the biological reactor, which may freeze to impede free liquid transfer or operation of the decanting apparatus (Carucci *et al*., 1995). Other difficulties include a tendency for the mixed liquor solids in the reactor to migrate into the decanter during the aeration and mixing reaction period of operation, such that the initial portion of the decanted liquid contains a significant amount of waste water solids which must be rerouted to the input end of the reactor to avoid contamination of the clarified discharge liquid (Mikkel, 1986).

To optimize the performance of the system, two or more batch reactors are used in a predetermined sequence of operations. SBR systems have been successfully used to treat both municipal and industrial wastewater. They are uniquely suited for wastewater treatment applications characterized by low or

intermittent flow conditions. Fill-and-draw batch processes similar to the SBR are not a recent development as commonly thought. Between 1914 and 1920, several full-scale fill-and thaw systems were in operation. Interest in SBRs was revived in the late 1950s and early 1960s, with the development of new equipment and technology. Improvements in aeration devices and controls have allowed SBRs to successfully compete with conventional activated sludge systems (TJSEPA, 1999).

In SBR operation, each reactor in the system has five basic operating modes or periods. The periods are fill, react, settle, draw and idle. SBR technology has gained more and more importance in wastewater treatment plants (Schiegl *et al*., 1996; Franta *et al*., 1997). The main advantages are easy operation, low cost, handling hydraulic fluctuation, no need for settling tank and sludge recycling as well as organic load without any significant variation in removal efficiency (Kolb and Wildere, 1997; Keudel and Dichtl, 2000).

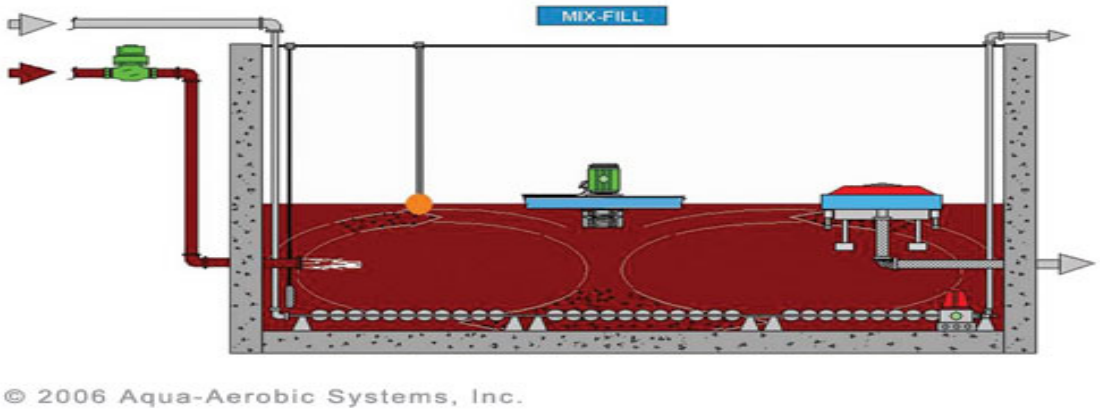


Figure 2.7: Mix Fill Process

MIX FILL:

- Influent enters reactor as Figure 2.7.
- Complete mix of contents is achieved without use of aeration
- Controls filamentous organisms
- Essential for systems requiring phosphorus removal

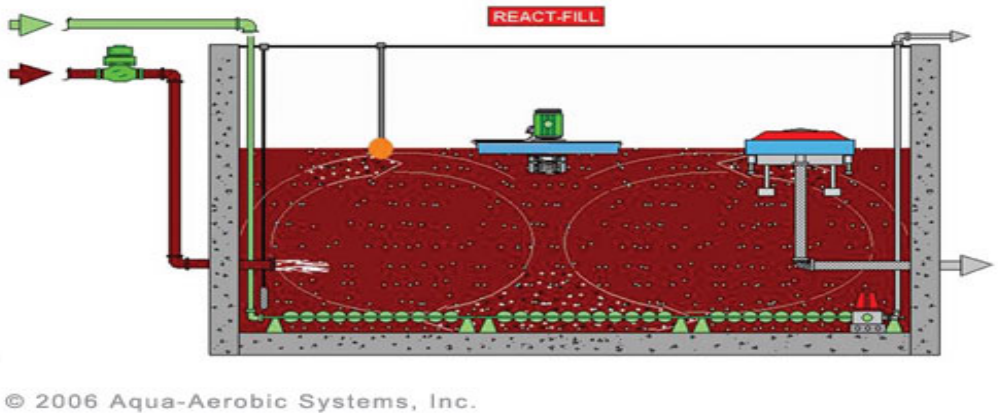


Figure 2.8: React Fill Process

REACT FILL:

- Influent flow continues under mixed and aerated conditions as Figure 2.8.
- Aeration may be intermittent to promote aerobic or anoxic conditions
- Nitrification and denitrification is easily managed
- Aeration source may also be turned down during low flow conditions to conserve energy

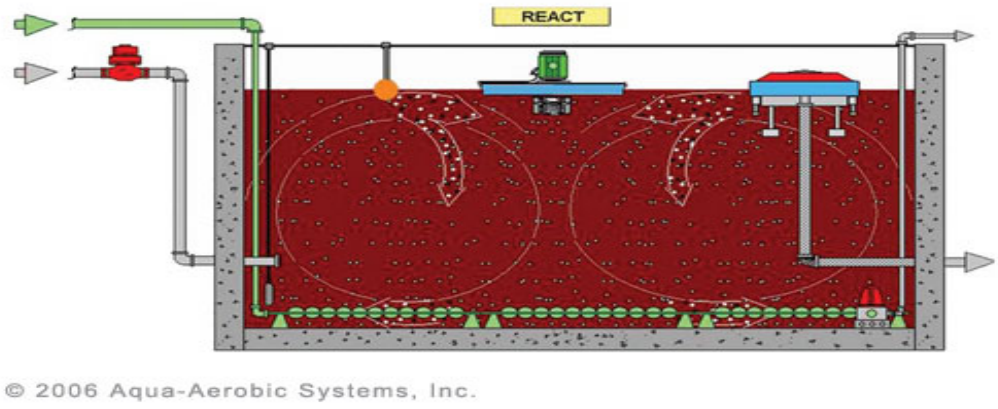


Figure 2.9: React Process

REACT:

- Influent flow is terminated as Figure 2.9.
- Mixing and aeration continue in absence of raw waste

- Dissolved oxygen probes can be used to deliver oxygen on "as needed" basis without loss of mixing
- Provides a treatment barrier that separates the Fill phases from the Settle and Decant Non-Fill phases

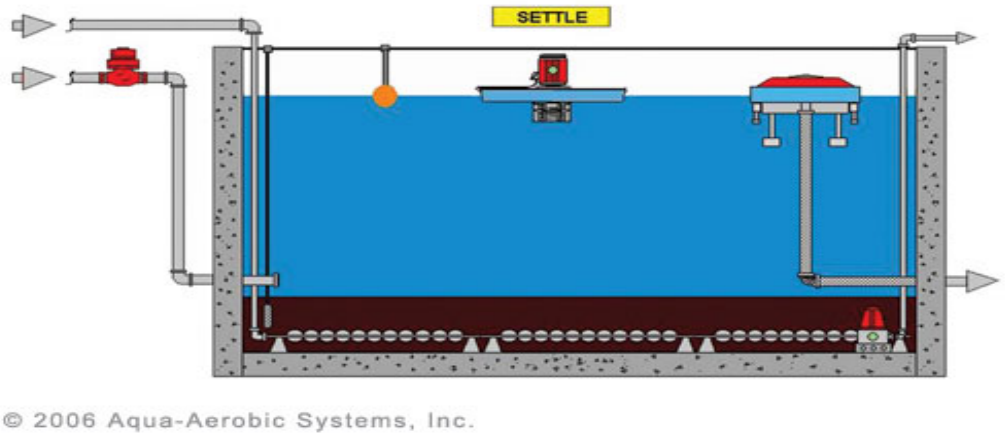


Figure 2.10: Settle Process

SETTLE:

- Influent flow does not enter reactor as Figure 2.10.
- Mixing and aeration cease
- Ideal solids/liquid separation is achieved due to perfectly quiescent conditions
- Adjustable time value allows settling time to match prevailing process needs

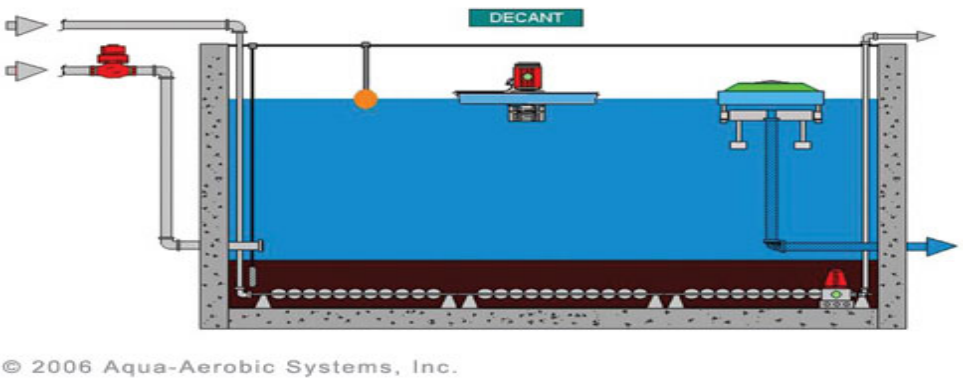


Figure 2.11: Decant Process

DECANT/SLUDGE WASTE:

- Influent flow does not enter reactor as Figure 2.11.
- Mixing and aeration remain off
- Decantable volume removed by subsurface withdrawal
- Reactor is immediately ready to receive next batch of raw influent
- A small amount of sludge is wasted near end of each cycle

2.5.4 Selection of sequencing batch reactor

The Sequencing Batch Reactor (SBR) has been chosen as the reactor in this experiment because SBR technology has gained more and more importance in wastewater treatment plants (Schiegl *et al*., 1996; Franta *et al*., 1997). The second reason is because SBR can optimize the performance of the system, two or more batch reactors are used in a predetermined sequence of operations. SBR systems have been successfully used to treat both municipal and industrial wastewater.

