MANGANESE REMOVAL USING NATURAL MICROFLORA

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of the requirements for the award of the degree of
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DECLARATION

I declare that this thesis entitled “Manganese removal using natural microflora” is the result of my own research except as cited in references. The thesis has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.”

Signature : ..................................................................
Name : Mohd Kamarul Syahid bin Salleh
Date : 23rd April 2009
DEDICATION

Special Dedication to

Pn Norhizan Ahmad, my beloved mother, you are everything to me,
En Salleh Hj. Ahmad, my beloved father, you are my inspiration,
Nurul Shareena Agmar Mohd Sharif, I do believe in you and me,

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

Thank you.
ACKNOWLEDGEMENT

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

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

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

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

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

The removal process of heavy metals from wastewater and industrial wastes has become a very important environmental issue. The main scope of this study is to monitor the removal of manganese concentration in the biological treatment of simulated manganese wastewater using mixed culture from soil. The study is carried out in two phase consist of acclimatize phase and treatment phase. In the acclimatize phase, mixed culture from soils is inoculated in a 10 L reactor and the growth of the mixed culture is monitored. In the mean time, the mixed culture is fed with glucose solution and a trace amount of manganese solution. In the treatment phase, the mixed culture is used to treat simulated manganese wastewater. The experiments are carried in 15 days of period at 8 different loading rates which has been decided by the Design-Experts software. The degradation of manganese was monitored at three different water quality parameters which are the suspended solids, the chemical oxygen demand (COD) and the manganese concentration respectively. The experimental results show that, LR 3.0 mg/L.d shows the highest percentage of manganese removal at 87.50% with mean removal efficiencies of 77.70%. Also, LR 3.0 mg/L.d shows the highest percentage of COD removal at 83.77% with mean removal efficiencies of 73.45%. Based on Design-Expert analysis, it shows that, in order to achieve maximum of manganese concentration and COD removal, the loading rate need to be lowered. As the conclusion, the mixed culture in soils is capable of degrading manganese at which the effective loading rate for this study is 3.0 mg/L.d.
ABSTRAK

Proses penyingkiran logam berat daripada air sisa dan sisa industri telah menjadi suatu isu alam sekitar yang penting. Skop utama kajian ini adalah untuk memantau proses penyingkiran kepekatan mangan di dalam rawatan biologi air sisa mangan buatan dengan menggunakan kultur campuran daripada tanah. Kajian ini telah dijalankan di dalam dua fasa terdiri daripada fasa penyuaian dan fasa rawatan. Di dalam fasa penyuaian, kultur campuran daripada tanah dibiakkan di dalam sebuah reaktor 10 liter dan tumbbesaran kultur tersebut dipantau. Pada masa yang sama, kultur campuran tersebut diberi larutan glukosa dan mangan sebagai nutrien. Di dalam fasa rawatan, kultur campuran tersebut digunakan untuk merawat air sisa mangan buatan. Eksperimen dijalankan di dalam tempoh 15 hari pada lapan (8) kadar beban (LR) yang berbeza yang mana kadar beban ini telah ditentukan oleh perisian Design-Expert. Proses penguraian mangan dipantau pada tiga parameter kualiti air iaitu kepekatan pepejal terampai, permintaan oksigen kimia (COD) dan mangan. Keputusan eksperimen menunjukkan bahawa, kadar beban 3.0 mg/L.h memberikan peratusan penyingkiran mangan yang tertinggi iaitu 87.50% dengan purata kecekapan sebanyak 77.70%. Di samping itu, kadar beban 3.0 mg/L.h juga memberikan peratusan penyingkiran COD tertinggi iaitu 87.77% dengan purata kecekapan sebanyak 73.45%. Berdasarkan analisis perisian Design-Expert, ia menunjukkan, untuk mencapai penyingkiran kepekatan mangan dan COD yang maksimum, kadar beban perlu direndahkan. Sebagai kesimpulan, kultur campuran daripada tanah mampu untuk mengurai mangan di mana kadar beban yang efektif untuk kajian ini adalah 3.0 mg/L.h.
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<th>Description</th>
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<tr>
<td>COD</td>
<td>Chemical Oxygen Demand</td>
</tr>
<tr>
<td>GPM</td>
<td>gallon per million</td>
</tr>
<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
</tr>
<tr>
<td>LR</td>
<td>Loading Rate</td>
</tr>
<tr>
<td>Mn</td>
<td>Manganese</td>
</tr>
<tr>
<td>Mn$^{2+}$</td>
<td>Manganese ion</td>
</tr>
<tr>
<td>SBR</td>
<td>Sequencing Batch Reactor</td>
</tr>
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<td>SS</td>
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CHAPTER 1

INTRODUCTION

1.1 Background

Removal of heavy metals from wastewater and industrial wastes has become an important environmental issue. Manganese dioxide is used to: manufacture ferroalloys; manufacture dry cell batteries; to "decolorize" glass; to prepare some chemicals, like oxygen and chlorine; and to dry black paints. Manganese sulfate (MnSO₄) is used as a chemical intermediate and as a micronutrient in animal feeds and plant fertilizers. Manganese metal is used as a brick and ceramic colorant, in copper and aluminum alloys, and as a chemical oxidizer and catalyst. Potassium permanganate (KMnO₄) is used as a bactericide and algicide in water and wastewater treatment, and as an oxidant in organic chemical synthesis.

