BIODEGRADATION OF MANGANESE IN SEQUENCING BATCH REACTOR

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MOHAMAD ZARQANI BIN YEOP

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 *"Biodegradation of manganese in sequencing batch reactor"* is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree."

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Name : Mohamad Zarqani bin Yeop

Date : 2 May 2009

DEDICATION

Special Dedication to

Pn Fadidatol Sa'adiah Hj Siraj, my beloved mother, you are everything to me,
En Yeop Mohamad, my beloved father, you are my inspiration,

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

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

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ABSTRACT

This thesis is about the biodegradation of manganese in sequencing batch reactor. Biodegradation of manganese in sequencing batch reactor (SBR) is a new invention treatment method that gives a lot of advantage in term of lowering operation and maintenance cost. The works done in this research explore the effects of different loading rate (LR) on the water quality parameter. For this research, biofilm process was selected as a treatment method which is develop in the SBR. At the beginning of the experiment, the mixed cultured was collected from drain and acclimatized in 10 liter reactor and monitoring the growth by using suspended solid (SS) test. The mixed cultured was acclimatized within two months by fed with 1mg/L manganese. For the treatment process, the mixed culture from acclimatization reactor will be transferred to the treatment reactor. The manganese treatment process will be carried out by controlling the loading rate. The hydraulic retention time (HRT) selected for this research is 5 days. The experiment will be run within two weeks. Within this period, the collection of the sample will be done for everyday as well as the addition of the simulated wastewater to the treatment reactor. The data shown that the lowest organic loading rate which is 3mg/L.d giving the highest percent of manganese removal. The highest manganese removal is 65% while the highest value of chemical oxygen demand (COD) removal is 72% at 3.5 mg/L.d. By using the Design Expert, the predicted value will represent the optimization results for this research. The value for optimization is at lowest LR which is 3 mg/L.d. By using lowest LR, the treatment will get 43% of COD removal and 57% manganese removal. The optimization of concentration of suspended solid is 381 mg/L.

ABSTRAK

Tesis ini adalah berkaitan dengan penguraian mangan secara biologi di dalam reaktor sesekumpul berjujukan. Penguraian secara biologi di dalam reaktor sesekumpul berjujukan adalah satu kaedah rawatan baru yang memberikan banyak kelebihan dari aspek pengurangan kos operasi dah kos penyelenggaraan. Kerja-kerja yang dijalankan di dalam kajian ini menyingkap perbezaan kesan kadar beban terhadap kualiti parameter air. Untuk kajian ini, kaedah rawatan yang dipilih adalah biofilem digabungkan dengan reaktor sesekumpul berjujukan. Pada permulaan eksperimen ini, kultur campuran diambil dari longkang dan dimasukkan dalam reaktor 10 liter untuk proses aklimitasi seterusnya pertumbuhan kultur campuran itu dipantau melalui ujian pepejal terampai. Proses penyesuaian kultur campuran tersebut di jalankan untuk tempoh masa dua bulan dengan memberi makan mangan kepada kultur campuran itu pada kepekatan 1 mg/L. Untuk proses rawatan, kultur campuran itu dipindahkan dari reaktor aklimitasi ke reaktor rawatan. Proses rawatan mangan dijalankan dengan mengawal nilai kadar beban. Masa penahanan hidraulik yang dipilih untuk kajian ini adalah 5 hari. Eksperimen ini dijalankan selama dua minggu. Dalam tempoh ini, sampel diambil dan tambahan sisa air buatan juga dilakuakan ke dalam reaktor yang mana proses ini dijalankan setiap hari. Data yang diperolehi menunjukkan bahawa kadar beban yang terendah iaitu 3 mg/L.h memberikan peratus penyingkiran mangan yang paling tinggi. Peratus penyingkiran paling tinggi adalah 65% manakala peratus penyingkiran tertinggi untuk permintaan oksigen kimia yang tertinggi adalah 72% pada 3.5 mg/L. Semua data yang diperolehi dimasukkan ke dalam perisian Design Expert untuk meramal nilai optimum bagi kajian ini. Kajian ini memperolehi nilai optimum pada kadar beban 3 mg/L.h. Dengan menggunakan kadar beban yang paling rendah, rawatan ini akan memperolehi penyingkiran permintaan oksigen kimia sebanyak 43% dan penyingkiran mangan sebanyak 57%. Nilai optimum untuk kepekatan pepejal terampai pula adalah 381 mg/L.

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LIST OF ABREVIATIONS

ACGIH	-	American Conference of Governmental Industrial Hygienists
COD	-	Chemical oxygen demand
CSTR	-	Continuous stirred tank reactor
EPA	-	Environmental Protection Agency
ESP	-	Electrostatic precipitator
FDA	-	Food and Drug Administration
Fe	-	Ferum
HR	-	High range
HRT	-	Hydraulic retention time
LR	-	Loading rate
Mn	-	Manganese
MSW	-	Municipal solid waste
OSHA	-	Occupational Safety and Health Administration
SBR	-	Sequencing batch reactor
SS	-	Suspended solid

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CHAPTER 1

INTRODUCTION

1 Background

Source water (ground and/or surface) may contain microbiological, chemical and radiological contaminants that must be removed or inactivated during water treatment in order to produce potable drinking water of sufficient quality (Nishijima and Speital, 2004; Kameya *et al*., 1997; Keinanen *et al*., 2004). Although ingestion of manganese (Mn) through drinking water of concentrations up to 500 µg/L has no harmful effects upon human health (WHO, 1993), its presence in drinking water at concentrations above 100 µg/L is undesirable to customers due to discoloration of the water and the subsequent staining of laundry and plumbing fixtures. The European Commission recommends an upper limit of 50 µg/L for manganese in water for drinking (Drinking Water Quality Intended for Human Consumption Belgium, 1998).

Several treatment methods to remove manganese from waste water have been reported such as trickling filter, electrodialytic process, biosorption and biofilm process. Each of these methods has some limitation in practice (Pehlivan, 2007). This research is done to prove that treating manganese by using microflora in drain is among an economically way. It also helps to determine the most applicable method in term of time and efficiency of each experiment that give more advantages and benefit. For example, modelling biological filter processes at the laboratory scale allowed the effects of defined parameter changes to be transferred to full-scale biofilters (Rowan *et al.*, 2002). By successfully modelling biofiltration and studying manganese (Mn)

bioaccumulation rates (i.e. the decrease in Mn concentration in water passing through the filter) in these filters we hoped to elucidate optimum operating parameters for the process and reduce filter maturation times from months to days. The advantages of biological treatments compared with conventional physicochemical treatments can be summarized as follows: no use of chemicals, higher filtration rates, the possibility of using direct filtration and lower operation and maintenance costs (Mouchet, 1992).

2 Objective of This Study

The main objectives of this research are:

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

3 Scope of Research

The scope of study in this research is to treat manganese in waste water that contains manganese was simulated with appropriate nutrients for microflora. Then, the experiments were conducted separately in reactor with loading rate. Each reactor has working volume 5 liter. The efficiency of treatment for different loading rate concentration was evaluated Mn concentration. Besides that, the effects of Mn concentration on biomass growth are defined in terms of suspended solid.

1.4 Problem Statement

Iron (Fe) and manganese cause aesthetic, organoleptic and operating problems when they are present in groundwater. These metals consume chlorine in the disinfection process and promote biofouling and microbiological induced corrosion in water networks. In groundwater, Fe and Mn are present as Fe (II) and Mn (II). The processes available for their removal are either physico-chemically or biologically based. In the absence of oxygen, iron and manganese would be reduced into soluble Fe^{2+} and Mn^{2+} states. Their presence has long been a serious problem in planning the water sources, determining the method of treatment and maintenance of the water supply system. This is evident in water treatment works with raw water source extracted from aquifers. Elevated levels of iron and manganese in drinking water will not pose health hazards apart from undesirability due to precipitation which stains clothes and utensils, corrosion of cast-iron and steel pipelines which produces "red-water" (Sawyer and McCarty, 1967). They may also impart a metallic, bitter, astringent or medical taste to water.

Manganese compounds exist naturally in the environment as solids in the soil and as small particles in water. Manganese may also be present in small dust-like particles in the air. These Mn containing particles usually settle out of the air within a few days depending on their size, weight, density, and the weather conditions. Manganese exists naturally in rivers and lakes, and is also naturally present in some underground water. Manganese can lead to the toxicity that will effect and give some problem to the crop (Bould *et* al., 1983). Algae and plankton in the water can consume some Mn and concentrate it within themselves.

In addition to occurring naturally in the environment, Mn can be introduced by human activity. Manganese can be released into the air by industry and by the burning of fossil fuels. More specifically, sources of airborne Mn include iron and steel producing plants, power plants, coke ovens, and dust from uncontrolled mining operations. Manganese released from burning a gasoline additive may also be a source of manganese in the air. Manganese from these human-made sources can enter surface water, groundwater, and sewage waters. Small Mn particles can also be picked up by

water flowing through landfills and soil. The chemical state of Mn and the type of soil determine how fast it moves through the soil and how much is retained in the soil. Maneb and mancozeb, two pesticides that contain manganese, may also add to the amount of Mn in the environment when they are applied to crops or released to the environment from packaging factories. There is information on the amount of maneb and mancozeb released into the environment from facilities that make or use these pesticides. This pesticide will induce the toxicity to the environment (Bould *et* al., 1983). However, the amount of Mn in the environment because of the release and use of these pesticides is not known.

Soluble Mn is often found in considerably greater concentrations in mine drainage waters than in unpolluted streams and groundwater (Banks *et al*., 1997). Even though there are uncertainties regarding the toxicity of manganese, recent research has shown that elevated concentrations of manganese are highly correlated to the toxicity of lake sediment pore water (Boucher and Watzin, 1999; Doyle *et al*., 2003). Besides its potential toxicity, the removal of this metal from surface and ground waters is desirable for several reasons.

In addition Mn in sources of water that are used for human consumption is undesirable because it imparts a metallic taste to water, stains laundry and water fixtures and, as Mn (IV) readily precipitates, Mn can block water distribution networks. For these reasons, the U.S. Environmental Protection Agency (EPA) has set a secondary maximum contaminant level for Mn of 0.05 mg/L. The EPA has also established guidelines limiting the concentration of Mn in acidic waters discharging from mines at maximum of 4 mg/L, as long as average discharges for a 30-day period do not exceed 2 mg/L. In the European Union, legislation under consideration may establish an Environmental Quality Standard for manganese of 0.03 mg/L.

Treatment of waste waters is essential to protect aquatic communities, and focuses on the removal of chemical and particulate contaminants. The treatment also to decrease as many as affect that can get from toxicity of manganese.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The word Mn comes from the Latin word magnes which means magnet. In ancient time, two black mineral from magnesia were thought to differ gender which is male and female. The male magnes is attracting iron and now we know as lodestone or magnetite also gives us the term magnet. In other hand, the female magnes did not attract ore and use to decolorize glass and now known as pylorusite or manganese dioxide (Wikipedia, 2008).

In 16th century, this compound was called as magnesium by glassmaker. After that an alchemist and the glassmaker eventually had to differentiate magnesia alba (white ore) from magnesia negra (black ore). Finally the metal was isolated and called manganese. The name magnesia was then used to refer only on white magnesia alba which provide the name magnesium for that free element. In 1774, the Swedish chemist Scheele was the first person recognizes that manganese was the element and his colleague, Johan Gotties Gahn isolated the pure element by reduction of dioxide with carbon (Wikipedia, 2008).

Manganese is naturally found in many type of rock. Pure Mn is a silver-colored metal, similar to iron in its physical and chemical properties. Manganese does not exists as pure metal in environment, but is a component of more than 100 minerals, including sulfides, oxides, carbonates, silicates, phosphates, and borates. It is

combined with other elements or chemicals such as oxygen, sulfur, and chlorine to make compounds that do not evaporate (Wikipedia, 2008).

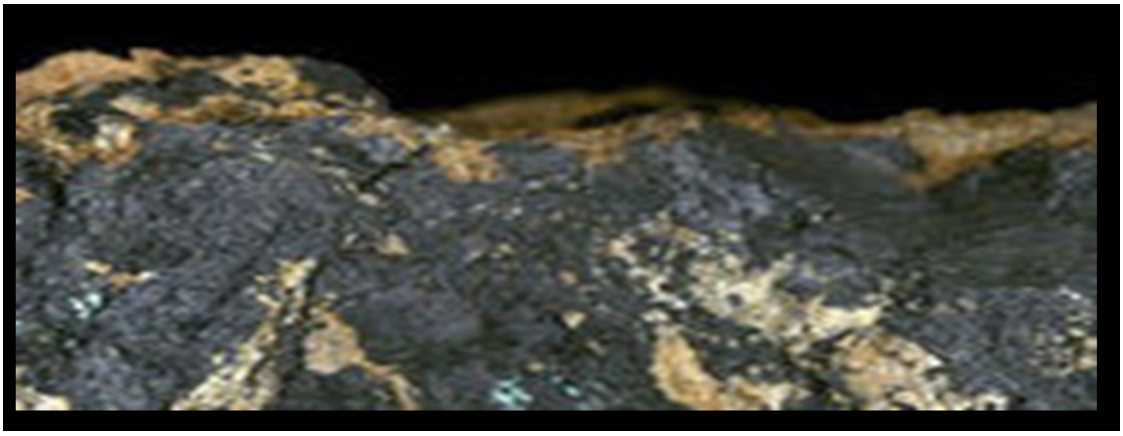

Figure 2.1: Psilomelane (manganese ore)

2.2 Metal

2.2.1 Properties of Manganese

The main ore mineral for Mn is pyrolusite (MnO_2). When Mn is alloyed with other metals like aluminum and copper, the end product is magnetic. Manganese is chemically reactive element. It easily can combine with ion in air and water. Manganese is a gray-white metal or silver, resembling iron. It is a hard metal and is very brittle, fusible with difficulty, but easily oxidized

The basic information about manganese is symbol used which is Mn and has atomic number of 25. It is transition metal in group 7, period 4 and block d. The

standard atomic weight is 54.93805 g/mole and consists of [Ar]$4s^2 3d^5$ for it electron configuration. Manganese has two different main properties which are physical properties and chemical properties which are summarize in Table 2.1, 2.2 and 2.3 (Qivx Inc.Integral Scientist Periodic Table, 2003).