CHAPTER 3

METHODOLOGY

3.1 Sampling Technique

Mixed culture from drain is selected to be used in the process of removing the phosphorus. Take 4L of drain water from the drain in University Malaysia Pahang. Fill the drain water into the acclimatization reactor. Before filled the drain water, the acclimatized reactor must first been filled with rocks as support media for the bacteria. After that, fill the reactor with 6L of deionized water that have been autoclave. Then, mixed the acclimatized reactor by shaking it. The mixed cultures must been acclimatized in the acclimatization reactor for 1month to make it growth and adapt to the new environment. For the 1st week, take suspended solid test every day without feeding the mixed culture. After 1week the acclimatize reactor been mixed and everyday been tested the suspended solid, the mixed cultured have to be feed by glucose solution that have been autoclave. The suspended solid test must to do every day until the suspended solid stable and can degrade the phosphorus in the treatment reactor. Before starting the treatment reactor, the bacteria in the acclimatization reactor must first been fed with small amount of phosphorus in order to get them adapt with the new environment and make sure they can degrade the phosphorus.

3.2 Preparation Of Glucose Stock Solution

Get 10g glucose powders. Then, mixed the glucose powder into 1L distilled water to give the concentration of 1g/L. The mixture is autoclaved at 121°C and then cooled until it reached room temperature. Then, the stock solution is stored in the chiller. For feed the mixed culture, 1L solutions from the acclimatization reactor are discarded. Then, 10ml solution that has been discarding is taken for suspended solid test and the remaining solution is let for 20minutes until the precipitate settled down. After it settled down, about 650 ml solutions are discarded and assured the precipitate is not discarded together. Then, 20ml glucose stock solution are measured and mixed with remaining solution above. Distilled water that has been autoclaved 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.

3.3 Reactor System

For this research, sequencing batch reactor (SBR) has been selected as reactor system. In this study, 8 reactors with each capacity of 5 liter will be used as treatment reactor as shown in Figure 3.1. For acclimatization reactor, a 10 liter reactor will be used.

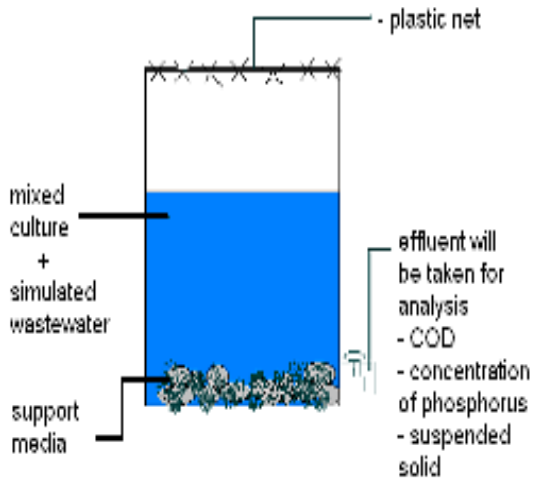


Figure 3.1: Sequencing batch reactor

This reactor can be filled with influent through the hole at the top. The effluent will be taken through a discharge pipe at the side bottom of the reactor for analysis. The rocks that have diameter in range of 1.5 to 2.0 cm are placed in the reactor as a support media for the mixed culture.

3.4 Operational Condition

This research will be conducted in two stages processes, which are acclimatization process and treatment process. In acclimatization process, the mixed culture isolated from drain is acclimatized in acclimatization reactor for two months period. Within this period, trace amount of phosphorus is added to the reactor in order to acclimatize the mixed culture with the treatment environment. By then, the mixed culture is fed with glucose as well.

For the treatment process, the mixed culture from acclimatization reactor will be transferred to the treatment reactor. The phosphorus treatment process will be carried out by controlling the loading rate. The hydraulic retention time (HRT) selected for this research is 2 days. The experiment will be run for eight (8) different loading rates will be run simultaneously in each cycle. The collection of the sample (effluent) will be done for each day within the two weeks period as well as the addition of the simulated wastewater (influent) to the treatment reactor. The details about the treatment process are summarizes in Table 3.1.

Table 3.1: Details of phosphorus treatment process

Loading Rate (mg/L.day)	Concentration (mg/L)	Hydraulic Retention Time(day)	Flowrate (L/ Day)
5.00	10	5	1
4.50	9	5	1
4.00	8	5	1
3.00	6	5	1

4.00	8	5	1
3.50	7	5	1
5.00	10	5	1
3.00	6	5	1

3.5 Chemical Composition

For this research, phosphorus powder will be dissolved into deionized water to produce stock solution of phosphate in which the solution will be the simulated wastewater for this research. The concentration of phosphate ion (PO_4^{3-}) used are based on the loading rate of the mixed culture that has been decided as shown in Table 3.3.1. The stock solution with concentration of 1g/L is provided for two weeks treatment and supply for four different loading rates. From this stock solution, it will dilute in deionized water to produce 4L phosphate solution for start the experiment and 1L phosphate solution to add into the reactor everyday. There are also glucose stock solution that must be prepared to fed the bacteria everyday. 20ml of glucose solution must be fed to the bacteria everyday.

3.6 Analytical Method

In this research, HACH Spectrophotometer will be used to analyze the parameters which are Chemical Oxygen Demand (COD), Suspended Solid (SS) and the phosphorus concentration. For suspended solids test, two sample cells are used. 25 ml 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. 2 ml sample is added to the first vial and 2 ml 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. 5 ml sample is added to the vials and then tested using the barcode program in the HACH Spectrophotometer DR 2800.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Biomass concentration in Acclimatization Reactor

The graph in Figure 4.1 shown that the trend for suspended solid concentration is increasing but in fluctuation form. During day 1 until 8 the value of suspended solid decreased. This happen due to the porous media. It creates a space for the bacteria to attach themselves in the porous support media (Dupin *et al*., 2000; Paulsen *et al*., 1997; Vayenas *et al*., 2002). Starting from day 8, the concentration of suspended solid increase. This is because at the first 7 day, the bacteria were not been fed but it started to been fed on the 8th day. After a certain period, during unfed part, the formation will decrease and then it will increase again when the microorganism being fed again (Zacheus, 1999). Bacteria will attach to solid surfaces to take advantage of the organic molecules adsorbed there. After being attached for some time, the microorganisms start to consume nutrients, grow, reproduce, and also produce extracellular polymeric substances which bind the cells together which will increase the concentration of suspended solid (Characklis and Marshall, 1990; Costerton and Scott, 1995).

Based on point in Figure 4.1, it shows that from day 8 until 88, the concentration of suspended solid is in the increasing trend but fluctuation. This is due to the bacteria itself. The bacteria are capable to swim in their own and random direction and make the reading unstable and fluctuation (Murphy *et al*., 2000; Ginn *et al*., 2002). From day 38, the value of suspended solid slowly increased and then become constant. During this period, the mixed culture starts to adapt with the new

environment and colonies. But as overall in the acclimatization reactor, the trend and the formation of biofilm is increase. This is because the bacterial activity in the meadows examined increased significantly in response to nutrient additions means as long as the bacteria been fed, the concentration of suspended solid will increase (López *et al*., 1998).