For human cases, the bad effects of long-term overexposure to manganese compounds may affect the central nervous system as well as lung tissue. Neurotoxicity is evidenced by the physical and psychological symptoms of manganism as well as the neurochemical changes in the brain. Other effects of overexposure to manganese in a work environment include Parkinson’s disease, impotence, insomnia, paralysis, flu-like symptoms, tight chest, malaise and fatigue. Hence, it is essential to remove manganese ion from industrial wastewater before discharges into natural’s water sources. Several treatment methods for removal of metals ions from aqueous solutions are ion exchange, solvent extraction, chemical precipitation and electrochemical reduction. They are in this case either economically
unfavorable or technically complicated and are thus used in special case. Each of these methods has some limitations in practice.

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

1.2 Problem Statement

Nowadays, world has become aware about the seriousness of one of the major effect of the waste discharged by the industrial activities, that is the quantity and diversity of hazardous waste. Manganese is used in industrial processes and in various consumer products. The major man-made sources of environmental manganese include municipal wastewater discharge, sewage sludge, emissions generated during alloy, steel and iron production and to a lesser extent by emissions from the combustion of fuel additives.

So, there is a need for controlling the discharge of manganese into environment as it is harmful to aquatic life as well as to human beings. This research is proposed because the method that will be used is quite economical since it only uses the microorganism from soil which is readily available in surroundings. These microorganisms are assumed can treat the wastewater contains manganese metal.
1.3 Objective of The Study

The main objectives of this study are:

- To study the degradation of manganese by microflora in soil.
- To study the effect of different loading rate on manganese removal.
- To study the effect of manganese concentration to the growth of mixed culture.

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

1.4 Scope of the Study

The scope of this study is to acclimatize the bacteria in order to treat manganese from wastewater and to monitor the mixed culture growth using suspended solid test. The wastewater that contains manganese was simulated with the appropriate nutrients for mixed culture. The experiment was conducted in aerobic sequencing batch biofilm reactor with different loading rate. According to several researchers (Mouchet, 1992; Gislette and Mouchet, 1997), the mixed culture requires a fully aerobic environment to degrade the manganese. The efficiency of the treatment for different loading rate was evaluated in terms of chemical oxygen demand (COD) and the manganese concentration. Besides, the effect of the manganese concentration on microorganism growth is measured in terms of suspended solid that exist throughout the experiment.
CHAPTER 2

LITERATURE REVIEW

2.1 Manganese

Manganese is a reactive element that easily combines with ions in water and air. In the Earth, manganese is found in a number of minerals of different chemical and physical properties, but is never found as a free metal in nature (Wikipedia, 2008).

Over 80% of the known world manganese resources are found in South Africa and Ukraine. Other important manganese deposits are in China, Australia, Brazil, Gabon, India, and Mexico. The United States imports manganese ore because the manganese resources in the U.S. are relatively low in manganese content per ton of ore. Importing these ores is presently more economical than mining them locally. Most manganese ore imported to the United States is used to manufacture intermediate manganese ferroalloy products and electrolytic manganese for use in dry-cell batteries. Only a small amount of the ore is directly used in the steel making process (Wikipedia, 2008).

2.1.1 Manganese Sources in Industrial Wastewater

Manganese is used in industrial processes and in various consumer products. The major man-made sources of environmental manganese include municipal wastewater discharge, sewage sludge, emissions generated during alloy, steel and
iron production and to a lesser extent by emissions from the combustion of fuel additives. Worldwide anthropogenic input of manganese to freshwater is summarized in Table 2.1 (Nriagu et al., 1993):

**Table 2.1**: Anthropogenic sources of manganese to freshwater (Nriagu et al., 1993)

<table>
<thead>
<tr>
<th>Source</th>
<th>Estimated Input (10^3 tonnes/year)</th>
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<td>Iron/Steel Refining</td>
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<td>&lt;0.1-1.5</td>
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<td>Non-ferrous Metal Refining</td>
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<td>Base Metal Mining/Dressing</td>
<td>0.8-12</td>
<td>Atmospheric Fallout</td>
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2.1.2 Effects of Manganese to The Environment

Basically, the present of manganese plays important role to the environment. This includes good effects as well as bad effects. The affected target group will be the living creature likes animals and plants as well as human beings.