Table 2.1: Physical properties of manganese

Physical properties	
Phase	solid
Density (near r.t.)	7.21 $g \cdot cm^{-3}$
Liquid density at m.p	5.95 $g \cdot cm^{-3}$
Melting point	1519 K (1246 °C)
Boiling point	2334 K (2061 °C)
Critical Temperature	4327.0 K (4054 °C)
Heat of fusion	12.91 $kJ \cdot mol^{-1}$
Heat of vaporization	221 $kJ \cdot mol^{-1}$
Specific heat capacity (25 °C)	26.32 $J \cdot mol^{-1} \cdot K^{-1}$

Table 2.2: Chemical properties of manganese

Chemical properties	
Oxidation states	+2, +3, +4, +6, +7
Electronegativity	1.55 (Pauling scale)
Atomic radius	140 pm
Covalent radius	139 pm
Vanderwaals radius	126 pm

Table 2.3: Standard reaction of manganese

Standard reactions	
oxidation	reduction
$MnO^{4-} + 4H^{+} + 3e^{-}$	$MnO_2 + 2H_2O$
$MnO^{4-} + 8H^{+} +$	$Mn^{+2} +$

5e-	$4H_2O$
$Mn^{+3} + e^-$	Mn^{+2}
$MnO^2 + 4H^+ + 2e^-$	$Mn^{+2} + 2H_2O$
$Mn^{+2} + 2e^-$	Mn

2.2.2 Environmental and Quality Issues

Toxic hazard has two types of effect which is acute (short term) exposure and chronic exposure (long term).The effects of exposure to any hazardous substance depend on the dose, the duration and how you are exposing.

Health effects from Mn are not a concern until concentrations are approximately ten times higher. The levels of Mn in groundwater from natural leaching processes can vary widely, depending upon the types of rock and minerals present at the water table. Typically, Mn concentrations from natural processes are low but can range up to 1.50 mg/L or higher. Sources of pollution, rich in organic matter (e.g., run off from landfills, composts, brush or silage piles, or chemicals such as gasoline), can add to the background level by increasing Mn release from soil or bedrock into groundwater (Division of Environmental Epidemiology and Occupational Health of Connecticut, 2001).

Manganism or Mn poisoning is a toxic condition resulting from chronic exposure to Mn and first identified in 1837 by James Couper. In initial stages of manganism, neurological symptoms consist of reduced response speed, irritability, mood changes, and compulsive behaviors. Manganism has become an active issue in workplace safety as it has been the subject of numerous product liability lawsuits

against manufacturers of arc welding supplies. In these lawsuits, welders have accused the manufacturers of failing to provide adequate warning that their products could cause welding fumes to contain dangerously high Mn concentrations that could lead welders to develop manganism (Couper, 1837).

Classical manganism presents with generally irreversible Parkinson-like symptoms (Jiang *et al*., 2006), which often progress even after exposure ends (Huang *et al*., 1993). Little is known about the potential health effects resulting from excessive ingestion exposures. Early stages of Mn toxicity include muscle tremors and can sometimes resemble Parkinson disease, which has been proposed as a sentinel among selected occupational groups. When the information got from hospital separation data for Parkinsonism between 1991 and 1995 in which New Brunswick rates were similar to other Canadian provinces (Kontakos and Stokes, 2000).

Leachates emanating from old mine workings often contain high concentrations of dissolved metals (such as Fe, Mn, Al and Zn) and can have a high acidity (Younger *et al.*, 2002; Manzano *et al.*, 1999). When these waters enter streams and rivers, Fe can rapidly become oxidized and hydrolyzed to form characteristic Fe oxyhydroxide precipitates that cover the stream bed thus suffocating much of the natural aquatic life in water courses (Jarvis and Younger, 1997). These Fe oxyhydroxides are capable of removing Mn from solution by sorption (Stumm and Morgan, 1981).

In September 2000, Mn has been found in at least 603 of the 1,517 National Priorities List (NPL) sites. It means 40% of the waste consists of manganese. As more sites are evaluated, the value will probably increase. When a substance released to the environment it will effects to biology system by breathing, eating, or drinking the substance or by skin contact.

2.2.3 Level Measurement to Discharge Manganese

Because of its potential to cause adverse health effect to the exposure, some regulations and guidelines was established for manganese. The EPA recommends that the concentration of Mn in drinking water not to be 0.005 ppm and followed by the Food & Drug Administration (FDA) for bottled water. This concentration is believed to be more than adequate to protect human health. The EPA has also established rules that set limits on the amount of Mn that factories can dump into water. EPA requires factories that use or produce Mn to report how much they dump in the environment. The Occupational Safety and Health Administration (OSHA) has set limits of 5 mg/m^3 for fume and 0.2 mg/m^3 for particulate matter as the average amounts of Mn in workplace air over 8-hour workday (OSHA, 1998). Similarly, the American Conference of Governmental Industrial Hygienists (ACGIH) has set a limit of 1 mg/m^3 for Mn fume and 0.2 mg/m^3 for the average amount of Mn, either elemental or as inorganic compounds that can be present in the air over an 8 hours workday (ACGIH, 1998).

The Food and Nutrition Board of the National Research Council (NRC) has not established a Recommended Daily Allowance for Mn because too little is known about the dietary requirements of this trace element. However, an Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for Mn has been estimated as 0.3–0.6 mg/d for infants from birth to 6 months, 0.6–1 mg/d for infants aged 6 months to 1 year, 1–1.5 mg/d for children aged 1–3 years, 1–2 mg/d for children aged 4–10 years of age, and 2–5 mg/d for children aged 10 years to adult. Offices within the EPA that issue regulations for Mn are Air Quality Planning and Standards, Water Regulation and Standards, Emergency and Remedial Response, Solid Waste, and Toxic Substances.

The FDA regulates Mn concentrations in bottled water. The Department of Justice's Drug Enforcement Agency ranks potassium permanganate as an essential chemical in illegal drug production; records of sales and uses are required for amounts over 500 kg. Under Section 313 of the Emergency Planning and Community Right to

Know Act of 1986, releases of more than one pound of Mn into the air, water, and land must be reported annually and entered into the Toxic Release Inventory (TRI).

2.3 Manganese Removal Method

1 Trickling Filter

Mn(II) is readily soluble in water while Mn(III) is more unstable and has a tendency to precipitate or dissociate to Mn(II) or Mn(IV) unless chelated to another molecule. Mn(IV) is insoluble and can be detected by the presence of a visible brown or black precipitate in neutral solutions. The oxidation of Mn(II) to Mn(IV) by aeration alone is a slow process unless the pH is raised above neutrality (Cleasby, 1975; Wilson, 1980; Diem and Stumm, 1984; Hem, 1981). Therefore, Mn cannot be removed by simple aeration and precipitation. Current Mn removal methods generally required the use of strong oxidizing agents such as potassium permanganate, chlorine, hypochlorite, chlorine dioxide or ozone.

The role of microorganisms in the oxidation of Mn in drinking water production plants has attracted considerable attention (Czekalla *et al.*, 1985; Seppanen, 1988; Tamara *et al.*, 2004). A wide variety of bacteria are known to catalyze the oxidation of Mn(II). In particular, Mn oxidation can be mediated by species of several genera such as *Leptothrix, Crenotrix, Hyphomicrobium, Siderocapsa, Bacillus sp. strain SG-1, and Metallogenium* (Mouchet, 1992; Brock *et al.*, 1994; Chris *et al.*, 2001). Mn(II) oxidation rates have been shown to increase by at least 3–5 orders of magnitude in the presence of Mn(II) oxidizing bacteria (Hastings and Emerson, 1986; Tebo, 1991; Brouwers *et al.*, 1998), and several researchers have suggested that microbial oxidation is the principal pathway in the marine environment (Tebo and Emerson, 1986; Henry *et al.*, 1994). Microbial Mn(II) oxidation proceeds through indirect or direct mechanisms.

Indirect mechanisms include the production of O_2 (in photosynthesis) and of alkaline or oxidizing metabolites. Direct Mn(II) oxidation involves the microbial production of specific macromolecules (polysaccharides or proteins) catalyzing the process. The various oxidizing systems differ in many respects. For instance, the process can be catalyzed by metabolically inert spores, by cellular outer membrane components, or by bacterial sheaths. Also, according to the literature, bacterial Mn oxidation appears to be confined to outer surface coverings (Brouwers *et al.*, 2000).

Most of the work related to bacterial Mn oxidation has been focused on investigation during batch experiments (Michael and Ania, 1997; Zhang *et al.*, 2002; Boogerd and Vrind, 1987) or in natural environments (Moffett and Ho, 1996; Peter *et al.*, 1997; Alan and Tracey, 2005). Detailed information about the biological oxidation of Mn and the products produced has been also reported for the case of in situ groundwater treatment plants (Rott and Lamberth, 1993; Mettler *et al.*, 2001; Rott, Meyer and Friedle, 2002).

Several approaches have been used to express the rates of biological Mn oxidation. Studies on Mn oxidation reported in the literature use first order (Peter, 1997; Ioannis and Anastasios, 2004) or Michaelis–Menten type rate expressions (Zhang *et al.*, 2002; Moffett and Ho, 1996; Alan and Tracey, 2005) to describe the rate of Mn(II) removal, while there are only limited references concerning modeling of biological filters (Štembal *et al.*, 2005; Ioannis and Anastasios, 2004).

During Mn(II) oxidation the oxidized Mn is deposited as MnO_2 in the form of a black precipitate coating of the sand surface. It is well known that the MnO_2 layer has a catalytic effect on Mn(II) oxidation by dissolved oxygen, accelerating the process of Mn removal (Morgan, 1967). Thus, in spite of the great importance of biological processes, the chemical ones cannot be neglected.

Two pilot-scale trickling filters with fractions of various sizes of silica gravel (monolayer and multilayer filters) were constructed and tested for the biological oxidation of Mn in potable water. A series of experiments were carried out in order to study the effect of the operating parameters, namely of the Mn feed concentration and of the volumetric flow rate, as well as, the effect of the support media (monolayer and multilayer) on filter efficiency. A novel mathematical model was developed to describe the biological and autocatalytic Mn oxidation in the trickling filter. The proposed model is based on three assumptions: (a) the bacterial Mn oxidation is confined to outer surface coverings(Brouwers, 2000), (b) the bacterial cells attach on the frame of the biofilm forming a monolayer (this assumption has been also applied to the case of iron oxidation (Karamaneve, 1991; Nemati and Webb, 1999; Tekerlekopoulou, 2006) and, (c) the biofilm surface density varies at the different gravel layers due to the aggregated biomass which is deposited on the monolayer surface (this assumption was verified experimentally). Finally, mixed culture experiments were conducted in flasks to determine the net rate of biological Mn(II) oxidation and to estimate the kinetic parameters. The general working of trickling filter is represent by the Figure 2.2 as below.

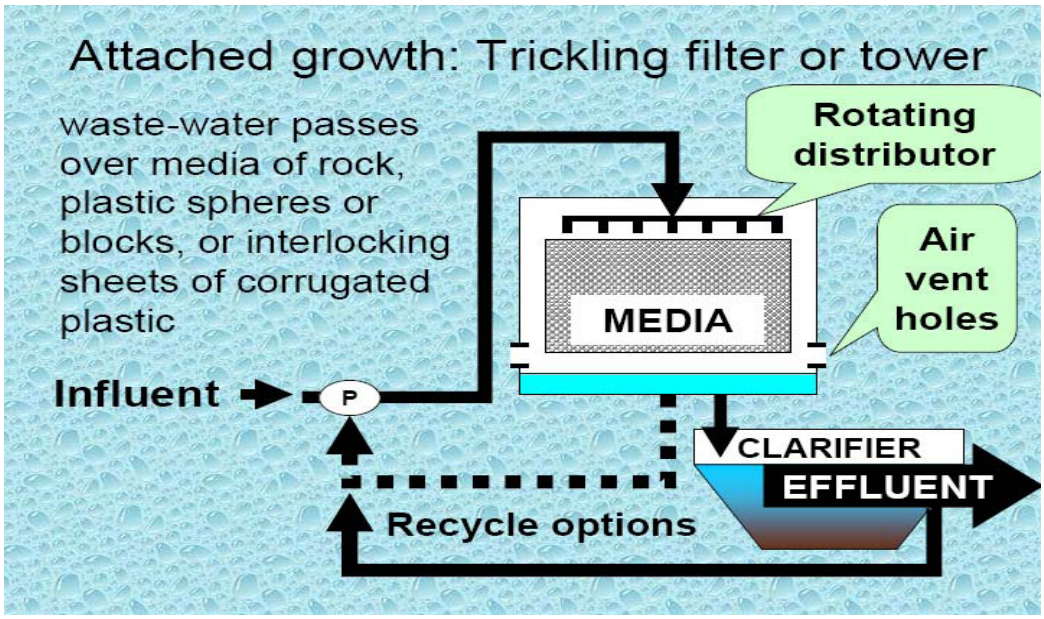

Figure 2.2: Trickling Tower

2 Electrodialytic Process

The electrodialytic remediation method was originally developed at the Technical University of Denmark (Ottosen and Hansen, 1992) for remediation of contaminated soil. The waste to be treated is saturated with water (or a solution of specific substances called "assisting agents") and placed inside the electrodialytic cell. An electric field is applied across the cell, causing the metal ions to migrate towards the electrodes, according to their charges. Selective ion exchange membranes placed between the compartments prevent the metal ions from reaching the electrodes. Instead, they are accumulated in "concentration" compartments from where they can be removed. This technique has also been applied with success to the treatment of wood waste (Ribeiro *et al.*, 2000) and biomass ash (Pedersen, 2002). Recently, some studies have been presented on the use of this technique for the treatment of electrostatic precipitator (ESP) fly ash from incineration of municipal solid waste (Pedersen, 2002).