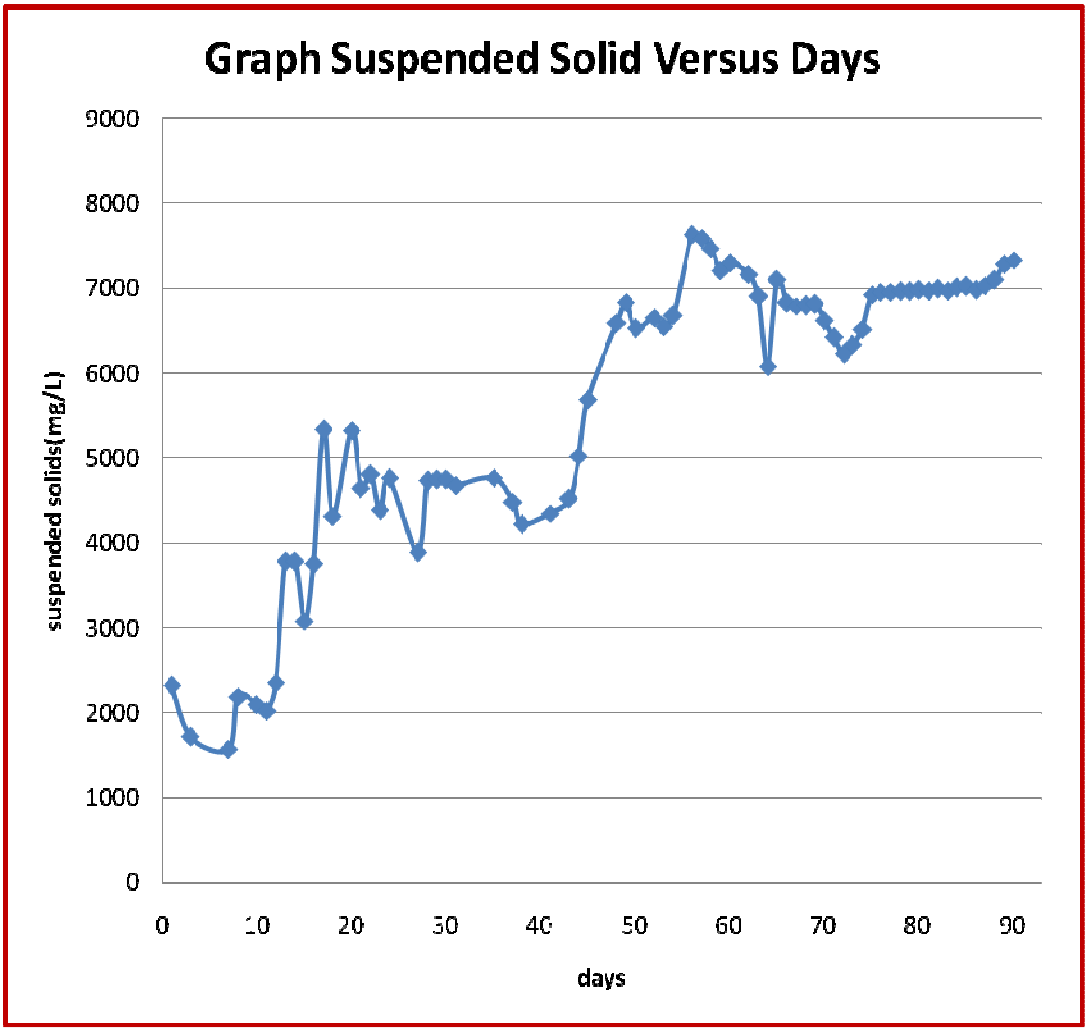


Figure 4.1: Suspended solid of acclimatization reactor

4.2 Effect of Loading Rate on Biomass Growth

From Figure 4.2, the highest value of suspended solid is 846.24 mg/L at loading rate 5.0 mg/L.d. The lowest value of suspended solid is 653.93 mg/L at loading rate 3.0 mg/L.d. It can be conclude that the highest concentration of phosphorus give the highest reading in suspended solid concentration. At initial of the treatment it shown that the concentration of suspended solid is decreased. This is because of the porous media. It creates a space for the bacteria to attach themselves in the porous support media (Dupin *et al*., 2000; Paulsen et al., 1997; Vayenas *et al*., 2002). Starting from day 3, the concentration of suspended solid increase. This is because after being attached for some time, the microorganisms start to consume nutrients, grow, reproduce, and also produce extracellular polymeric substances which bind the cells together which will increase the concentration of suspended solid (Characklis and Marshall, 1990; Costerton and Scott, 1995). The higher the loading rate, the higher the concentration of suspended solid. This is because the bacteria from drain such as Pseudomonas adapt to eat the phosphorus even at concentration of 5.0 mg/L and the higher the concentration, the higher amount of food and it can be eat by the microorganism and this makes the concentration of suspended solid increase (Gersberg and Alien, 1985; Suresh *et al*., 1985; Buchan, 1981; Lotter, 1985).

From day 7 until 16, the concentration of suspended solid is in the increasing trend but fluctuation. Because of the capability of the bacteria to swim in their own and random direction, it creates the unstable and fluctuation reading (Murphy *et al*., 2000; Ginn *et al*., 2002). Starting from day 3, the mixed culture starts to adapt with new environment and colonies. But as overall in the treatment reactor, the trend and the formation of biofilm is increase. This is because the bacterial activity is depend on the food means as long as the bacteria been fed, the concentration of suspended solid will increase (López *et al*., 1998).

In Figure 4.3, it shows that the loading rate 5.0 mg/L.d will given the highest suspended solid concentration. So, the higher the loading rate the higher the concentration of suspended solid. So, it can be conclude that the data from the

experiment is acceptable because it create same trend with the trend in Design Expert.