As a vital micronutrient for plants, manganese prevents a yellowing of the leaves and helps leaves grow properly. In plants manganese ions are transported to the leaves after uptake from soils. When too little manganese can be absorbed from the soil this causes disturbances in plant mechanisms. For instance disturbance of the division of water to hydrogen and oxygen, in which manganese plays an important
part. Domestic animals need an adequate supply of manganese in their food to avoid reduced reproduction and an increased risk of deformed or poorly maturing young (Online Lawyer Source, 2008).

Exposure to manganese can come through the air, in fumes from steel, iron and power plants; through water, where it can leak from waste disposal; through the soil, and through water plants. Manganese can cause both toxicity and deficiency symptoms in plants. When the pH of the soil is low, manganese deficiencies are more common. For human cases, the bad effects of long-term overexposure to manganese compounds may affect the central nervous system as well as lung tissue. Neurotoxicity is evidenced by the physical and psychological symptoms of manganism as well as the neurochemical changes in the brain. Based on a study on 36 welders diagnosed with the symptoms of typical Mn poisoning in Beijing, the onset of symptoms is between 2 and 34 years (average 16.3 years), the welders having average working duration of 24.4 years (4–40 years). The symptoms include headache and insomnia (88%), memory loss (75%), emotional instability (35%), exaggerated tendon reflexes (83%), hyper-myotonia (75%), hand tremor (23%), speech disturbances (6%) and festinating gait (3%) (Janelle et. al., 2004). Other effects of overexposure to manganese in a work environment include Parkinson’s disease, impotence, insomnia, paralysis, flu-like symptoms, tight chest, malaise and fatigue (Online Lawyer Source, 2008).

2.1.3 Manganese Level of Discharge

As stated previously, exposure to manganese can come through three ways which is through water, through the and through the soils as well. Since the exposure to manganese could caused harmless to living creatures, some regulations had been made regarding the level of discharge of manganese to the environment in order to protect living creature as well as to ensure its species autonomy.
Some of the international organizations that involved making the regulations are World Health Organization (WHO), Environmental Protect Agency (EPA) and National Institute for Occupational Safety and Health (NIOSH). In Malaysia, the regulations are Environmental Quality Act 1974, Environmental Quality (Sewage and Industrial Effluents) Regulations 1978 and Environmental Quality (Prescribe Activity) (Environmental Impact Assessment) Order 1989 (Malaysia Environmental Quality Report. 2006. Department of Environment, Ministry of Natural Resources and Environment Malaysia).

The WHO has established rules that set guidelines value in drinking water for aesthetic quality which is 0.1 mg/L (WHO1984a). WHO also recommends for exposure limits in workplace air regarding respirable manganese particles which is 0.3mg/m$^3$ (WHO1986). In Malaysia, under Environmental Quality (Sewage and Industrial Effluents) Regulations 1978 stated that the manganese limits of effluent for standard (a) is 0.2 mg/L and for standard (b) is 1.0 mg/L (Environmental Quality Act 1974 (Act 127) & Subsidiary Legislation, 2006).

2.2 Manganese Removal Method

2.2.1 Trickling Filter

A trickling filter consists of a fixed bed of rocks, gravel, slag, polyurethane foam, sphagnum peat moss, or plastic media over which sewage or other wastewater flows downward and causes a layer or film of microbial slime to grow, covering the bed of media. Aerobic conditions are maintained by splashing, diffusion, and either by forced air flowing through the bed or natural convection of air if the filter medium is porous. The process mechanism, or how the removal of waste from the water happens, involves both absorption and adsorption of organic compounds within the sewage or other wastewater by the layer of microbial slime. Diffusion of the wastewater over the media furnishes dissolved air, the oxygen which the slime layer
requires for the biochemical oxidation of the organic compounds and releases carbon dioxide gas, water and other oxidized end products. As the slime layer thickens, it becomes more difficult for air to penetrate the layer and an inner anaerobic layer is probably formed. This slime layer continues to build until it eventually sloughs off, breaking off longer growth into the treated effluent as a sludge that requires subsequent removal and disposal (Wikipedia, 2008).

Trickling filters are rugged and easy to operate. They can be very simple to build. Trickling filters are completely scalable; they can be built to handle water flows from 4 to 4 million GPM. They have the ability to treat a wide variety of nutrient levels. Properly designed systems can handle solids very well. One of the big advantages of a trickling filter is that the water can leave with more oxygen than it entered. Because trickling filters have a large-air water interface, they also act as strippers to remove CO$_2$, H$_2$S, N$_2$ or other undesirable volatile gases. Very few other types of biofilters perform all these functions. There are only two minor disadvantages to trickling filters. One is the energy cost required to pump the water to the top of the filter. The other disadvantage to trickling filters is their size. They are larger and take more space than some other types of biofilters. The major disadvantage of this type of filter is the high capital cost to build as well as its maintenance costs (L. S. Enterprises, 2003).