Theoretically, the electrodialytic remediation of municipal solid waste (MSW) fly ash is possible. Fly ash particles have a porous structure and a solubility of almost 40% (w/w) (Ferreira *et al.*, 2003). A promising aspect is that the majority of the toxic metals are presumably found on the surface, as chloride compounds. This assumption is based on the fact that during combustion and gas transport, the thermodynamic conditions necessary for the formation of such compounds have occurred (Ferreira *et al.*, 2003). Since the great majority of chlorides are extremely soluble, the electrodialytic remediation may present good results.

Several conditions must be met for the successful application of this method to this material. Firstly, the solution used to prepare the initial slurry is important. The use of assisting agents during electrodialytic remediation is different for each different

media: soils and waste wood (Ottosen *et al.*, 1998), bioash and ESP fly ash (Pedersen, 2002). Different types of assisting agents can be used. These agents can normally be defined as solubilisation enhancement compounds, which form stable complexes with the substances to be removed. In order to work with the electrodialytic technique, the complexes must be electrically charged, so that they can be mobilized by the electric field. In a previous investigation by Ferreira *et al*. (2002), several assisting agents were evaluated for heavy metal removal from MSW fly ash, ammonium acetate, ammonium citrate and Na-gluconate. According to that study, Na-gluconate presented the best removal efficiencies for both zinc and lead, while performing well for the other metals (cadmium and copper). The same study showed that Na-gluconate presents additional characteristics such as non-toxicity, good performance at high pH values, formation of charged complexes with the metals and reasonable price. Although this pointed out the possibility of using Na-gluconate as an assisting agent in the electrodialytic remediation of fly ash, this substance has not been tested so far in electrodialytic cells. This testing is conducted in the current work.

Another important aspect is the physical design of the electrodialytic cell. Cells can have varying numbers of compartments as well as use different membrane types. This flexibility allows for the development of process specific designs. For example, migrating ions may either be allowed to deposit on the electrodes, or collected in a chamber by changing from passive membranes to ion selective membranes. Further design possibilities were recently to solve issues such as slow remediation rates, formation of precipitates and self-hardening of fly ash, which happened when working with ESP fly ash. This new design consists of a cell in which the sample is continuously stirred.

2.3.3 Biofilm Process

A biofilm is a structured community of microorganisms encapsulated within a self-developed polymeric matrix and adherent to a living or inert surface. Biofilms are

also often characterized by surface attachment, 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 structures such as pili (Wikipedia, 2008).

A filter able to remove manganese from groundwater by biological oxidation and accumulation (bioaccumulation) without the addition of chemicals is said to be a mature filter (Vandenabeele *et al*., 1995) . A mature biological filter capable of Mn removal is achieved by passing aerated water vertically through a column of filter sand, in the absence of chlorine, and allowing the benthic microorganisms to proliferate as biofilms within the void spaces (Bourgine *et al*., 1994). Biological treatment, for the bioaccumulation of Mn in slow sand filters has been reported to lower Mn concentrations successfully from an input of 350 µg/L to an output of 20 µg/L (Seppänen, 1992). Maturation times for these filters can vary from weeks to months, even after seeding with filter medium from other mature filters (Mouchet, 1992). At the Saints Hill water treatment plant, having raw groundwater Mn concentrations between 500 and 600 µg/L, Mn biofilters were operated for 3 months before Mn was removed by biological processes (Bourgine *et al*., 1994). The success of Mn biofilter maturation can differ greatly between two water treatments sites, even though the chemical and biological characteristics of the incumbent groundwater may be very similar (Ward, 1994).

There is an urgent need to improve the ability to establish mature manganese biofilters in a reasonable time, at suitable water treatment sites. In order to overcome some of the difficulties associated with this requirement a pilot biofilter was designed and operated using a monoculture of *Leptothrix discophora*, thereby allowing a direct comparison of the effects of changing process variables to be made. The principal objective of the work was to establish the conditions which would reduce filter maturation times to a minimum.

L. discophora is one of the predominant organisms in Mn biofilters (Czekalla *et al*., 1985; Vandenabeele *et al*., 1995) and is characterized by its ability to oxidize Mn^{2+} (Ghiorse, 1984) and to form a sheath incorporating manganic oxides (Mulder *et al*., 1989). It is this ability to oxidise Mn as well as iron that distinguishes *Leptothrix* species from other sheath forming genera, such as *Sphaerotilus*, which can only oxidize Fe (Corstjens *et al*.,1992).

L. discophora strain SS-1 (ATCC 43182) is a Mn oxidizing, aerobic, heterotrophic gram negative short rod, which has lost is sheath forming ability after successive inoculations onto laboratory media at high growth rates. Its inability to form a sheath is possibly due to the loss of a plasmid encoding sheath genes (Hope, 1999). This strain has received the most attention in terms of its growth kinetics, metabolism and Fe/Mn oxidation (Adams and Ghiorse, 1986; Adams and Ghiorse, 1987; Boogerd and Vrind, 1987; Zhang *et al*., 2002).

'Wild-type' *L. discophora* are most probably the sheath forming strain SP-6 (ATCC 51168). Wild-type *L. discophora* appear as two indistinct forms; motile (sheathless) cells and immotile (sheathed) cells. Sheath-forming strains of *L. discophora (SP-6)* can be maintained indefinitely in slow growing conditions at temperatures between 20°C and 25°C in combination with careful culture management at 4°C and −80°C (Emerson and Ghiorse, 1992).

Biofiltration of groundwater was modelled in batch cultures of *L. discophora SP-6* grown in laboratory fermenters containing a side arm loop passing medium, supplemented with Mn, through a biofilter. The decrease in the amount of detectable Mn over time in these systems represented the bioaccumulation of Mn by the biofilms of *L. discophora* within the filter matrix. Modelling biological filter processes at the laboratory scale allowed the effects of defined parameter changes to be transferred to full-scale biofilters (Rowan *et al*.,2002). By successfully modelling biofiltration and studying Mn bioaccumulation rates (i.e. the decrease in Mn concentration in water

passing through the filter) in these filters we hoped to elucidate optimum operating parameters for the process and reduce filter maturation times from months to days.

2.3.4 Selection of Biofilm Process

For this experiment, biofilm is the best method to use because it provide many advantages than others method. For instant, it elucidates operating parameter for process and reduces filter maturation from month to days. The microorganism use is flexible and has ability to oxidise other heavy metal such as iron. Compare to trickling method, the success of the method depend on the chemicals and biological characteristics.

2.4 Mixed Culture

2.4.1 Mixed Culture in Drain

Pedomicrobium is a ubiquitous bacterium dominant in biofilms of man made aquatic environments such as water distribution systems and bioreactors. Due to their abilities to oxidise Mn, they are found to be the main culprits of Mn related “dirty water” (Sly *et al*., 1988a). *Pedomicrobium* are budding hyphal bacteria that can be found in both terrestrial and aquatic environments (Sly *et al*., 1988a).

The dimorphic mode of reproduction results in a non-motile form, which has the ability to adhere strongly to surfaces and form biofilm (Sly *et al*., 1988b). The attached cells take advantage of the nutrients and soluble manganous ions attracted to the solid-liquid interface, which are continually replenished by the water flow (Sly *et*

al., 1988a). Manganese oxidizing bacteria in biofilms have been shown to greatly enhance the rate of Mn oxidation (Sly *et al*., 1988b).

The association of Mn oxides with the surfaces of microbial cells is well known (Larsen *et al*., 1999). Oxidation of Mn by *Pedomicrobium* has been shown to occur enzymatically, and the deposition of the manganous oxide occurs on extracellular acidic polysaccharides (Sly *et al*., 1990a; Larsen *et al*., 1999). The mechanism of Mn (II) oxidation by *Pedomicrobium* is a two-step process involving adsorption of Mn through surface charges and ionic attraction, and subsequent oxidation to Mn oxide (Larsen *et al*., 1999). Larsen *et al*. (1999) showed that the Mn oxidizing enzyme was located in the outer cell membranes and that enzyme activity was copper dependent.

Leptothrix discophora is a freshwater bacterial species known for its ability to oxidize iron (Fe) and manganese (Mulder, 1972; Veen *et al*., 1978). In Fe and Mn rich natural waters, sheath-covered cell strings and empty sheaths of this species encrusted with ferromanganese oxides and oxyhydroxides are often found (Ghiorse, 1984). Extracellular sheath macromolecules are believed to be involved in Fe and Mn oxidation. The strain *L. discophora SS-1* was isolated and characterized (Adams *et al*., 1985; Adams *et al*., 1986). This strain does not form a well structured sheath anymore, but at least one factor that is able to catalyze the oxidation of Mn is excreted into the culture medium (Adams *et al*., 1987; Boogerd and Vrind, 1987).

2 Mixed Culture in Soil

Aspergillus niger is a fungus and one of the most common species of the genus *Aspergillus*. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions, and peanuts, and is a common contaminant of food. It is ubiquitous in soil and is commonly reported from indoor environments, where its black colonies can be confused with those of Stachybotrys (species of which

have also been called "black mold").Microbial viability is essential for biotransformation as these reactions are enzyme mediated. Generally metal ions are converted into insoluble form by specific enzyme mediated reactions and are removed from the aqueous phase (Park *et al*., 2000). There are reports of live microbial systems for the purpose of remediation of contaminated soils and waters (Cervantes and Silver, 1992). Higher fungi (mushrooms), yeast, bacteria, seaweed and plant bark materials are abundantly available in nature and can be a source of low cost biosorbents (Cervantes *et al*., 2001; Shrivastava and Thakur, 2003).

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

One of the *Brevibacillus* species which is *Brevibacillus sp*, found very efficient in degradation of dye-contaminated wastewater. It can growth in the air but cannot growth anaerobically, the structure of *Brevibacillus sp* was rod, can react with Aminopeptidase and can oxidize. From the experimental result, the strain could degrade 50 mM as well as 125 mM Toluidine Blue solutions quite efficiently. However, the strain apparently could not handle the higher concentrations of the dye. The best pH for dye degradation is around pH 7 (Alhassani *et al*., 2005).

Another bacterium was *Nocardia Globerula*. Some studies show that it can degrade the pollution in petroleum. The characteristic of Nocardia were listed which were non motile, the shape of the bacteria was rod, slightly pellicle, heavy in mannitol

with ammonium sulohate and it was good growth at 25^{o}C but poor growth at 37^{o}C (Azarowicz *et al*., 1973).

3 Mixed Culture in 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 cause 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 were not any report about the infection by this bacterium.

4 Selection of Mixed Culture in Drain

For this study, mixed culture in drain is selected to be used in removal of manganese. This is supported by fact which is drainage mixed culture have more advantages instead of disadvantages than mixed culture in soil and river. The supported fact which is Bacillus spp has bad effect in our health. Earlier, the most obvious/discussed problem concerning drainage of wetlands was the physical distortion of the archaeological wooden objects when drying out (Bjordal and Nelson, 2002)

2.5 Reactor

2.5.1 Batch 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 continuous operation (Fogler, 2006).

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). 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).

Figure 2.3: Batch reactor with single external cooling jacket

2 Continuous Stirred Tank Reactor(CSTR)

These types of reactors employ a stirred tank, to which reactants are continuously added and products continuously withdrawn. In a continuous stirred tank reactor (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 (Wikipedia, 2008).

The CSTR is an easily constructed, versatile and cheap reactor, which allows simple catalyst charging and replacement. Its well -mixed nature permits

straightforward control over the temperature and pH of the reaction and the supply or removal of gases. The CSTR tend to be rather large as the: need to be efficiently mixed. Their volumes are usually about five to ten time the volume of the contained immobilized enzyme. This, however, has the advantage that there is very little resistance to the flow of the substrate stream, which may contain colloidal or insoluble substrates, so long as the insoluble particles are not able to sweep the immobilized enzyme from the reactor. The mechanical nature of the stirring limits the supports for the immobilized enzymes to materials which do not easily disintegrate to give 'fines' which may enter the product stream. However, fairly small particle (down to about 10 mm diameter) may be used, if they are sufficiently dense to stay within the reactor. This minimizes problems due to diffusional resistance (Chaplin, 2004)

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 plug flow reactor (PFR).

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 CSTR 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).

2.5.3 Sequencing Batch Reactor (SBR)

In recent years, SBR has been employed as an efficient technology for wastewater treatment, especially for domestic wastewaters, because of its simple configuration (all necessary processes are taking place time- sequenced in a single basin) and high efficiency in biological oxygen demand (BOD) and suspended solids removal. SBRs could achieve nutrient removal using alternation of anoxic and aerobic periods (Eli *et al*., 1997). The SBR has received considerable attention since Irvine and Davis described its operation (Irvine and Davis, 1971) and studies of SBR process were originally conducted at the University of Notre Dame, Indiana (Irvine and Busch, 1979). The sequencing batch reactor is a fill-and thaw activated sludge system for wastewater treatment. In this system, wastewater is added to a single "batch" reactor, treated to remove undesirable components, and then discharged. Equalization, aeration, and clarification can all be achieved using a single batch reactor.

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 (JJS EPA, 1999). 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 filling, 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).