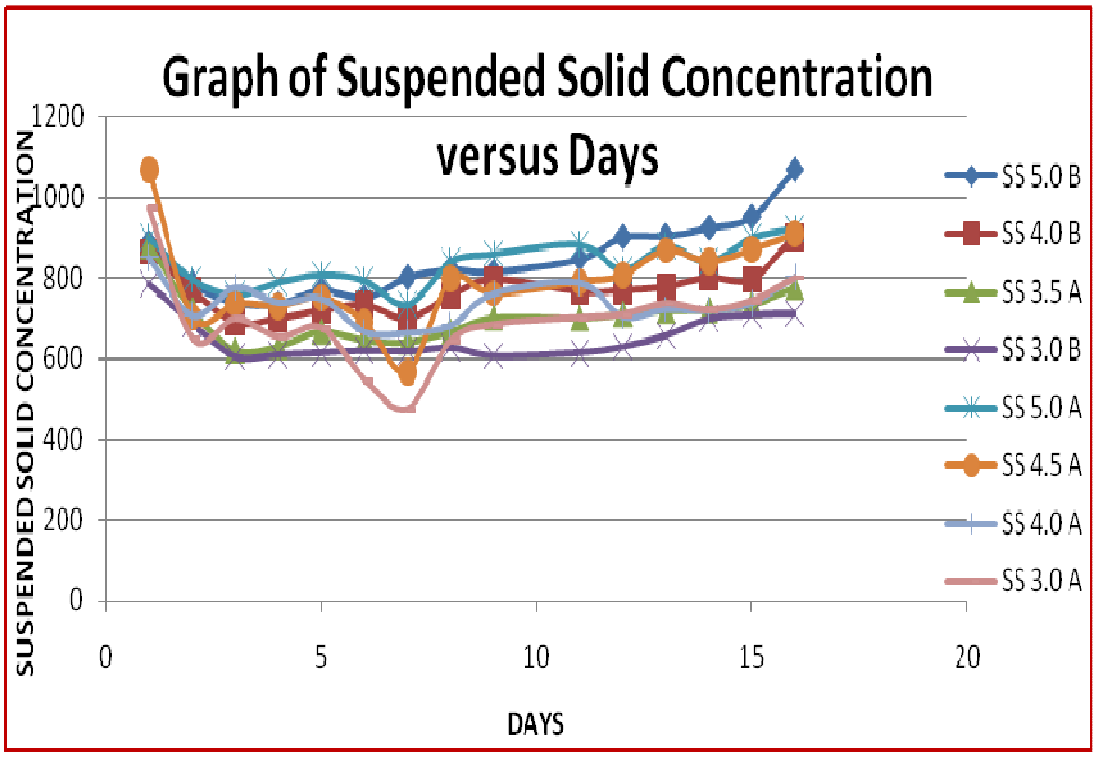


Figure 4.2: Suspended Solid in Treatment Reactor

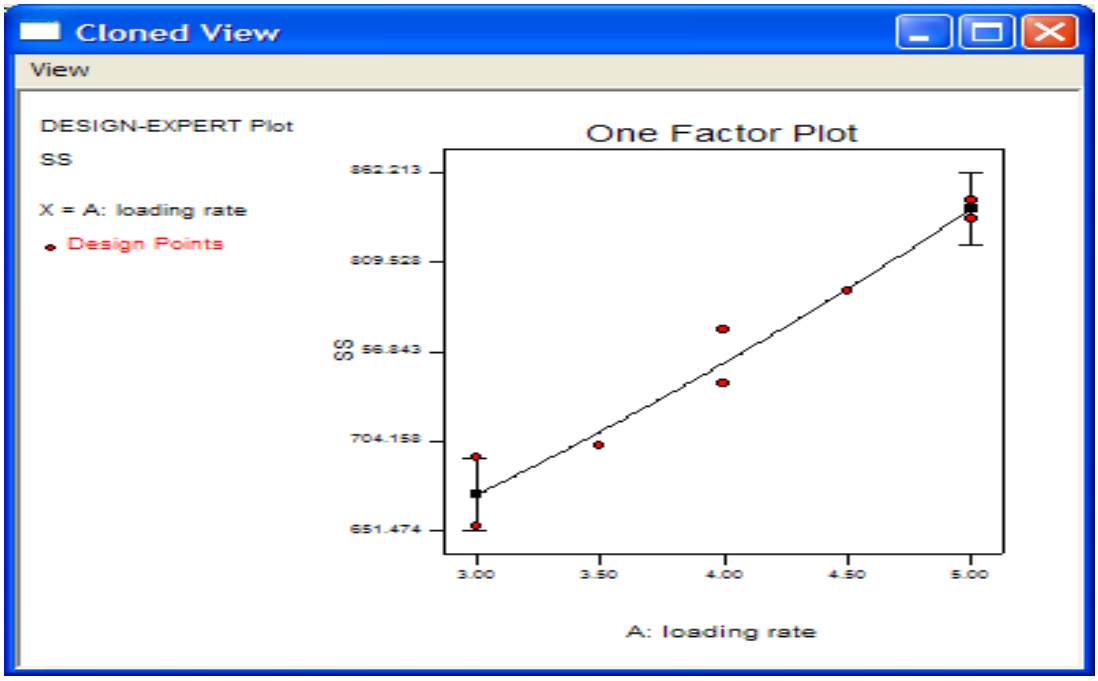


Figure 4.3: Suspended solid using Design Expert software

4.3 Effect of Loading Rate on Phosphorus Removal

From the graph in Figure 4.4, it shown that the bacteria from drain can remove the phosphorus in wastewater. The highest removal achieved is 57.38% at loading rate 5.0 mg/L.d in the second cycle followed by the loading rate 5.0 mg/L.d in the first cycle with 56.46% removal. The lowest removal done by the loading rate 3.0 mg/L.d with only 45.64% removal. It has been said that the removal of phosphorus is done because of the growth of the microorganism (Janssen *et al*., 2002). Because of that, it can be relate to the suspended solid concentration as shown in Figure 4.2, the highest concentration is at loading rate 5.0 mg/L.d and this make the microorganism in that reactor is highest and it create the highest phosphorus removal (Janssen *et al*., 2002). The removal of phosphorus is also depends on the type of bacteria in the biofilm. It has been discovered that bacteria such as Acinetobacter and Pseudomonas is high in microorganism from drain and this type of bacteria is good in removing phosphorus (Gersberg and Alien, 1985; Suresh *et al*., 1985; Buchan, 1981; Lotter, 1985; Cloet and Steyn, 1998).

Figure 4.5 which is the Design Expert calculation to conclude the phosphorus removal shown that the loading rate 5.0 mg/L.d give the highest removal of phosphorus. It can be conclude that in order to find higher removal, higher loading rate is required. The treatment was done successfully because it shows the same trend with the design-expert which is the higher loading rate will create higher removal.

Based on Figure 4.4, the highest value of removal is 69.79% and based on the Table 4.1, the removal of phosphorus is not much different with the removal from the other treatment. As in Table 4.1, the removal of phosphorus is among 70% to 80%. So, it can be conclude that the treatment has be done successfully.

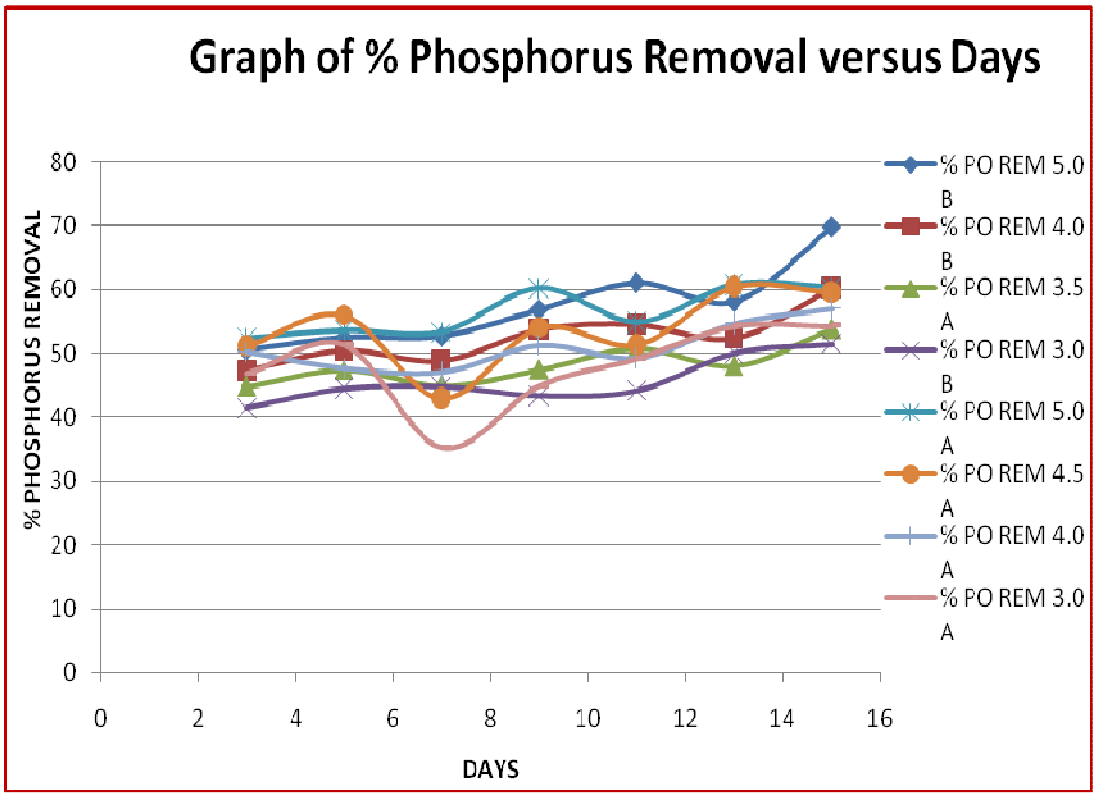


Figure 4.4: Phosphorus removal in treatment reactor

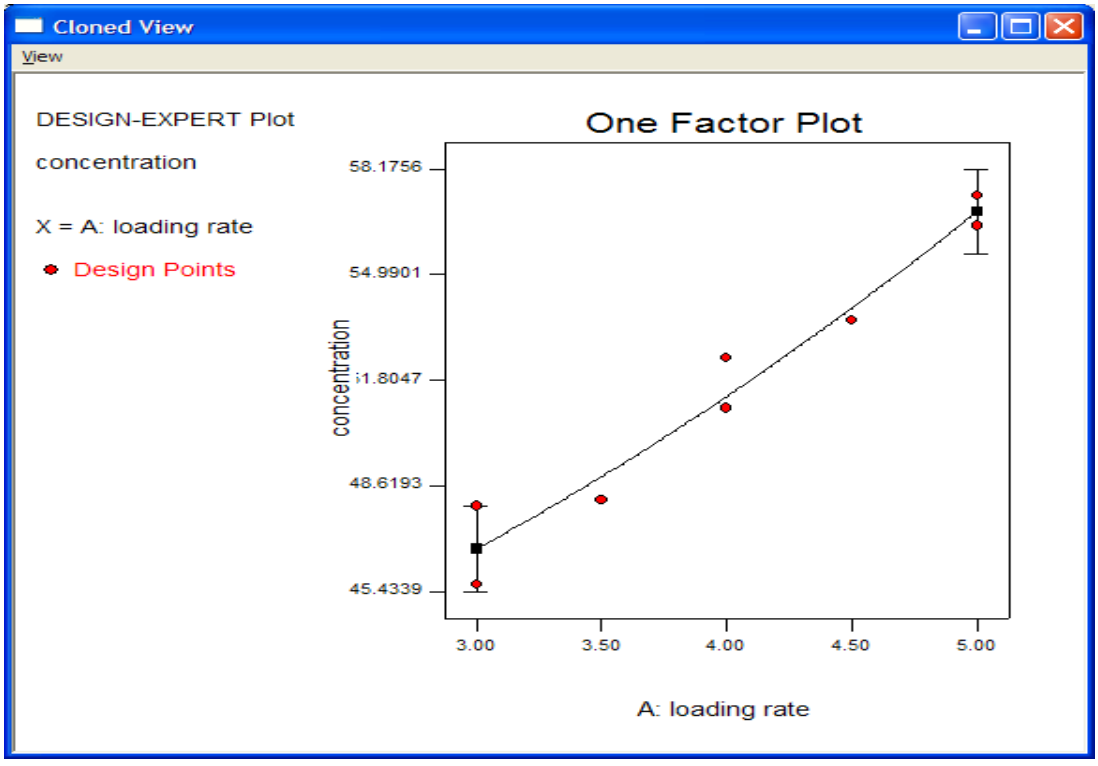


Figure 4.5: Phosphorus removal using Design Expert software

Table 4.1: Comparison of phosphorus removal with other study

NO	STUDY	CONCENTRATION	% REMOVAL
1	Sequencing batch reactor with single-stage oxic process Wang et al. 2 August 2007	INITIAL - About 15-20 mg/L	Percentage of phosphorus removal more than 72%
2	Activated Sludges Process Cai et al. 20 April 2007	INITIAL - 10 mg/L	Phosphorus removal 78.8%
3	Sequencing Batch Reactor Obaja et al. 25 May 2001	INITIAL - 144mg/L of phosphate	Percentage of phosphate removal higher than 80.6%
4	This Research (Sequencing Batch Reactor using microflora from drain)	INITIAL - 25 mg/L	Phosphorus removal 69.79%

4.4 Effect of Loading Rate on COD Removal

From Figure 4.6, the highest value of COD removal done at loading rate 5.0 mg/L.d with 64.33% removal and the lowest removal done by the loading rate 3.0 mg/L.d with 48.12% removal. The graph is in the fluctuation n unstable trend. This is because COD need long period of time to reach the steady state condition (Shao et al., 2008). The COD removal is proportional to the phosphorus removal as we can see in Figure 4.4, the highest removal done by the loading rate 5.0 mg/L.d. This happen because of the oxygen demand is decrease when the phosphorus concentration decrease.