2.2.2 Roughing Filtration

Roughing filtration is increasingly becoming accepted as a viable and efficient natural pre-treatment alternative in both developed and developing countries (Collins et al., 1994; Galvis et al., 1993; Wegelin, 1996). The performance of roughing filters is based on a combination of transport, attachment and purification mechanisms, including screening, sedimentation, absorption, biochemical oxidation and bacteriological activity (Galvis et al, 1993). Various types of roughing filters have been developed: horizontal-flow (HRF), downflow (DRF) and upflow roughing filters in series (URFS) and in layers (URFL). The main selection criteria for
roughing filtration are based upon raw water quality characteristics: turbidity, suspended solids, colour, iron, manganese and faecal coliform levels (Galvis et al., 1993; Wegelin, 1996)

Roughing filtration technology is a filtration process through a coarse medium using low filtration rates. It is mainly used as pretreatment in order to retain solid matter before slow filtration (Wegelin, 1988; Boller, 1993). This process has been used successfully as pretreatment to remove turbidity, being subsequently followed by slow sand filtration (Ingallinella et al., 1992). Given the high solid retention capability of roughing filtration, this process was considered likely to be an appropriate treatment for the removal of iron and manganese from groundwater by means of biological processes by biotic and abiotic mechanisms. This system combines simple, low-cost operation and maintenance with high iron and manganese removal efficiencies, thus constituting a technology which is particularly suited to small waterworks (Ingallinella et al., 1992).

2.2.3 Biofilm Process

The biofilm has been described as a “porous tangled mass of slime matrix” (Weber et al., 1978). It consists of microbial cells either immobilized at the surface of the granular activated carbon, GAC (substratum) or embedded in an extracellular microbial organic polymer matrix (Ghosh et al., 1999). Bacterial and fungi cells in the biofilms secrete extracellular polymeric substances to form a cohesive, stable matrix in which cells are held in dense agglomeration (Branda et al., 2005; Lazarova and Manem, 1995). The extracellular matrix is comprised of polysaccharides, proteins, nucleic acids and lipids (Goodwin and Forster, 1985; Horan and Eccles, 1986).

Information regarding the composition of a biofilm is commonly gained by estimating the total biofilm amount. This amount can be represented by physical (i.e. biofilm thickness, total dry weight) or physico-chemical (total organic carbon (TOC),
chemical oxygen demand (COD)) parameters (Lazarova and Manem, 1995). The thickness of the biofilm determines its respective mass transfer properties such as nutrient diffusion/flux from the bulk liquid to the biofilm and frictional resistance (de Beer et al., 1994). Furthermore, the increasing biofilm thickness observed during growth can be described by a sigmoid curve where the plateau indicates the critical biofilm thickness (Bryers and Characklis, 1981). Total dry weight depends on the type and volume of microorganisms present in the biofilm, but, overestimates the mass since the measure also includes the weight of inert mass, exopolymers and adsorbed organic matter (Lazarova and Manem, 1995). TOC that represents approximately 50 per cent of cell biomass (Harris and Kell, 1985) is measured via photometric methods used to quantify the free-living biomass and indirectly quantify total biofilm amount (Lazarova and Manem, 1995). The measurement of the oxidizable biofilm matter, expressed by COD, constitutes another indirect method to estimate fixed biomass with high precision (Bryers and Characklis, 1981).

The composition of a biofilm can also be described in more detail by measuring specific biofilm components (i.e. exopolysaccharides, proteins, peptidoglycane, lipopolysaccharides and lipids) (Lazarova and Manem, 1995). Depending on the physiological state and environmental conditions, these components will vary in size and structure. For example, micro-organisms, such as biofilm bacteria, produce new proteins during periods of extremely low nutrient availability (Atlas, 1982). A colorimetric analysis of total protein would reveal this change in protein composition (Atlas, 1982). As well, an “ideal” method for estimating cellular bacteria mass in biofilms has been proposed which involves the simultaneous measurement of three bacteria components including peptidoglycane, lipopolysaccharides and lipids. It is believed that these three components remain relatively constant irregardless of bacterial strain and physiological condition (Lazarova and Manem, 1995).
2.2.4 Selection of Biofilm Process as Treatment Method

For this research, biofilm process has been selected as treatment method. The main advantages of this method are low cost of operation and simple principle of methodology. In the other hand, the trickling filters need high capital cost to build as well as its maintenance costs which is economically unfavorable. For roughing filtration, the major problem would be the filter medium which is commonly gravel. Gravel may be unavailable in some locations and is difficult to transport long distances because of its weight (Kapranis and Reep, 1998).

2.3 Mixed Culture

2.3.1 Mixed Culture from 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 manganese (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).

Manganese oxidising 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.*, 1988a; 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).