SBRs can be used to deal with variations in flow and composition of wastewater (Mace and Mata, 2002). These reactors are based on the fill-and-draw principle EPA (1999) and commonly operated with fixed time intervals. There are five process phases which is summarized in Figure 2.4 and in the following sequence:

- Idle: the reactor is not in use.
- Re-aerate: the biomass in the reactor is re-aerated to saturation, to ensure oxygen is available when the substrate enters the reactor.
- Fill and react: the working volume of the reactor is filled with wastewater and the activated sludge within the reactor degrades organic and nitrogen compounds through metabolic activity.
- Settle: the activated sludge sediments to the bottom of the tank
- Draw: the working volume of treated water is removed from the reactor (effluent).

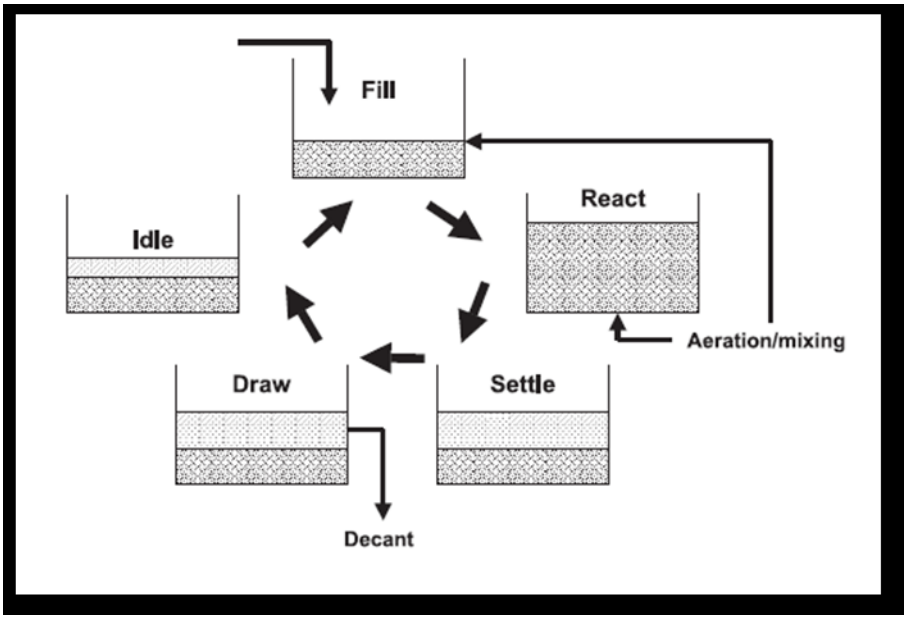

Figure 2.4: Five process of SBR

2.5.4 Selection of Sequencing Batch Reactor

In this study, sequencing batch reactor has been selected because the flexibility of operating, potential capital cost savings by eliminating clarifiers and other equipment. Other than that this reactor has high efficiency in COD and suspended solids removal and need minimal footprint. The Equalization, primary clarification (in most cases), biological treatment, and secondary clarification can be achieved in a single reactor vessel.

CHAPTER 3

METHODOLOGY

1 Development of Mixed Culture

Take 4 liters sample from the selected drain to develop the mixed culture. Put the sample into 10 liters reactor which is provided with support media from rocks that have diameter in range of 1.5cm to 2cm. The rest of 10 liters reactor was filled with autoclave distilled water.

The reactor was used for the growth of mixed culture from drain. The mixed culture was fed with manganese to make it acclimatized in order to treat manganese from waste water. The mixed culture also fed with 200 mg/L glucose as their nourishment every day after one week. Suspended solid test was used to keep monitoring the growth of mixed culture everyday for at least one month. The hydraulic retention time for the acclimatization is 5 days.

2 Reactor System

In this research,SBR has been selected as reactor system. For acclimatization reactor, a 10 liters reactor was used while 8 reactors with each capacity of 5 liters was used as treatment reactor as shown in Figure 3.1.

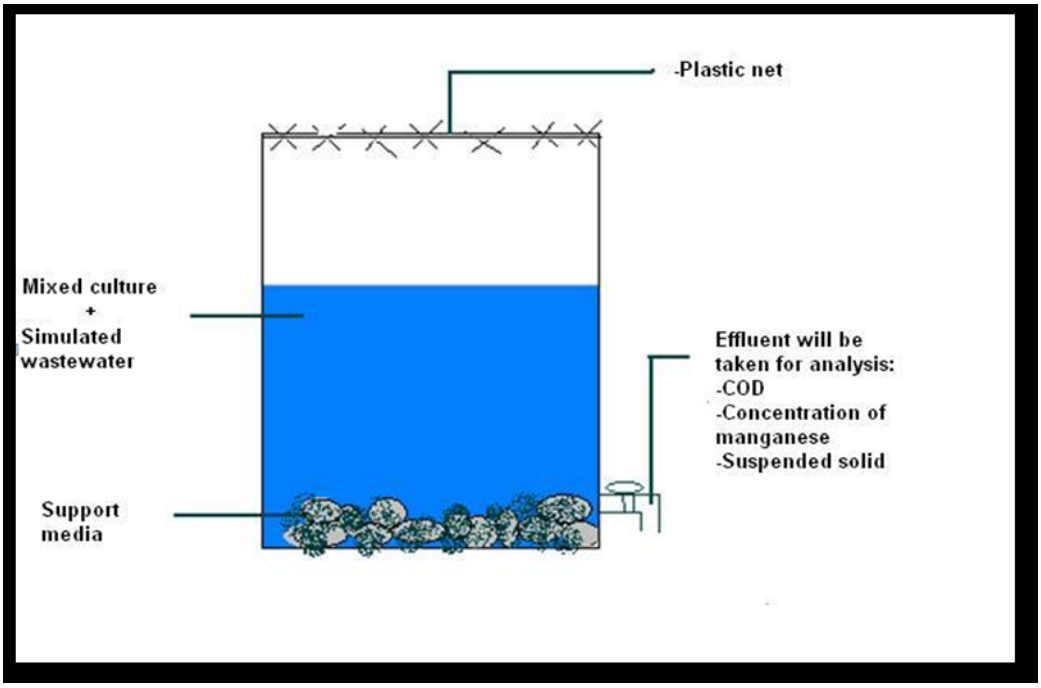

Figure 3.1: Sequencing batch reactor

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

3 Operational Condition

This experiment consists of two stages processes, which are acclimatization process and treatment process. In acclimatization process, the mixed culture from drain is acclimatized in acclimatization reactor for one month. Within this period, some small amount of manganese is added to the reactor in order to acclimatize the mixed culture with the treatment environment and fed with glucose as well.

For the treatment process, the mixed culture from acclimatization reactor will be transferred to the treatment reactor. The manganese treatment process will be carried out by controlling the loading rate. The hydraulic retention time (HRT)

selected for this research is 5 days. The experiment will be run within two weeks. Within this period, the collection of the sample will be done for everyday as well as the addition of the simulated wastewater to the treatment reactor. The details about the treatment process are summarizes in Table 3.1.

Table 3.1: Details of treatment

Loading Rate (mg/L.d)	Concentration (mg/L)	Hydraulic Retention Time(day)	Flowrate (L/ d)
5.00	25.0	5	1
4.50	22.5	5	1
4.00	20.0	5	1
3.00	15.0	5	1
4.00	20.0	5	1
3.50	17.5	5	1
5.00	25.0	5	1
3.00	15.0	5	1

4 Chemical Composition

For this research, manganese (II) carbonate will be dissolved into deionized water to produce manganese solution in which the solution will be the simulated wastewater for this research. The concentration of manganese ion (Mn^{2+}) used are based on the selected loading rate as shown in Table 3.1.

3.5 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 manganese concentration as well.

3.5.1 Determining Total Manganese

. For manganese concentration analysis, the sample is placed in a 10 ml sample cell. Then, the Buffer Cytrate Type for Manganese Powder Pillow is placed in the cell before being well-mixed. After that, a Sodium Periodate Powder Pillow is placed in the cell. Then, the solution is left for 2 minute reaction. Another 10ml sample cell is filled with the sample which acts as blank. The sample then is tested using the HACH Spectrophotometer DR2800. Example of the sample is shown in Figure 3.2.

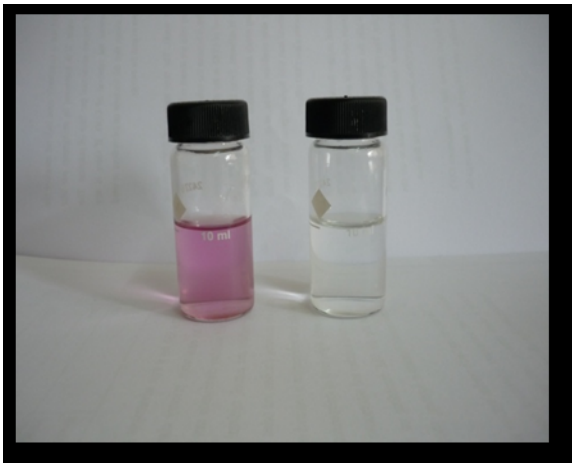

Figure 3.2: Sample cell for manganese concentration analysis

3.5.2 Chemical Oxygen Demand Test

For COD analysis, COD high range (HR) reagents are used. 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. The example is shown in Figure 3.3.

Figure 3.3: COD vials for analysis

3.5.3 Suspended Solids Test

For suspended solids analysis, the sample taken is placed in 25 ml sample cell. Another sample cell is filled with deionized water which acts as the blank. The sample cells are wiped out for removing fingerprints. The sample then is tested using the HACH Spectrophotometer DR2800.

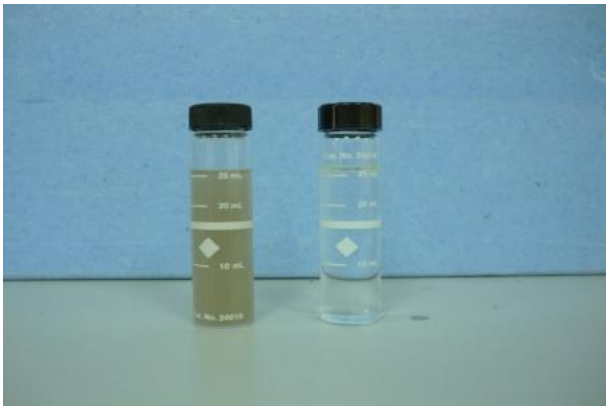

Figure 3.4: Sample cell for suspended cell analysis

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Suspended Solid Concentration

4.1.1 Suspended Solid Concentration for Acclimatize Reactor

The Figure 4.1 shows the graph of concentration of suspended solid within 72 days during cultivated period. During 1st day until 11th day period the mixed cultures try to acclimatize to the new environment. There are several distinct segments in the biomass curve that warrant further examination. The microorganisms must first become acclimated to their surrounding environment and the food provided and the period will vary in length, depending on the history of the seed organisms (Howard, 1985). It starts to increase higher at 11th because it was acclimatize with new environment.

On the 12th day until 15th the concentration increases slowly because it being feed with glucose every day. From 16th day until 18th day the concentrations become unstable again but the range of the data still in the range after being feed. The formation of biofilms on grain surfaces is the outcome of the complex interaction between several physical, chemical and biological processes (Bryers 2000, Characklis 1990). Many bacteria are capable of "swimming" on their own in random or specific directions, for example in response to the concentration gradient of a nutrient or a

toxin. It means some of the bacteria can adapt and eat the glucose. Some of the bacteria deaths because within this period it is starve and cannot eat glucose. Time of the bacteria death make the data unstable. During 20th day the concentration decreases drastically. It is because that the mixed culture does not having enough nutrient so that some of them were died of starving. From 21th day until 27th the concentrations of the suspended solid increase every day. It is because that the mixed culture is well acclimatizing with the environment and the nutrient as well. During 28th day until 38th the concentration is fluctuation but it still within the high range value. The morphology of biofilms in porous media depends strongly on the bacterial species, the structure of the porous medium and the prevailing hydrodynamic and nutritional conditions and ranges from continuous, smooth films to discontinuous, highly irregular colonies (Rittmann, 1993). It is because some of the bacteria were adhered and some of the bacteria were lived in the pore of the support media. In addition to this adhesion, a consecutive cellular multiplication and the associated production of extracellular polymeric substances (EPS) must also be considered (Michaud *et al*., 2005). It will allow irreversible adhesion of the microorganisms to the surface of the carrier.

At 41th day the concentration decreases drastically. The main reason is because it does not have enough nutrients for their food. It is proved by the next day from 43th until 45th that the concentration increases gradually while at 48th the concentration decrease again. On the 49th the concentration decrease and after that it increase at high value. From 50th day until 62th day the concentration become fluctuation again but within high value. Indeed, in this kind of processes, detachment is mainly due to shocks between the colonized particles (Trinet *et al*., 1991). Attrition involves the erosion of the weakest fixed biofilm fragments. Thus, once this fraction of the biofilm detached, and if the growth is sufficient to compensate for the detachment, the system tends towards a steady-state. From 63th day until 72th day the concentration is increase gradually except at 67th day. The graph shown that the

mixed culture is well acclimatizing with the environment and the nutrient as well and also the porous media is well fullfill.