Figure 4.7 which is the Design Expert calculation to conclude the COD removal shown that the loading rate 5.0 mg/L.d give the highest removal of phosphorus. It can be conclude that in order to find higher removal, higher loading

rate is required. The treatment was done successfully because it shows the same trend with the design-expert which is the higher loading rate will create higher removal.

Based on Figure 4.6, the highest value of removal is 90.8% and based on the Table 4.2, the removal of phosphorus is not much different with the removal from the treatment. The highest COD removal from other treatment is about 90%. So, it can be conclude that the treatment has be done successfully.

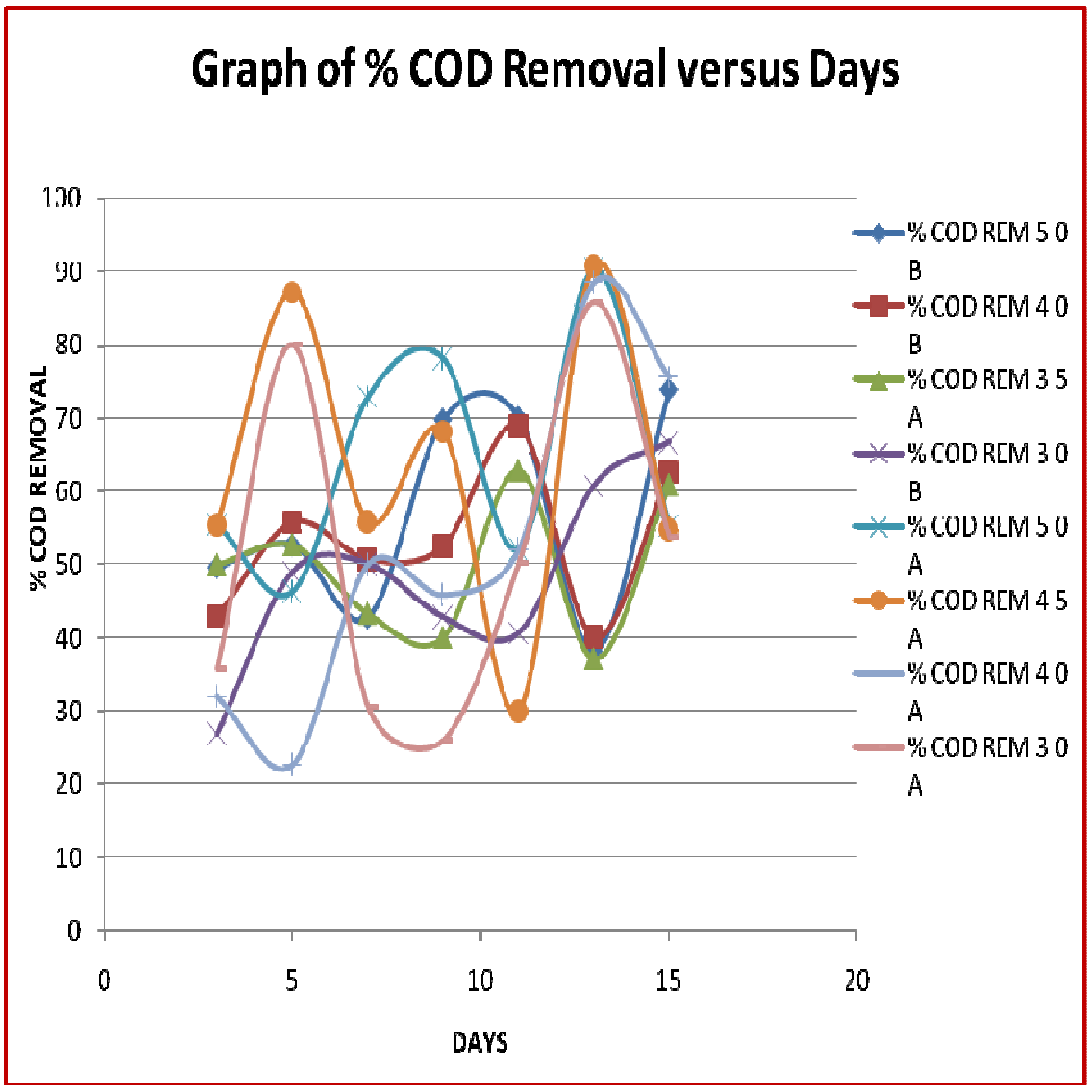


Figure 4.6: COD removal in treatment reactor

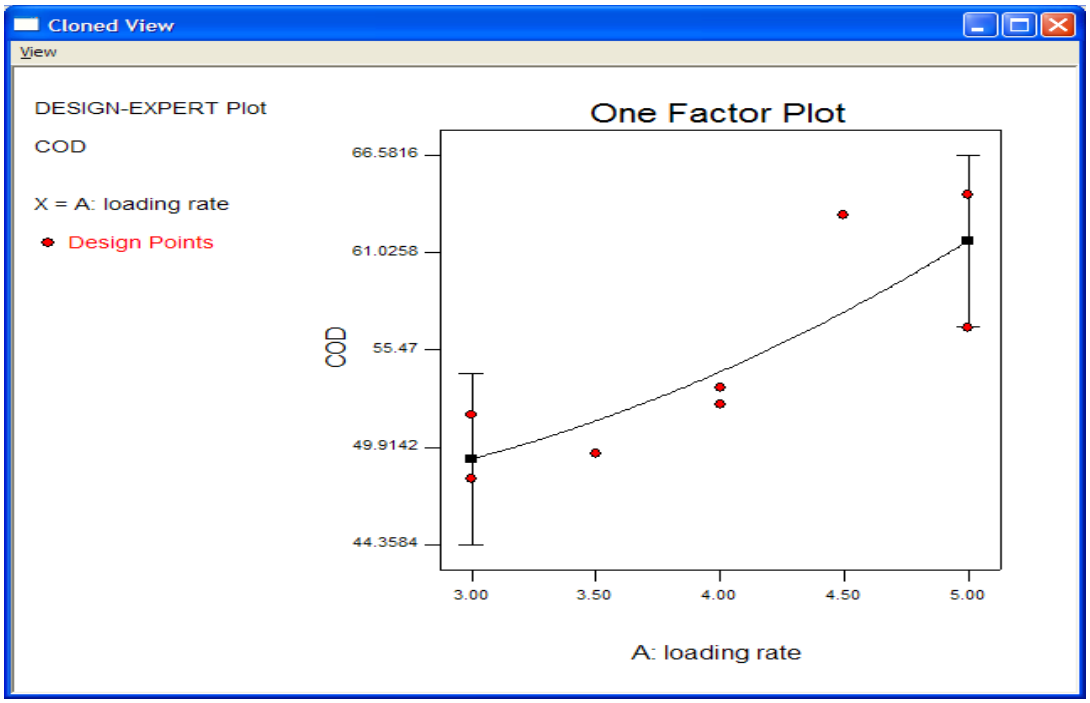


Figure 4.7: COD removal using Design Expert software

Table 4.2: Comparison of COD removal with other study

NO	STUDY	CONCENTRATION	% REMOVAL
1	Sequencing batch reactor with single-stage oxic process Wang et al. 2 August 2007	INITIAL COD - 400 mg/L	COD removal about 87.5 %
2	Sequencing batch reactor in piggery Obaja et al. 25 May 2001	INITIAL COD - 3744 mg/L	70.2 % of COD reduction observes
3	A^2O process with excess aeration Peng et al. 20 september 2004	INITIAL COD - 342.8 mg/L	>90% COD removal About 79% removal occur in the anaerobic phase More than 11% removal in the anoxic zone
4	This Research (Sequencing Batch Reactor using microflora from drain)	INITIAL COD - Depends on LR with range from 50 mg/L to 300 mg/L	Highest removal of 90.8%

4.5 Optimum Loading Rate

As shown in Figure 4.8, the Design Expert software has been run to find in range loading rate, maximum COD, maximum concentration and in range suspended solid. From the Design Expert, the optimum loading rate is 5.0 mg/L.d. The optimum COD removal is 61.685%. The optimum phosphorus removal is 56.89% and the optimum suspended solid concentration is about 840.923 mg/L.