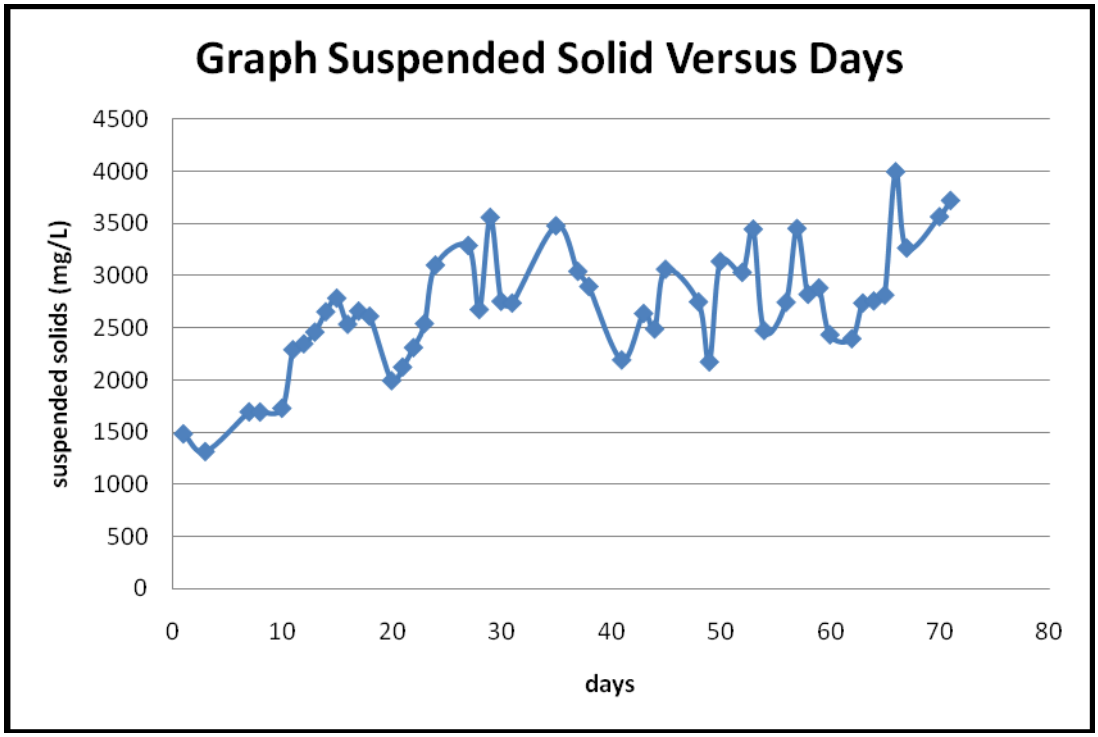

Figure 4.1: Suspended solid concentration for acclimatization reactor

4.1.2 Effect of Loading Rate on Biomass Growth

The Figure 4.2 shows the graph suspended solid versus day for the treatment reactor. The data represent for eight LR which is 5 mg/L.d (A), 5 mg/L.d (B), 4.5 mg/L.d (A), 4 mg/L.d (A), 4 mg/L.d (B), 3.5 mg/L.d (A), 3.5 mg/L.d (B) and 3 mg/L.d. The highest value of suspended is 648 mg/L at 5 mg/L.d (A). The lowest value of suspended solid is 301 mg/L at 3.5 mg/L.d. For the same LR, it shown some differs in value because of some error such as lab handling and equipment efficiency.

The trend of the entire graph is decrease over the time. It is shows that the dying mixed culture surpasses the growth of them because the concentration of manganese being fed for acclimatizing is extremely different. The microorganisms must first become acclimated to their surrounding environment and the food provided and the period will vary in length, depending on the history of the seed organisms (Howard, 1985).This behavior coincides with the finding of Seppänen (1988) who concluded that the time necessary for Mn bacteria development is longest before the mixed culture became stable and the growth will increase.

There is fluctuation trend because some of the bacteria were adhered and some of the bacteria were lived in the pore of the support media. In previous researcher reported that the problem would have been exacerbated by biofilms of bacteria growing within the void spaces (Miltner, 1993 and tsotsis, 2001). In addition, many bacteria are capable of “swimming” on their own in random or specific directions. The trend of the graph is decreasing but in fluctuation. It is because the morphology of biofilms in porous media depends strongly on the bacterial species, the structure of the porous medium and the prevailing hydrodynamic and nutritional conditions and ranges from continuous, smooth films to discontinuous, highly irregular colonies (Rittmann, 1993).

According to the Design-Expert in Figure 4.3, the results show that the maximum suspended solid concentration is at the highest LR which is 5 ml/L.d. Both of the treatment and Design Expert graph show the same trend for each LR. So, the conclusion is the data of the experiment is acceptable.

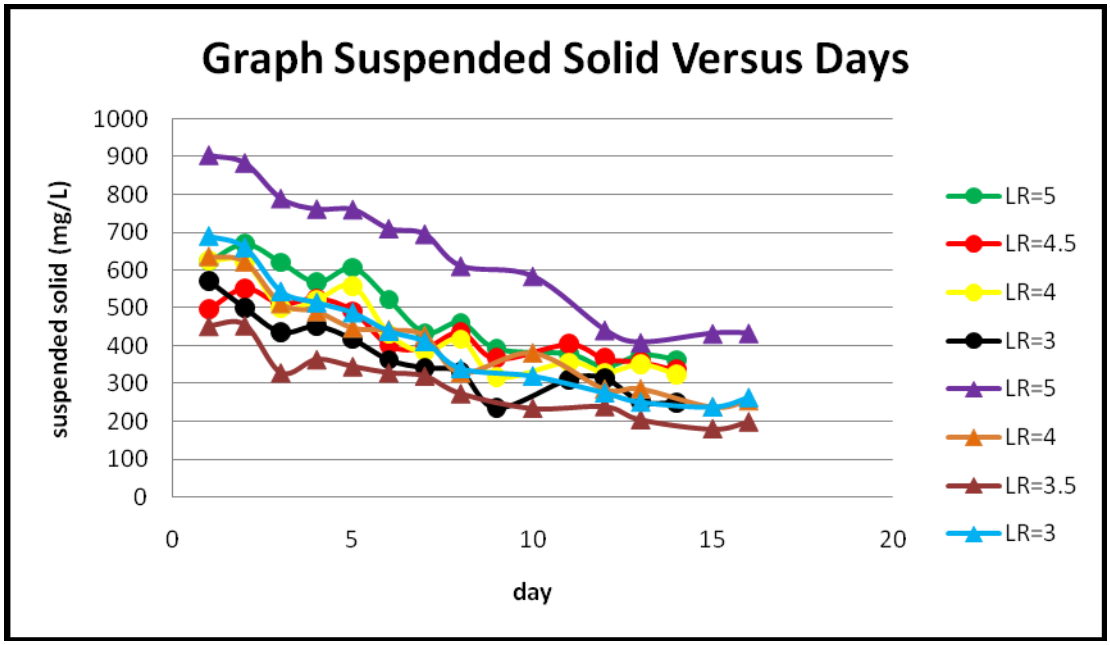

Figure 4.2: Suspended solid concentration for treatment reactor

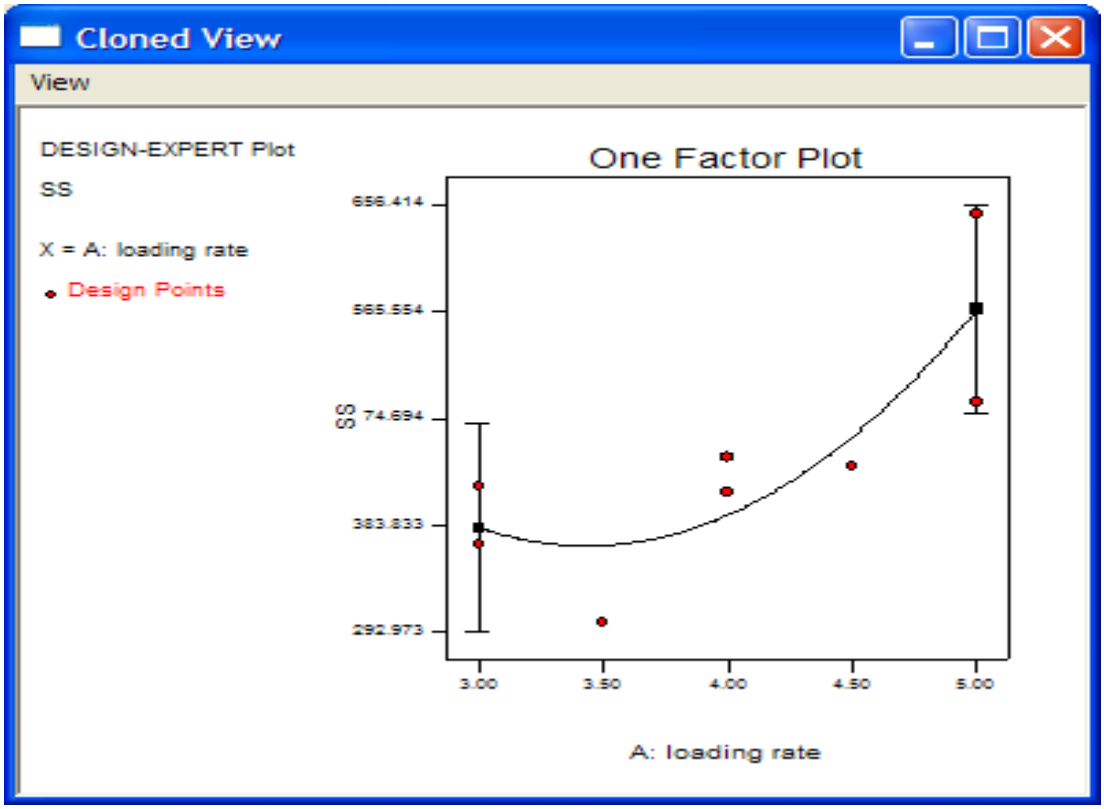

Figure 4.3: Design-Expert for suspended solid concentration

4.2 Effect of Loading Rate on Manganese Removal

The Figure 4.4 shows the graph manganese removal versus day for treatment process. The data represent for eight LR which shown that the mixed cultured from drain have an ability to degrade Mn from wastewater. The highest manganese removal is 65% at 3 mg/L.d of LR. The lowest value of manganese removal is 23% at 5 mg/L.d (A) of LR. The overall trend of the graph is fluctuation because the value of Mn removal is inconsistently and unstable. For Mn treatment, the period needed to reach the Mn removal efficiency was 8 weeks, which is in accordance with the findings of several other researchers (Hatva, 1988; Gislette and Mouchet, 1997). In addition, the biofilm configured system integrated with periodic operation imposes regular variations in substrate concentration on biofilm organisms (Woolard, 1997). Therefore, organisms throughout the film achieve higher growth rates which results in improved reaction potential leading to stable and robust system well suited for treating highly variable wastes.

From the results shown that the mixed cultured can remove highest percentage at lowest manganese concentration. It is because the mixed culture is acclimatized with low concentration simulated wastewater. The microorganisms must first become acclimated to their surrounding environment and the food provided and the period will vary in length, depending on the history of the seed organisms (Howard, 1985).

Based on Design-Expert in Figure 4.5, it shows that the lowest LR which is 3 mg/L.d giving the highest percentage of manganese removal for the treatment. In other hand, the highest LR which is 5mg/L.d giving the lowest manganese removal. It means that the data is acceptable because the trend of each parameter is consistent for both graphs.

From the Table 4.1, there are some of the comparison percentage manganese removals using different removal technique. The SBR technique with 65% manganese removal does not represent good efficiency of removal. It is because by using roughing filtration and bench-scale filtration, the removal can achieve 95% and 90% respectively. In other hand, trickling filter can achieve 63% manganese removal. It shows that SBR treatment in this research is acceptable but need the further research

to make it achieve the highest removal that can be advantage for the treatment applied to the wastewater in industry.