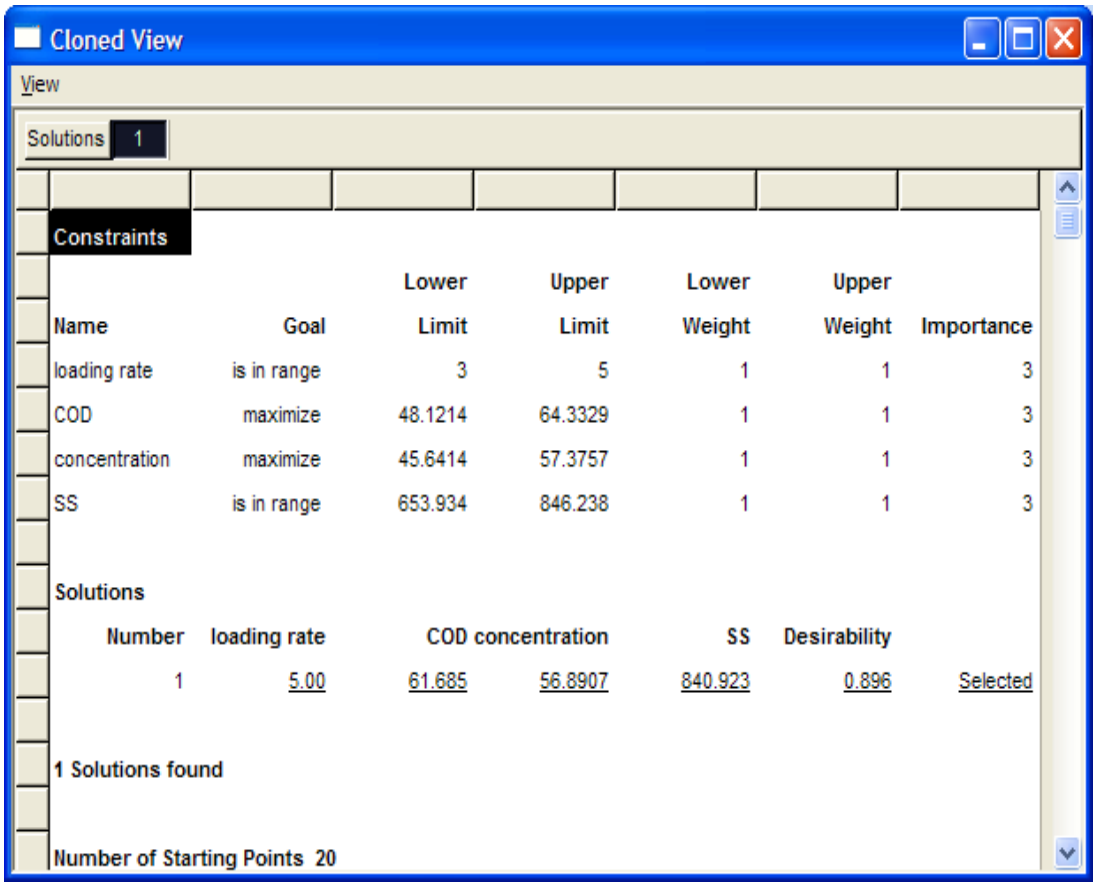


Figure 4.8: Optimum loading rate from Design Expert

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 CONCLUSION

As conclusion, this mixed culture from drain can degrade phosphorus. Based on result, 5 mg/L of loading rate gives the highest value of phosphorus removal and COD removal. The different LR will give different phosphorus concentration and it can affect the microorganism growth. The higher LR, the higher value of food and will create the higher value of SS, phosphorus removal and also COD removal. The % of removal is proportional to the loading rate. Means, the higher the loading rate, the higher the concentration of phosphorus and the higher the removal for COD and phosphorus. The highest phosphorus removal is 57.38% and the highest COD removal is 75.6%. The amount of phosphorus in the wastewater will affect the growth of microflora at the beginning but the microflora tend to adapt the new environment and can consume as high as 5.0 mg/L concentration of phosphorus. Finally, it can be conclude that the amount of suspended solid is increase from day to day and this value is because of the food that been supply and also the food that the microflora can get because of consuming the phosphorus in the solution. We can see the value of SS increase until 7333 mg/L in the acclimatization reactor shows that suspended solid growth well in the acclimatization reactor and also in the treatment reactor because the SS concentration increase from day to day.

5.2 RECOMMENDATION

Numerous additional works can be done to further improve the reliability of the research treatment. Some of the recommendations are as follows:

- The experiment period should be prolonged to avoid limited result and in order to create more stable result.
- Set bigger different loading rate in order to create bigger different in result in order to simplify the process of choosing optimum loading rate.
- Use the single culture and give the specific micronutrient in order to maximize the growth of biofilm.
- Fixed the other conditions such as pH and temperature of acclimatize and treatment reactor. It is because, according to previous findings (Mouchet, 1992), the biological removal of manganese requires more stringent conditions.

REFERENCES

Allison, D. (2000). *Community Structure and Co-Operation in Biofilms*. Cambridge: Cambridge University Press. http://en.wikipedia.org/wiki/Biofilm[14 August 2008].

Buchan, L., 1981. The location and nature of accumulated phosphorus in seven sludges from activated sludge plants which exhibited enhanced phosphorus removal. Water SA. 7, 1-7.

Benedict, R. G. and Carlson, D. A. 1971. "Aerobic Heterotrophic Bacteria in Activated Sludge," Water Research, v. 5, pp. 1023-1030.

Bentor, Yinon. Chemical Element.com-Phosphorus

http://www.chemicalelements.com/elements/p.html [14 April 2009].

Bouwer, E.J. (1989). Transformation of xenobiotics in biofilms. In: W.G. Charcklis and P.A. Wilderer, Editors, *Structure and Function of Biofilms* , John Wiley, New York: 251–256.

Characklis, W.G. (1990). Biofilm processes. In: Characklis, W.G. and Marshall, K.C (1990). B*iofilms*, Wiley, New York.

Cloete, T. E., Smith, Z. and Saayman, G. (1999). A cooling water system as a biofilm reactor for the treatment of municipal wastewater. *Water SA*. 25: 371-376.

Dupin H.J. and McCarty P.L., Impact of colony morphologies and disinfection on biological clogging in porous media, *Environ Sci Technol* **34** (2000), pp. 1513–1520.

Franta, J. R. and Wildere P. A. (1997). Biological treatment of paper mil wastewater by sequencing batch reactor technology to reduce residual organic. *Wat Sci Tech*. 35 (3): 129-133.

Gersberg, R.M., Alien, D.W., 1985. Phosphorus uptake by Klebsiella pneumonia and Acitobacter calcoacetics. Water sci. Technol. 17,113-118.

Ginn T.R., Wood B.D., Nelson K.E., Scheibe T.D., Murphy E.M. and T.P. Clement, Processes in microbial transport in the natural subsurface, *Adv Water Resour* **25** (2002), pp. 1017–1042.

Howard, S., Donald, R. and George, T. (1985). *Environmental Engineering. Singapore:* McGraw Hill, Inc.

Janssen, P.M.J., Meinema, K., van der Roest, H.F., 2002. Biological Phosphorus Removal – Manual for Design and Operation. IWA Publishing, London.

Keudael ,L.O., Dichtl, N.J. Settling Characteristic of Activated Sludge in Sequencing Batch Reactors Obtained from Full-Scale Experiments. *2nd International Symposium on Sequencing Batch 2. Reactor Technology.* July 10-12, 2000, Narbone France.2000. 75-83.

Kim et al., 1997a W. Kim, W. Nishijima, E. Shoto and M. Okada, Pilot plant study on ozonation and biological activated carbon process for drinking water treatment, *Water Sci. Technol.* **35** (8) (1997), pp. 21–28.

Kulikowska, D., Klimiuk, Ewa. and Drzewicki, A. (2007). BOD_5 and COD removal and sludge production in SBR working with or without anoxic phase. *Bioresource Technology*. 98: 1426-1432.

Lenntech. 1998. Chemical Properties of Phosphorus-Health effects of Phosphorus-Environmental effects of Phosphorus. http://www.lenntech.com/Periodic-chart-elements/p-en.html [14 April 2009].

Lotter, L.H., 1985. The role of bacterial phosphate metabolism in enhanced phosphorus removal from the activated sludge process. Water Sci. Technol. 17, 127-138.

Metcalf & Eddy 2003, 'Wastewater Engineering', International Edition, http://www.lenntech.com/phosphorous-removal.htm [31 July 2008].

Murphy E.M. and Ginn T.R., Modeling microbial processes in porous media, *Hydrogeol J* **8** (2000), pp. 142–158.

Paulsen J.E., Oppen E. and Bakke R., Biofilm morphology in porous media, a study with microscopic and image techniques, *Water Sci Technol* **36** (1997) (1), pp. 1–9.

Rittmann, B.E. (1993). The significance of biofilms in porous media. *Water Resource. Res.* 29: 2195–2202.

Schmidt, Lanny D. (1998). *The Engineering of Chemical Reactions*. New York: Oxford University Press. http://en.wikipedia.org/wiki/Continuous_stirred-tank_reactor [16 August 2008].

Schwarz, B.C.E., Devinny J.S and Tsotsis, T.T. (2001). A biofilter network model—importance of the pore structure and other large-scale heterogeneities. *Chem Eng Sci*. 56: 475–483.

Scott Fogler, H., Andrea Acrivors, John Dahler, Thomas, Hanratty, J., John Prausnitz, J. M., Scriven, L.E. (2006). *Element of Chemical Reaction Engineering.* 4rd ed. United State: Pearson Education International.

Sorbie K.S. and Huang Y., Rheological and transport effects in the flow of low-concentration xanthan solution through porous media, *J Colloid Interf Sci* **145** (1991) (1), pp. 74–89.

Stewart T.L. and Kim D.S., Modeling of biomass-plug development and propagation in porous media, *Biochem Eng J* **17** (2004), pp. 107–119.