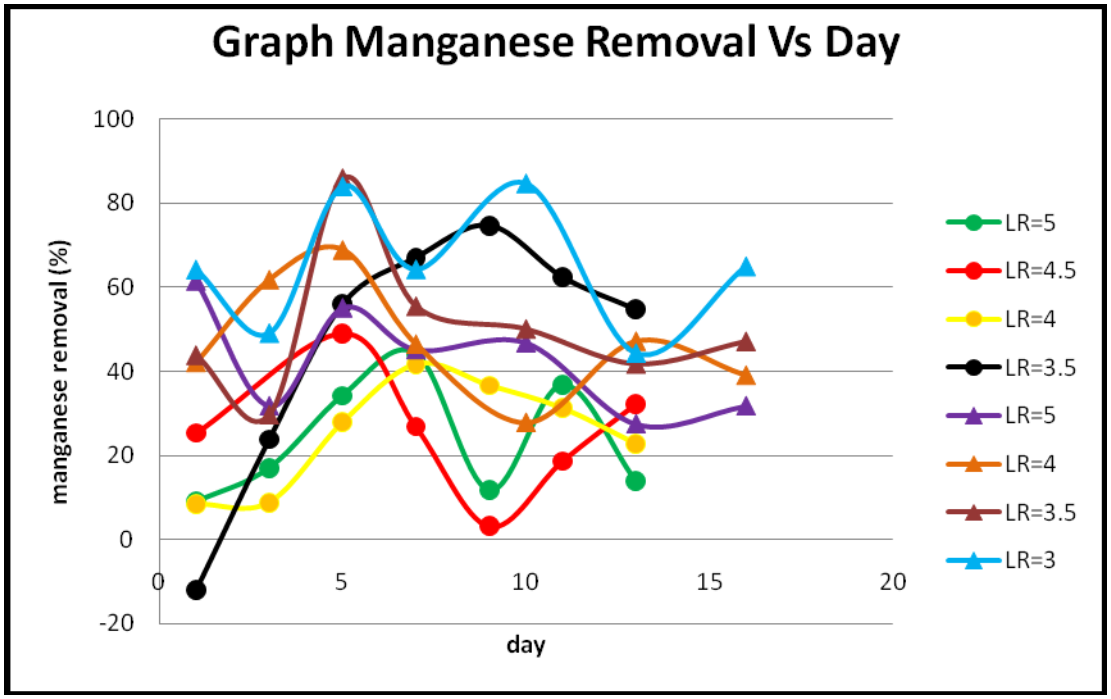

Figure 4.4: Percentage of manganese removal

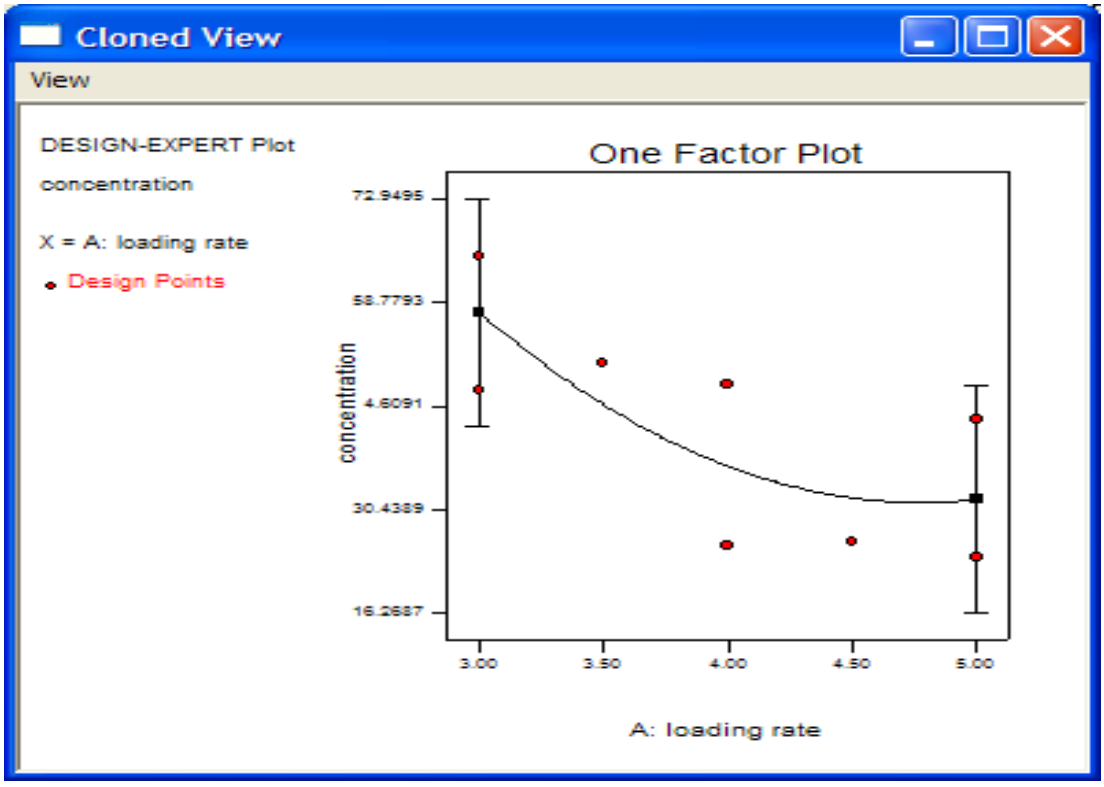

Figure 4.5: Design-Expert for percentage manganese removal

Table 4.1: Comparison for various techniques in manganese removal

Removal Technique (corresponding author)	**Maximum percentage of manganese removal**
Trickling Filter (Tekerlekopoulou and Vayenas, 2007)	63%
Bench Scale Filtration (Burger *et al*.,2008)	90 %
Roughing Filtration	95%

(Pacini *et al*.,2005)	
Sequencing Batch Reactor (This study)	65%

4.3 Effect of Loading Rate on COD Removal

The measurement of nonbiodegradable organics is usually by the chemical oxygen demand (COD) test which is the indirectly measurement. Most applications of COD determine the amount of organic pollutants found in surface water (e.g. lakes and rivers), making COD a useful measure of water quality. It is expressed in milligrams per liter (mg/L), which indicates the mass of oxygen consumed per liter of solution. In this experiment, the percent of COD removal shows that the amount of oxygen in simulated wastewater is being consumed.

The Figure 4.6 shows the graph manganese removal versus day for treatment process. The data represent two cycles for eight LR. The graph shows that highest value of COD removal is 72% at 3.5 mg/L.d of LR. The lowest value of COD removal is -3% at 4.5 mg/L.d of LR. The data for the same LR shown some differs in value because of some error such as lab handling and equipment efficiency.

The percent of COD removal is depending on biomass growth and ion Mn^{2+} in simulated wastewater. The unstable growth of biomass will also affect the COD result .The trend of the graph is unstable and fluctuation. From the literature review, the COD efficiency stabilize after reaching steady state condition where it need long period of time to reached the steady state condition (Shao, *et al*., 2008).

Based on Figure 4.7 that represent the Design-Expert, the data shows that the lowest LR which is 3 mg/L.d giving the highest percentage of COD removal. It is an acceptable and expected result because the manganese removal and COD removal

should be concordant because the COD measurement is depending on the manganese concentration. As a conclusion, to achieve the highest COD removal the treatment should be in lowest LR which 3 mg/L.d.

From the Table 4.2, there are some of the comparison percentage COD removals using different removal technique. The SBR technique in this research showed the second highest percentage of removal which is 72%. The highest percentage of COD removal is 83%, 66% and 42% respectively. This result revealed that SBR is the optional treatment technique with acceptable percentage removal. It mean, the treatment is success but need the further research to make it achieve the highest removal that can be advantage for the treatment applied to the wastewater in industry.

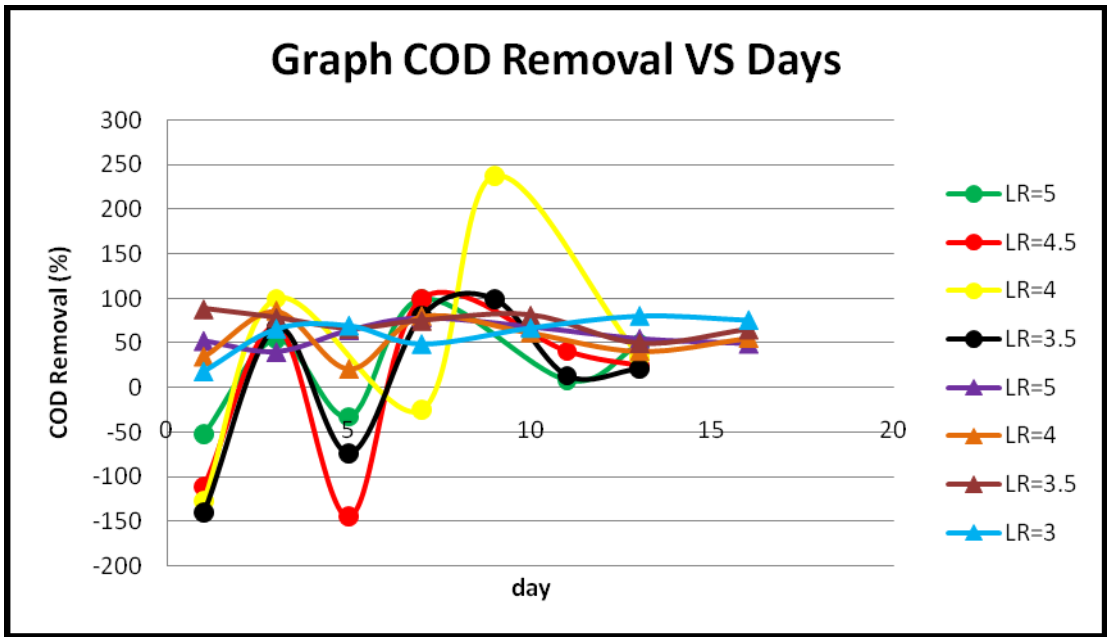

Figure 4.6: Percentage of COD removal

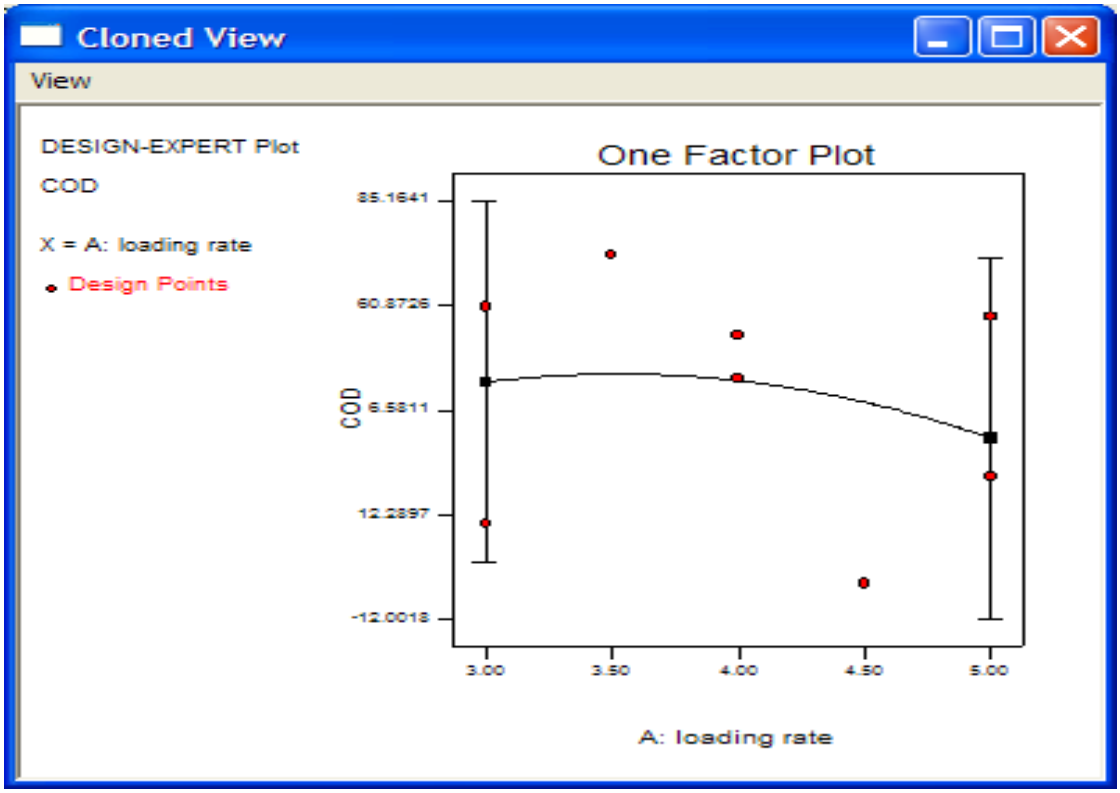

Figure 4.7: Design-Expert for percentage COD removal

Table 4.2: Comparison for various techniques in COD removal

Removal Technique (corresponding author)	**Maximum percentage of COD removal**
Biofilm Reactor (Cloete *et al.*,1999)	42%
Electrocoagulation (Wang *et al.*,2008)	66%
Sequencing Batch Reactor (Kulikowska *et al.*,2006)	83%
Sequencing Batch reactor (This study)	72%

4.4 Design-Expert for Optimization

The Figure 4.8 shows the graph of Design-Expert for optimization. The results represent the suggestion data for the treatment to get the optimization value. The value for optimization is at lowest LR which is 3 mg/L.d. By using lowest LR, the treatment will get 43% of COD removal and 57% manganese removal. The optimization of concentration of suspended solid is 381 mg/L. The desirability of the lowest parameter of LR is 0.707

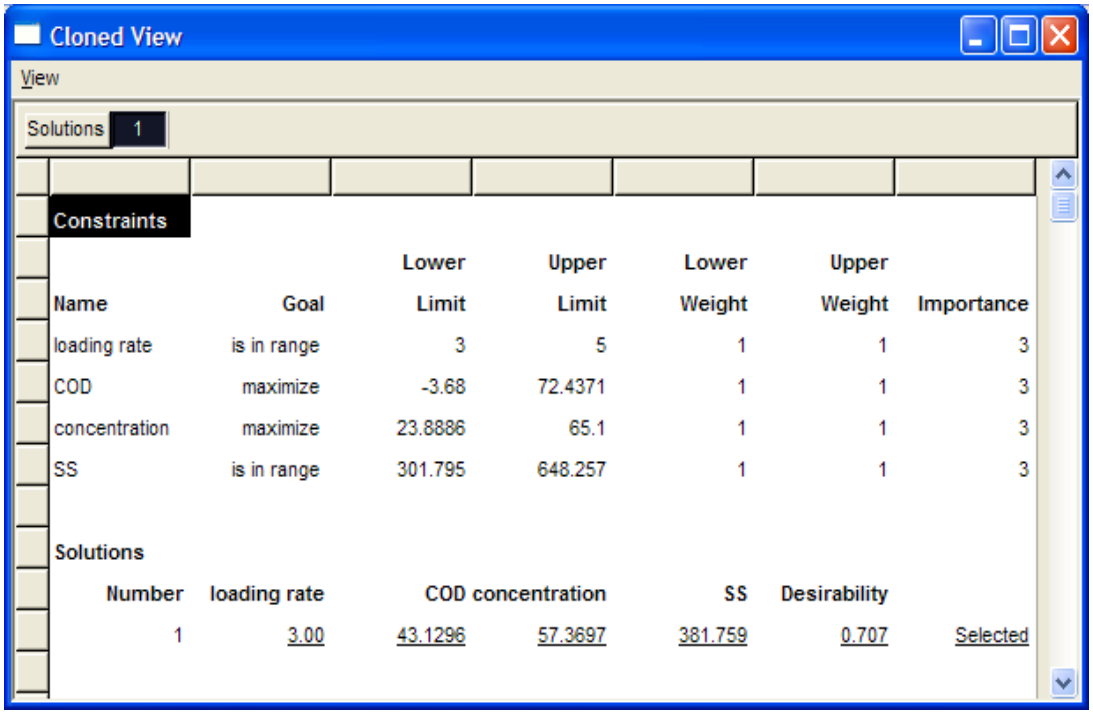

Figure 4.8: Design-Expert for optimization

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

Based on the results provided in this research treatment, the data proved that the mixed culture from drain can degrade manganese but in certain concentration. Based on result, 3 mg/L.d of LR gives the highest efficiency of manganese removal and COD removal. The different of manganese concentration can affect the growth of mixed culture. At the end of this experiment shows the highest manganese removal is 65% and the highest COD removal is 72%. By using the Design Expert, the predicted value will represent the optimization results for this research. The value for optimization is at lowest LR which is 3 mg/L.d. By using lowest LR, the treatment will get 43% of COD removal and 57% manganese removal. The optimization of concentration of suspended solid is 381 mg/L. Experimental data revealed that the biofilm configuration coupled with sequencing/periodic discontinuous batch mode operation appears to be promising option for the treatment of complex industrial wastewater containing poorly degradable compounds.