Suchomel B.J., Chen B.M. and Allen M.B., Macroscale properties of porous media from a network model of biofilm processes, *Transport Porous Med* **31** (1998), pp. 39–66.

Suresh, N., Warburg, R., Timmerman, M., Halvorson, H.O., 1885. New strategies for the isolation of microorganism responsible for phosphate accumulation. Water Sci. Technol. 17, 99-111.

Threlfall, R.E., (1951). *100 years of Phosphorus Making: 1851 - 1951*. Oldbury: Albright and Wilson Ltd file:///F:/PSM/Phosphorus/Phosphorus.htm [31 July 2008].

Van Loosdrecht, M.C.M., Eikelboom, D., Gjaltema, A., Mulder, A., Tijhuis, L. and Heijnen, J.J. (1995). Biofilm structures. *Water Sci. Technol.* 31: 163–171.

Van Veen, W. L., E. G. Mulder, and Deinema, M. H. (1978). The Sphaerotilus-Leptothrix group of bacteria. *Microbiol. Rev*. 42: 329-356.

Vayenas D.V., Michalopoulou E., Constantinides G.N., Pavlou S. and Payatakes A.C., Visualization experiments of biodegradation in porous media and calculation of the biodegradation rate, *Adv Water Resour* **25** (2002), pp. 203–219.

Wang, C. T., Chou, W. L., and Kuo Y. M. (2008). Removal f COD from laundry wastewater by electrocoagulation/electroflotation. *Journal of Hazardous Materials.* 195: 234- 241.

Wikimedia Foundation, Inc. 2008. Aspergillus

http://en.wikipedia.org/wiki/Aspergillus [20 August 2008]

Wikimedia Foundation, Inc. 2008. Batch Reactor

http://en.wikipedia.org/wiki/Batch_Reactor [2 August 2008]

Wikimedia Foundation, Inc. 2008. Biofilm

http://en.wikipedia.org/wiki/Biofilm [17August 2008]

Wikimedia Foundation, Inc. 2008. CSTR

http://en.wikipedia.org/wiki/CSTR [2August 2008]

World Health Organisation. *Guidelines for drinking water quality*. 2nd ed. Recommendations. 1993: 50–1.

APPENDIX A1

Determining Total Phosphorus

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 A2

Chemical Oxygen Demand

HACH DR/2400

Oxygen Demand, Chemical

Reactor Digestion Method*

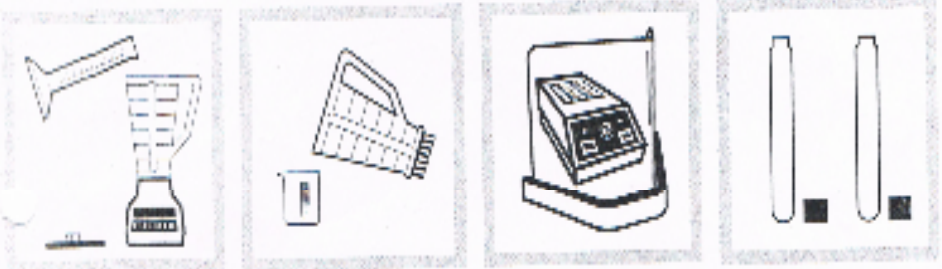
(3 to 150, 20 to 1500, and 200 to 15,000 mg/L COD)

*Method 8000

Scope and Application: For water, wastewater, and seawater; digestion is required;
3–150 mg/L and 20–1500 mg/L COD ranges are USEPA approved for wastewater analysis**;
200–15,000 mg/L COD range is not USEPA approved.

**Reference

- Some of the chemicals and apparatus used in this procedure may be hazardous to the health and safety of the user if inappropriately handled or accidentally misused. Please read all warnings and refer to *Waste Management and Safety* on page 67 of this manual.
- Wear appropriate eye protection and clothing for adequate user protection. If contact occurs, flush the affected area with running water. Follow instructions carefully.
- Use a safety shield in front of the COD reactor to prevent injury by exploding glass.
- The reagents are light sensitive. Keep unused vials in the opaque shipping container. Refrigerate if possible.
- Spilled reagent will affect test accuracy and is hazardous to skin and other materials. Wash spills with running water.
- Run one blank with each set of samples. Run all tests (the samples and the blank) with the same lot of vials. The lot number appears on the container label. See *Blanks in Colorimetric Determination* on page 4.
- For greater accuracy, analyze a minimum of three replicates and average the results.



1. Homogenize 100 mL of sample for 30 seconds in a blender. (For samples containing large amounts of solids, increase the homogenization time.)

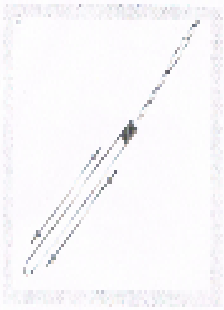
   Note: If the sample does not contain suspended solids, see step 1 and skip 2.

2. For the 200–15,000 mg/L range or to improve accuracy and reproducibility at 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 °C. Place the safety shield in front of the reactor.

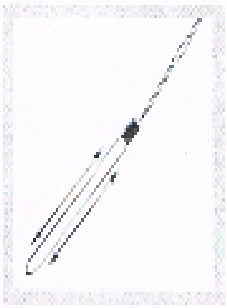
4. Remove the caps from two COD Digestion Reagent Vials. (Be sure to use vials for the appropriate range.)

Oxygen Demand, Chemical



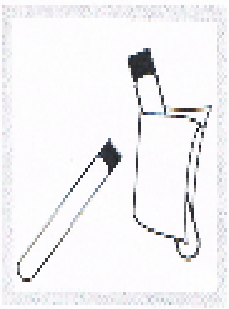
5. Hold one vial at a 15-degree angle. Use a clean volumetric pipet to add 2.00 mL of sample to the vial. This is the prepared sample.

Note: Use a TenSette pipet to add 0.20 mL for the 200–15,000 mg/L range.

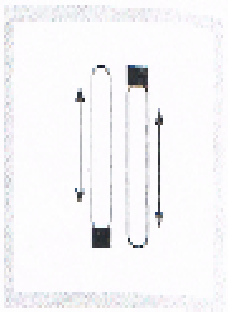


6. Hold a second vial at a 45-degree angle. Use a clean volumetric pipet to add 2.00 mL of deionized water to the vial. This is the blank.

Note: Use a TenSette pipet to add 0.20 mL for the 200–15,000 mg/L range.

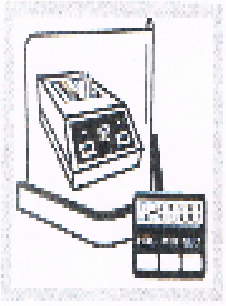


7. Cap the vials tightly. Rinse them with deionized water and wipe with a clean paper towel.

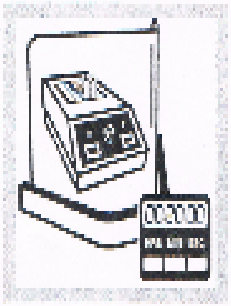


8. Hold the vials by the cap over a sink. Invert gently several times to mix. Place the vials in the preheated COD Reactor.

The sample vials will become very hot during mixing.

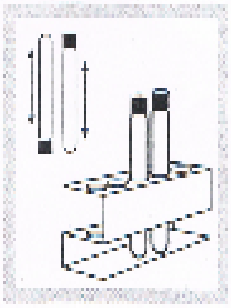


9. Heat the vials for two hours.



10. Turn the reactor off.

Wait about 20 minutes for the vials to cool to 120 °C or less.



11. Invert each vial several times while still warm. Place the vials into a rack and cool to room temperature.

12. Proceed to the Colorimetric Determination Method 8000 on page 3.

1. Touch

Hach Programs

Select program

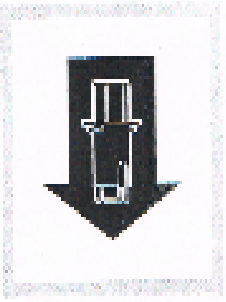
430 COD LR (Low Range)

or

435 COD HR (High Range/High Range Plus).

Touch Start.

2. Clean the outside of the vials with a damp towel followed by a dry one to remove fingerprints or other marks.



3. Install the 16-mm adapter.

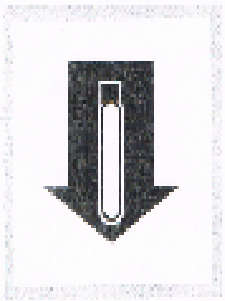
Note: See Section 2.5 in the Instrument Manual for Installation details.

Place the blank into the adapter.

4. Touch Zero.

The display will show:

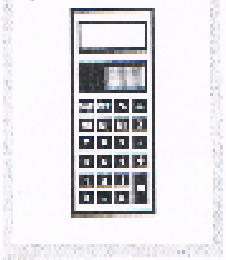
0 mg/L COD



5. When the timer beeps, place the sample vial into the adapter.

Touch Read.

Results will appear in mg/L COD.



6. If using High Range Plus COD Digestion Reagent Vials, multiply the result by 10.

Note: For most accurate results, all samples over 15,000 mg/L COD should be analyzed with a diluted sample.

Oxygen Demand, Chemical

Blanks for Colorimetric Determination

The blank may be used repeatedly for measurements using the same lot of vials. Store it in the dark. Monitor decomposition by measuring the absorbance at the appropriate wavelength (420 or 620 nm). Zero the instrument in the absorbance mode, using a vial containing 5 mL of deionized water and measure the absorbance of the blank. Record the value. Prepare a new blank when the absorbance has changed by about 0.01 absorbance units.

Interferences

Chloride is the primary interference when determining COD concentration. Each COD vial contains mercuric sulfate that will eliminate chloride interference up to the level specified in column 1 in the table below. Samples with higher chloride concentrations should be diluted. Dilute the sample enough to reduce the chloride concentration to the level given in column 3.

If sample dilution will cause the COD concentration to be too low for accurate determination, add 0.50 g of mercuric sulfate ($HgSO_4$) (Cat. No. 1915-20) to each COD vial before the sample is added. The additional mercuric sulfate will raise the maximum chloride concentration allowable to the level given in column 4.

[illegible]	[illegible]	[illegible]	[illegible]
Low Range (0–150 mg/L)	2000	1000	8000
High Range (20–1500 mg/L)	2000	1000	4000
High Range Plus (200–15,000 mg/L)	20,000	10,000	40,000

Sampling and Storage

Collect samples in glass bottles. Use plastic bottles only if they are known to be free of organic contamination. Test biologically active samples as soon as possible. Homogenize samples containing solids to assure representative samples. Samples treated with sulfuric acid (Cat. No. 979-49) to a pH of less than 2 (about 2 mL per liter) and refrigerated at 4 °C can be stored up to 28 days. Correct results for volume additions; see *Section 3.1.3 Correcting for Volume Additions* on page 29 for more information.

APPENDIX A3

Suspended Solids

HACH DR/2400

Suspended Solids

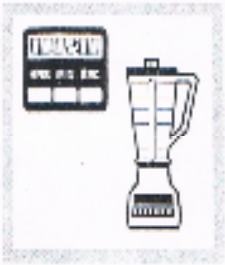
Method 8006

Photometric Method*
(0 to 750 mg/L)

Scope and Application: For water and wastewater

* Adapted from Sewage and Industrial Wastes, 31, 1159 (1959).

1. Touch
Hach Programs.
Select program
630 Suspended Solids.
Touch Start.



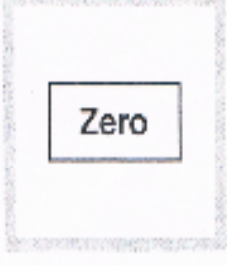
2. Blend 500 mL of sample in a blender at high speed for exactly two minutes.

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).

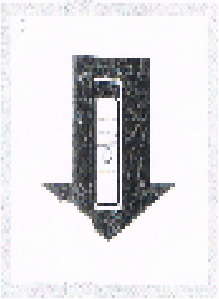
5. Fill a second sample cell with 25 mL of tap water or deionized water (the blank).
Remove gas bubbles in the water by swirling or tapping the bottom of the cell on a table.

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.

Interferences

Samples that absorb strongly at 810 nm, such as blue dyes, may give false, high-bias readings. A user-entered calibration is advised for these samples.

Calibration for this test is based on parallel samples using the gravimetric technique on sewage samples from a municipal sewage plant. For most samples, this calibration will provide satisfactory results. When higher accuracy is required, run parallel spectrophotometric and gravimetric determinations with portions of the same sample. The new calibration should be made on your particular sample using a gravimetric technique as a basis.

Sampling and Storage

Collect samples in clean plastic or glass bottles. Analyze samples as soon as possible after collection. The sample may be stored seven days by cooling to 4 °C (39 °F).

Summary of Method

This method of determining suspended solids is a simple, direct measurement which does not require the filtration or ignition/weighing steps that gravimetric procedures do. The USEPA specifies the gravimetric method for solids determinations, while this method is often used for checking in-plant processes. Test results are measured at 810 nm.

Required Apparatus

Description	Quantity Required Per Test	Unit	Cat. No.
Beaker, 600-mL, pyrex	1	each	1060-52
Blender, 1.2 L, 120 VAC	1	each	2616-03
Blender, 1.2-L, 240 VAC	1	each	2616-02
Cylinder, graduated, 500 mL, poly	1	each	1081-49
Pipet, serologic, 25-mL	1	each	2066-40
Pipet, Filler, safety bulb	1	each	1465-00
Sample Cells, 10-20-25 mL, w/cap	2	ea/pkg	2401-06

APPENDIX B

Data From Hach Spectrophotometer for Suspended Solid Concentration In Acclimatization Reactor

Day	Suspended Solid (mg/L)	Day	Suspended Solid (mg/L)	Day	Suspended Solid (mg/L)
1	2314.67	18	4312	37	4481
3	1711.67	20	5325.33	38	4216.67
7	1570	21	4640	41	4343.33
8	2191.67	22	4803.33	43	4516
10	2100.67	23	4382.89	44	5023
11	2023.33	24	4761.33	45	5688.67
12	2348.33	27	3882.67	48	6593.33
13	3781.67	28	4730.67	49	6833.33
14	3776	29	4744	50	6523.33
15	3077.33	30	4748.33	52	6656.67
16	3752	31	4680	53	6549.67
17	5336	35	4766.67	54	6676

Day	Suspended Solid (mg/L)	Day	Suspended Solid (mg/L)	Day	Suspended Solid (mg/L)
56	7623.33	71	6420	85	7020
57	7589	72	6225	86	6980
58	7467	73	6326	87	7033
59	7210	74	6518.67	88	7100
60	7300	75	6927.33	89	7288.67
62	7169	76	6955.67	90	7333.33
63	6903.33	77	6957.67		
64	6070	78	6960		
65	7096.67	79	6959		
66	6823.33	80	6978		
67	6790	81	6970		
68	6798	82	6989		
69	6812.67	83	6973		
70	6618.67	84	7008		

APPENDIX C1

Data From Hach Spectrophotometer for Suspended Solid Concentration

Days	SS 5.0 B	SS 4.0 B	SS 3.5 A	SS 3.0 B	SS 5.0 A	SS 4.5 A	SS 4.0 A	SS 3.0 A
1	886	866	880	786	900	1068	850	974
2	786	770	720	688.67	797	704	707	649
3	738	690	620	605.67	759	735	777	699
4	735.67	700.67	630	610.67	790	730.33	739	653.67
5	770	720	667	615.33	808.67	750.67	748.67	676
6	750.67	733.33	645	620.67	794	689.67	666.67	548
7	800.33	700	640.67	618.67	733.67	565.67	664.33	473.67
8	820.67	755.67	666.67	628.33	840	796.33	682.33	643.67
9	815.33	796	700.67	608	860	761.67	761.67	685
11	845.67	766.67	700.33	615.67	883	790	788.33	701.67
12	900.23	769	709.67	630.33	820	806	705	710
13	903	777.67	718.33	658	880	868.67	723.33	736.67
14	923.33	800.33	720.33	700	840	840	720	721
15	950.67	796.67	733.33	710	900	870	738	743.67
16	1068	903	777	713	923.33	908.67	803	800.67
average	846.238	769.6673333	701.9333333	653.934	835.2446667	792.312	738.2886667	694.3793333

APPENDIX C2

Data From Hach Spectrophotometer for Phosphorus Removal

Days	% PO REM 5.0 B	% PO REM 4.0 B	% PO REM 3.5 A	% PO REM 3.0 B	% PO REM 5.0 A	% PO REM 4.5 A	% PO REM 4.0 A	% PO REM 3.0 A
3	50.53	47.3	44.79	41.38	52.3	51.12	50.17	46.84
5	52.48	50.42	47.43	44.43	53.55	55.95	47.58	51.37
7	52.7	48.8	44.95	44.73	53.28	42.88	46.96	35.15
9	56.89	53.73	47.47	43.28	60.15	54.05	51.2	44.91
11	61.09	54.5	50.83	44.1	54.85	51.34	49.21	49.22
13	58.15	52.35	48.06	50.09	60.73	60.45	54.63	54.32
15	69.79	60.25	53.87	51.48	60.33	59.55	57.03	54.28
average	57.37571429	52.47857143	48.2	45.64142857	56.45571429	53.62	50.96857143	48.01285714

APPENDIX C3

Data From Hach Spectrophotometer for COD Removal

Days	% COD REM 5.0 B	% COD REM 4.0 B	% COD REM 3.5 A	% COD REM 3.0 B	% COD REM 5.0 A	% COD REM 4.5 A	% COD REM 4.0 A	% COD REM 3.0 A
3	49.66	42.87	50	26.86	55.46	55.47	31.95	35.83
5	52.68	55.69	52.67	48.97	46.21	87.15	22.6	79.97
7	42.79	50.69	43.33	50.08	72.85	55.88	50	30.7
9	69.77	52.46	40.08	42.8	78.14	68.15	45.85	26.06
11	70.08	68.84	62.86	40.67	52.07	30	52.04	50.2
13	38.27	40.08	37.18	60.77	90.32	90.8	88.58	85.83
15	73.84	62.48	60.86	66.7	55.28	54.82	75.6	53.85
Average	56.72714286	53.30142857	49.56857143	48.12142857	64.33285714	63.18142857	52.37428571	51.77714286