5.2 Recommendation

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

- The experiment period should be prolonged to avoid limited result
- 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.

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APPENDIX A

Table 1: Data for acclimatization

Date	Day	Suspended Solid (mg/L)	Date	Day	Suspended Solid (mg/L)	Date	Day	Suspended Solid (mg/L)
24-Dec	1	1486	16-Jan	24	3101.66	13-Feb	52	3031.67
26-Dec	3	1316.667	19-Jan	27	3288.33	14-Feb	53	3445.67
30-Dec	7	1696.667	20-Jan	28	2676.17	15-Feb	54	2475
31-Dec	8	1695	21-Jan	29	3558.33	17-Feb	56	2745
2-Jan	10	1731.166	22-Jan	30	2755	18-Feb	57	3451.67
3-Jan	11	2291.66	23-Jan	31	2738.33	19-Feb	58	2821.67
4-Jan	12	2348.33	27-Jan	35	3478.35	20-Feb	59	2883.33
5-Jan	13	2460	29-Jan	37	3041.66	21-Feb	60	2435
6-Jan	14	2655	30-Jan	38	2896.66	23-Feb	62	2396.67
7-Jan	15	2785	2-Feb	41	2193.31	24-Feb	63	2738.33
8-Jan	16	2535	4-Feb	43	2638.335	25-Feb	64	2758.33
9-Jan	17	2661.66	5-Feb	44	2490	26-Feb	65	2815
10-Jan	18	2611.66	6-Feb	45	3061.67	27-Feb	66	3995
12-Jan	20	1995	9-Feb	48	2750	28-Feb	67	3265
13-Jan	21	2125	10-Feb	49	2175	3-Mar	70	3565
14-Jan	22	2311.66	11-Feb	50	3136.67	4-Mar	71	3720
			15-Jan	23	2541.66			

APPENDIX B1

Table 2: Data for suspended solid in treatment reactor

Loading Rate=5			Loading Rate=4.5			Loading Rate=4			Loading Rate=3		
Date	Days	Suspended Solid	Date	Days	Suspended Solid	Date	Days	Suspended Solid	Date	Days	Suspended Solid
13-Feb	1	623.33	13-Feb	1	496.67	13-Feb	1	626.67	13-Feb	1	571.67
14-Feb	2	670.33	14-Feb	2	551.33	14-Feb	2	622.67	14-Feb	2	500
15-Feb	3	620.67	15-Feb	3	510.67	15-Feb	3	501.33	15-Feb	3	436
16-Feb	4	568	16-Feb	4	525	16-Feb	4	522.67	16-Feb	4	452
17-Feb	5	606.33	17-Feb	5	491.67	17-Feb	5	559	17-Feb	5	417
18-Feb	6	521.67	18-Feb	6	401.33	18-Feb	6	431.33	18-Feb	6	362.67
19-Feb	7	433.67	19-Feb	7	394.33	19-Feb	7	384	19-Feb	7	341.67
20-Feb	8	461	20-Feb	8	435.33	20-Feb	8	417.33	20-Feb	8	330.67
21-Feb	9	390.67	21-Feb	9	365.67	21-Feb	9	316.67	21-Feb	9	235.67
23-Feb	11	377.33	23-Feb	11	403.67	23-Feb	11	356.67	23-Feb	11	311.67
24-Feb	12	343.33	24-Feb	12	365.67	24-Feb	12	329	24-Feb	12	314.33
25-Feb	13	374.67	25-Feb	13	357	25-Feb	13	351	25-Feb	13	252.67
26-Feb	14	363	26-Feb	14	335	26-Feb	14	324.33	26-Feb	14	250

Table 3: Data for suspended solid in treatment reactor

Loading Rate=5			Loading Rate=4			Loading Rate=3.5			Loading Rate=3		
Date	Days	Suspended Solid	Date	Days	Suspended Solid	Date	Days	Suspended Solid	Date	Days	Suspended Solid
4-Mar	1	905.33	4-Mar	1	637	4-Mar	1	452.33	4-Mar	1	690
5-Mar	2	884.67	5-Mar	2	622.67	5-Mar	2	454.33	5-Mar	2	659.67
6-Mar	3	790.67	6-Mar	3	512	6-Mar	3	328.33	6-Mar	3	543.33
7-Mar	4	762.67	7-Mar	4	491.33	7-Mar	4	363.67	7-Mar	4	513.67
8-Mar	5	761.67	8-Mar	5	448	8-Mar	5	345	8-Mar	5	487
9-Mar	6	711	9-Mar	6	443	9-Mar	6	328.67	9-Mar	6	438
10-Mar	7	696.33	10-Mar	7	428	10-Mar	7	320	10-Mar	7	410.3
11-Mar	8	611.67	11-Mar	8	329.33	11-Mar	8	273.67	11-Mar	8	339
13-Mar	10	584.67	13-Mar	10	381.67	13-Mar	10	234.67	13-Mar	10	319
15-Mar	12	442.33	15-Mar	12	286	15-Mar	12	239.33	15-Mar	12	274.67
16-Mar	13	409.33	16-Mar	13	287	16-Mar	13	204.67	16-Mar	13	250
18-Mar	15	433	18-Mar	15	238.67	18-Mar	15	180	18-Mar	15	237
19-Mar	16	434	19-Mar	16	255.33	19-Mar	16	198.67	19-Mar	16	262

APPENDIX B2

Table 4: Data for manganese removal in treatment reactor

Loading Rate=5			Loading Rate=4.5			Loading Rate=4			Loading Rate=3		
Date	Days	% Removal of manganese	Date	Days	% Removal of manganese	Date	Days	% Removal of manganese	Date	Days	% Removal of manganese
13-Feb	1	9.03	13-Feb	1	25.4	13-Feb	1	8.5	13-Feb	1	-12
15-Feb	3	17.1	17-Feb	5	49.1	15-Feb	3	8.93	15-Feb	3	23.97
17-Feb	5	34.32	19-Feb	7	26.89	17-Feb	5	27.95	17-Feb	5	56.04
19-Feb	7	44.25	21-Feb	9	3.23	19-Feb	7	41.69	19-Feb	7	67.14
21-Feb	9	11.76	23-Feb	11	18.74	21-Feb	9	36.67	21-Feb	9	74.77
23-Feb	11	36.81	25-Feb	13	32.25	23-Feb	11	31.25	23-Feb	11	62.5
25-Feb	13	13.95				25-Feb	13	22.81	25-Feb	13	54.8

Table 4: Data for manganese removal in treatment reactor

Loading Rate=5			Loading Rate=4			Loading Rate=3.5			Loading Rate=3		
Date	Days	% Removal of manganese	Date	Days	% Removal of manganese	Date	Days	% Removal of manganese	Date	Days	% Removal of manganese
4-Mar	1	61.5	4-Mar	1	42.2	4-Mar	1	43.77	4-Mar	1	64.23
6-Mar	3	31.8	6-Mar	3	61.83	6-Mar	3	29.66	6-Mar	3	49.18
8-Mar	5	55.1	8-Mar	5	68.87	8-Mar	5	85.8	8-Mar	5	83.95
10-Mar	7	45.15	10-Mar	7	46.52	10-Mar	7	55.41	10-Mar	7	64.28
13-Mar	10	46.8	13-Mar	10	27.92	13-Mar	10	50	13-Mar	10	84.62
16-Mar	13	27.53	16-Mar	13	47.21	16-Mar	13	41.72	16-Mar	13	44.44
19-Mar	16	31.8	19-Mar	16	39.13	19-Mar	16	47.06	19-Mar	16	65

APPENDIX B3

Table 6: Data for COD removal in treatment reactor

Loading Rate=5			Loading Rate=4.5			Loading Rate=4			Loading Rate=3		
Date	Days	% of COD Removal	Date	Days	% of COD Removal	Date	Days	% of COD Removal	Date	Days	% of COD Removal
13-Feb	1	-52.86	13-Feb	1	-111.14	13-Feb	1	-127.9	13-Feb	1	-140.04
15-Feb	3	54.58	15-Feb	3	68.19	15-Feb	3	100	15-Feb	3	67.58
17-Feb	5	-33.1	17-Feb	5	-145.1	19-Feb	7	-25.39	17-Feb	5	-73.07
19-Feb	7	100	19-Feb	7	100	21-Feb	9	238.2	19-Feb	7	80.86
23-Feb	11	8.51	23-Feb	11	41.13	25-Feb	13	34.45	21-Feb	9	100
25-Feb	13	49.19	25-Feb	13	24.84				23-Feb	11	14.17
									25-Feb	13	21.73

Table 6: Data for COD removal in treatment reactor

Loading Rate=5			Loading Rate=4			Loading Rate=3.5			Loading Rate=3		
Date	Days	% of COD Removal	Date	Days	% of COD Removal	Date	Days	% of COD Removal	Date	Days	% of COD Removal
4-Mar	1	52.3	4-Mar	1	34.3	4-Mar	1	88.48	4-Mar	1	17.93
6-Mar	3	40.04	6-Mar	3	85.93	6-Mar	3	78.66	6-Mar	3	65.7
8-Mar	5	64.38	8-Mar	5	20.51	8-Mar	5	66.82	8-Mar	5	69.22
10-Mar	7	78.52	10-Mar	7	80.08	10-Mar	7	75.55	10-Mar	7	48.66
13-Mar	10	68.44	13-Mar	10	61.38	13-Mar	10	81.48	13-Mar	10	66.66
16-Mar	13	54.7	16-Mar	13	40.5	16-Mar	13	50.4	16-Mar	13	80.15
19-Mar	16	49.3	19-Mar	16	55.4	19-Mar	16	65.67	19-Mar	16	75.37

APPENDIX C1

HACH® DR/2400

Manganese

Method 8149

1-(2-Pyridylazo)-2-Naphthol PAN Method*

Powder Pillows

LR (0.007 to 0.700 mg/L)

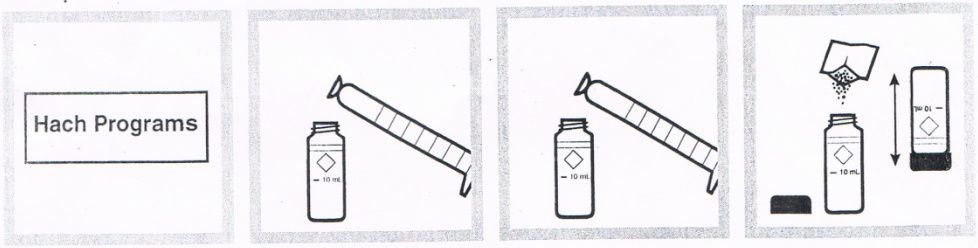
Scope and Application: For water and wastewater; digestion is required for determining total manganese (see *Section 2* for digestion procedure)

* Adapted from Goto, K., et al., *Talanta*, 24, 652-3 (1977)

Tips and Techniques

- Rinse all glassware with 1:1 Nitric Acid Solution. Rinse again with deionized water.
- Wipe the outside of sample cells before each insertion into the instrument cell holder. Use a damp towel followed by a dry one to remove fingerprints or other marks.
- The alkaline cyanide solution contains cyanide. Cyanide solutions should be collected for disposal as a reactive (D001) waste. Be sure cyanide solutions are stored in a caustic solution with pH >11 to prevent release of hydrogen cyanide gas. See *Section Section 4 Waste Management and Safety* on page *55* for more information on proper disposal of these materials.

Powder Pillows — Method 8149



1. Touch **Hach Programs.** Select program **290 Manganese LR.** Touch **Start.**

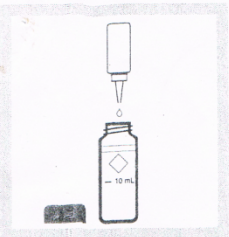
2. Pour 10.0 mL of deionized water into a round sample cell (the blank).

3. Pour 10.0 mL of sample into another round sample cell (the prepared sample).

4. Add the contents of one Ascorbic Acid Powder Pillow to each cell. Cap and invert gently to mix.

Figure 1: Manganese Test for HACH Spectrophotometer

Manganese

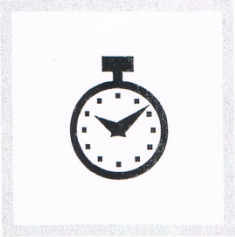


5. Add 15 drops of Alkaline-Cyanide Reagent Solution to each cell. Cap and invert gently to mix.

A cloudy solution may form. The turbidity should dissipate after *step 6*.

6. Add 21 drops of PAN Indicator Solution, 0.1%, to each sample cell. Cap and invert gently to mix.

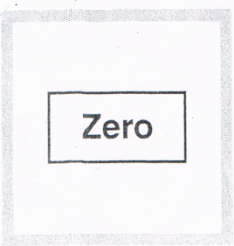
An orange color will develop in the sample if manganese is present.



7. Touch the timer icon. Touch **OK**.

A two-minute reaction period will begin.

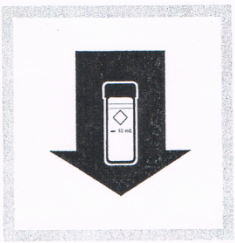
8. When the timer beeps, wipe the blank and place it into the cell holder.



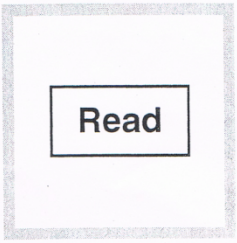
9. Touch **Zero**.

The display will show:

0.000 mg/L Mn



10. Wipe the prepared sample and place it into the cell holder.



11. Touch. **Read**.

Results will appear in mg/L Mn.

Interferences

Interfering Substance	Interference Levels and Treatments
Aluminum	20 mg/L
Cadmium	10 mg/L
Calcium	1000 mg/L as $CaCO_3$
Cobalt	20 mg/L
Copper	50 mg/L
Iron	25 mg/L (If sample contains more than 5 mg/L iron, allow a 10-minute reaction period in *step 7*.)
Lead	0.5 mg/L
Magnesium	300 mg/L as $CaCO_3$
Nickel	40 mg/L
Zinc	15 mg/L

Figure 1: Manganese Test for HACH Spectrophotometer

APPENDIX C2

HACH DR/2400

Oxygen Demand, Chemical

★Method 8000

Reactor Digestion Method*

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

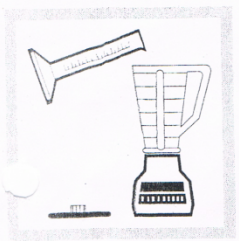
Scope and Application: For water, wastewater, and seawater; digestion is required; 3–150 mg/L and 20– 1500 mg/L COD ranges are USEPA approved for wastewater analyses**; 200–15,000 mg/L COD range is not USEPA approved.

* Jirka, A.M.; Carter, M.J., *Analytical Chemistry*, 1975, 47(8), 1397
** *Federal Register*, April 21, 1980, 45(78), 26811-26812

Tips and Techniques

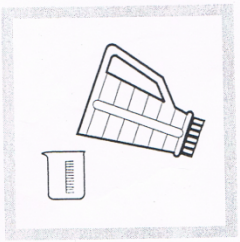
- Some of the chemicals and apparatus used in this procedure may be hazardous to the health and safety of the user if inappropriately handled or accidentally misused. Please read all warnings and refer to *Waste Management and Safety* on page 55 of this manual.
- Wear appropriate eye protection and clothing for adequate user protection. If contact occurs, flush the affected area with running water. Follow instructions carefully.
- Use a safetyshield in front of the COD reactor to prevent injury if splattering occurs.
- The reagent mixture is 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 for Colorimetric Determination* on page 4.
- For greater accuracy, analyze a minimum of three replicates and average the results.

Digestion Procedure — Method 8000

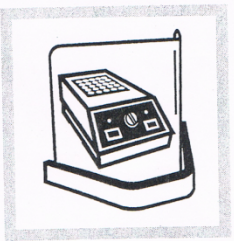


1. Homogenize 100 mL of sample for 30 seconds in a blender. (For samples containing large amounts of solids, increase the homogenization time.)

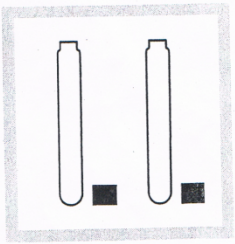
Note: If the sample does not contain suspended solids, omit step 1 and step 2.



2. For the 200–15,000 mg/L range or to improve accuracy and reproducibility of the other ranges, pour the homogenized sample into a 250-mL beaker and gently stir with a magnetic stir plate.

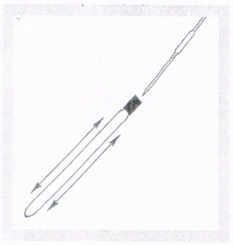


3. Turn on the COD Reactor. Preheat to 150 °C. Place the safety shield in front of the reactor.



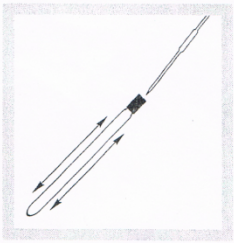
4. Remove the caps from two COD Digestion Reagent Vials. (Be sure to use vials for the appropriate range.)

Figure 3: Chemical Oxygen Demand Test for HACH Spectrophotometer



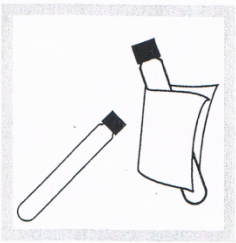
5. Hold one vial at a 45-degree angle. Use a clean volumetric pipet to add 2.00 mL of sample to the vial. This is the prepared sample.

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

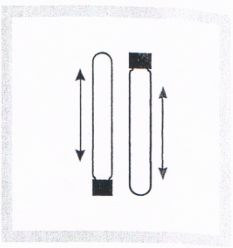


6. Hold a second vial at a 45-degree angle. Use a clean volumetric pipet to add 2.00 mL of deionized water to the vial. This is the blank.

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

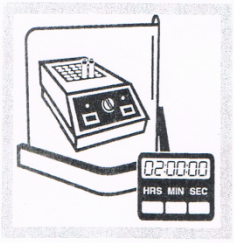


7. Cap the vials tightly. Rinse them with deionized water and wipe with a clean paper towel.

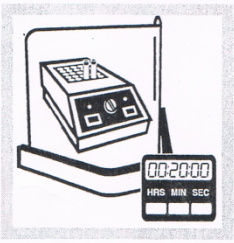


8. Hold the vials by the cap over a sink. Invert gently several times to mix. Place the vials in the preheated COD Reactor.

The sample vials will become very hot during mixing.

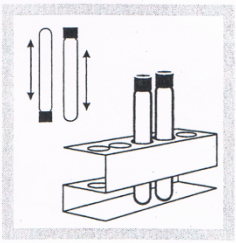


). Heat the vials for two hours.

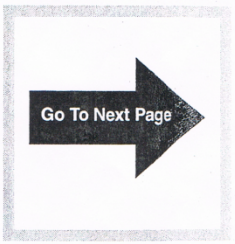


10. Turn the reactor off.

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

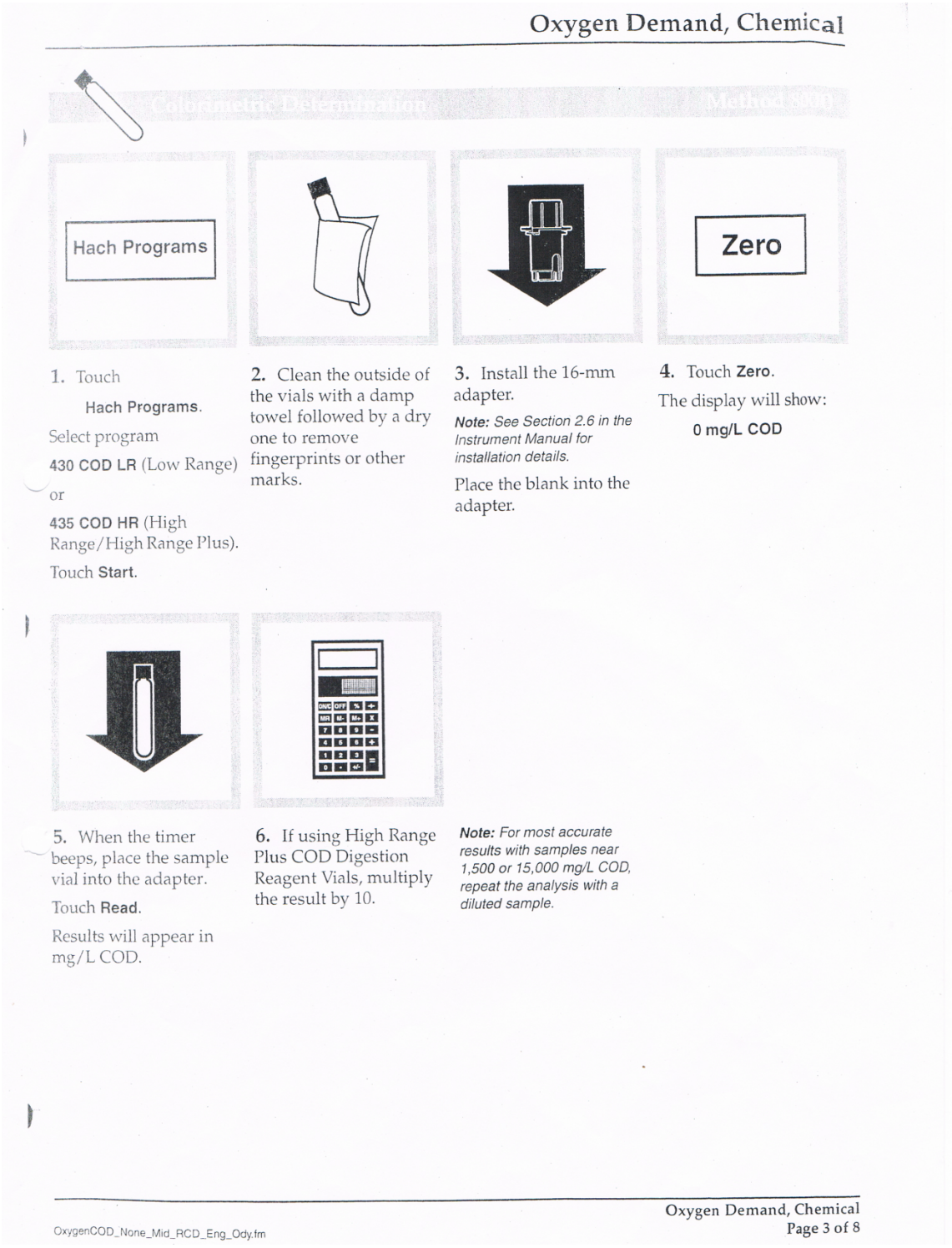


11. Invert each vial several times while still warm. Place the vials into a rack and cool to room temperature.



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

Figure 4: Chemical Oxygen Demand Test for HACH Spectrophotometer



Oxygen Demand, Chemical

Colorimetric Determination Method 8000

Hach Programs

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 2.6 in the Instrument Manual for installation details.*
Place the blank into the adapter.

Zero

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 with samples near 1,500 or 15,000 mg/L COD, repeat the analysis with a diluted sample.*

OxygenCOD_None_Mid_RCD_Eng_Ody.fm

Oxygen Demand, Chemical
Page 3 of 8

Figure 5: Chemical Oxygen Demand Test for HACH Spectrophotometer

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.

Vial Type Used	Maximum Cl^- concentration in sample (mg/L)	Suggested Cl^- concentration of diluted samples (mg/L)	Maximum Cl^- concentration in sample when 0.50 $HgSO_4$ added
Low Range (3–150 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.

Figure 6: Chemical Oxygen Demand Test for HACH Spectrophotometer

APPENDIX C3

HACH® DR/2400

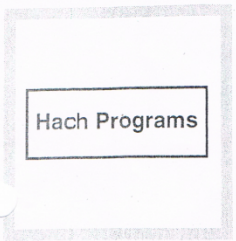
Suspended Solids

Photometric Method*
(0 to 750 mg/L)

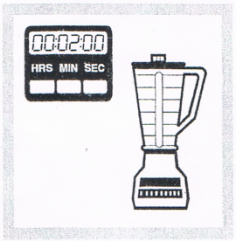
Method 8006

Scope and Application: For water and wastewater

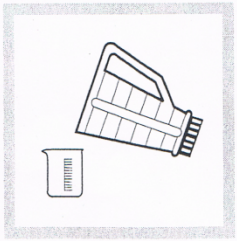
* Adapted from *Sewage and Industrial Wastes*, 31, 1159 (1959).



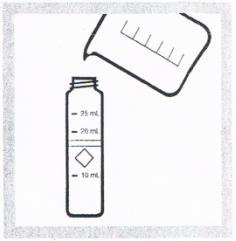
1. Touch **Hach Programs.**
Select program **630 Suspended Solids.**
Touch **Start.**



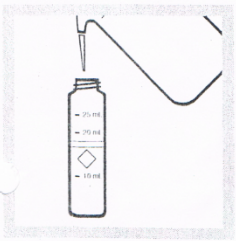
2. Blend 500 mL of sample in a blender at high speed for exactly two minutes.



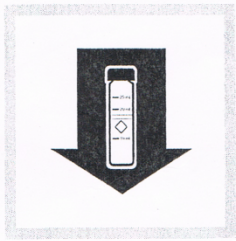
3. Pour the blended sample into a 600-mL beaker.



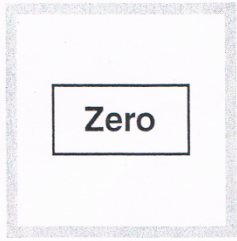
4. Stir the sample and immediately pour 25 mL of the blended sample into a sample cell (the prepared sample).



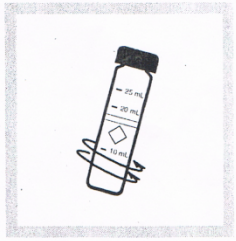
5. Fill a second sample cell with 25 mL of tap water or deionized water (the blank).
Remove gas bubbles in the water by swirling or tapping the bottom of the cell on a table.



6. Place the blank into the cell holder.



7. Touch **Zero.**
The display will show:
0 mg/L Susp.Solids



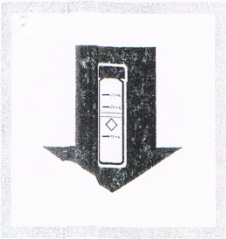
8. Swirl the prepared sample to remove any gas bubbles and uniformly suspend any residue.

SuspendedSolids_None_Other_PHO_Eng_Ody.fm

Suspended Solids
Page 1 of 2

Figure 7: Suspended Solids Test for HACH Spectrophotometer

Suspended Solids



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, poly	1	each	1080-52
Blender, 1.2-L, 120 VAC	1	each	26161-00
Blender, 1.2-L, 240 VAC	1	each	26161-02
Cylinder, graduated, 500-mL, poly	1	each	1081-49
Pipet, serologic, 25-mL	1	each	2066-40
Pipet, Filler, safety bulb	1	each	14651-00
Sample Cells, 10-20-25 mL, w/cap	2	6/pkg	24019-06

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SuspendedSolids_None_Other_PHO_Eng_Ody.fm

Figure 7: Suspended Solids Test for HACH Spectrophotometer