ZINC REMOVAL IN BIOFILM REACTOR WITH SUPPORT MEDIA

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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
I declare that this thesis entitled “Zinc removal in biofilm reactor with support media” 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 : Rahaiminullah Bin Razali
Date : 23th April 2009
DEDICATION

Special Dedication to

Razali Yatim, my beloved father,
Nipah Daud, my beloved mother,

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

Thank you.
ACKNOWLEDGEMENT

Praise be to god for His help and guidance that finally I am able to complete my final undergraduate project as one of a requirement before graduation.

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

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

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ABSTRACT

Zinc removal in biofilm reactor with support media by mixed culture, attract a lot of interest from the public and as well as chemical industry. In this research, biofilm process was selected as a treatment method with using sequencing batch reactor (SBR). This method is better choice when compared in cost of treatment. At the beginning of the experiment, the mixed culture was collected from the drain and acclimatized in 10 litres reactor and the growth of mixed cultured is monitored by take suspended solid test. The mixed culture is acclimatized within two months by feed trace amount of glucose and 1mg/L of zinc. The hydraulic retention time (HRT) selected for this research is 5 days. Three parameters were chosen during run the experiment which was suspended solid (SS) test for monitoring mixed culture growth while chemical oxygen demand (COD) removal and zinc removal for measure the water quality. This research will carry out by controlling the loading rate. The loading rate is choosing from 3 mg/L.d to 5 mg/L.d. The trend of results was determined. Based on result, increasing the loading rate make the all value of parameters decreased. The average value of SS in acclimatize reactor is 2645.39 mg/L. For the treatment reactor, the highest average value of SS is 566.21 mg/L. For COD removal and zinc removal, the highest average was at loading rate 3mg/L.d which is 41.49% and 75.01% respectively. Using Design-Expert programmed, the optimized data obtained. From the Design-Expert data, the zinc removal is 74.38% and the COD removal is 47.73%. The desirability of the lowest parameter of loading rate is 0.81.
Penyingkiran zink menggunakan bacteria di dalam reactor biofilm menarik minat orang ramai termasuk pihak industri kimia. Di dalam projek ini, proses biofilem digunakan dengan menggunakan “sequencing batch reactor” (SBR). Kaedah ini lebih bagus daripada kaedah lain jika dibandingkan dari aspek kos rawatan. Pada permulaannya, kultur campuran dikumpul dari longkang dan dibiak di dalam bekas 10 liter dan kadar pembiakan dipantau dengan melalui ujian pepejal terampai. Kultur campuran dibiak selama 2 bulan dengan diberi makan glukosa dan 1 mg/L. zink. “Hydraulic retention time” yang dipilih ialah selama 5 hari. Tiga parameter dipilih iaitu ujian pepejal terampai yang digunakan untuk memantau pertumbuhan kultur campuran dalam reaktor manakala penyingkiran permintaan oksigen kimia dan penyingkiran zink adalah untuk mengukur kualiti air. Projek ini dijalankan dengan mengawal kadar beban. Kadar beban yang dipilih adalah dari 3 mg/L.h hingga 5 mg/L.h. Berpandukan kepada data diperoleh, semakin tinggi kadar beban, semakin rendah nilai kesemua parameter. Purata nilai pepejal terampai untuk reaktor penyusain iklim adalah 2645.39 mg/L. Untuk reaktor rawatan pula, purata nilai pepejal terampai adalah 566.21 mg/L. Untuk penyingkiran permintaan oksigen kimia pula, purata nilai tertinggi adalah pada kadar beban 3 mg/L.h iaitu 41.19% dan purata nilai tertinggi bagi penyingkiran zink adalah 75.01%. Dengan menggunakan program “Design-Expert”, data optimum bagi setiap parameter diperoleh. Daripada data yang diperoleh, penyingkiran zink optimum adalah 74.38% manakala penyingkiran permintaan oksigen kimia optimum adalah 47.73%. Keberjayaan untuk parameter kadar beban yang terendah adalah 0.81.
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LIST OF ABBREVIATIONS

COD - Chemical Oxygen Demand
HRT - Hydraulic Retention Time
LR - Loading Rate
Zn - Zinc
Zn$^{2+}$ - Zinc ion
SBR - Sequencing Batch Reactor
SS - Suspended Solid
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CHAPTER 1

INTRODUCTION

1.1 Background

Heavy-metals are present in industrial wastewaters discharged from industries such as battery, paint, chemical manufacturing and also from mining industry in acid-mine drainage, posing significant disposal problems that require urgent solution to avoid serious environmental contamination (Tunay and Kabdasli, 1994). Heavy metal removal from wastewater usually involves precipitation, ion exchange or acidification; methods that require the use of expensive chemicals and resins and often, as with acidification, create disposal problems (Brierley, 1990).

The use of biofilms in the treatment of various types of hazardous wastes has attracted considerable interest as an alternative to the conventional suspended growth system. In biofilm reactors, microorganisms are immobilized on solid support media. The solid support media commonly used in biofilms are rock, sand, charcoal, diatomaceous earth, activated carbon, polyurethane foam, and wood. When a solid support is placed in a suspended cell system, organic molecules are adsorbed on the clean solid surface to form a conditioning layer. Some of the cells strike and adsorb to the surface for some finite time and then desorb. Some of the desorbed cells begin to make preparations for a lengthy stay by forming structures which may permanently adhere the cell to the surface. These cells excrete extracellular polymeric substances or sticky polymers, which hold the biofilm together and cement it to the solid surface. In addition, these polymers trap scarce nutrients and protect bacteria from biocides. As nutrients accumulate, the pioneer cells proceed to
reproduce microcolonies and soon a thriving colony of bacteria is established forming a biofilm (Borja et al., 2003).

1.2 Problem Statement

The long-term accumulation of heavy metals in the soil environment is a concern because they potentially have important consequences for the quality of the human food chain, toxicity to plants and soil microbial processes and once applied they have very long residence times in soil (Smith, 2008). Risk of diseases of metabolism such as atherosclerosis and adult onset diabetes mellitus is increased by fetal malnutrition. Deficiencies of micronutrients essential for methylation are believed to contribute to the phenomenon in part through epigenetic abnormalities. Zinc is one of the nutrients essential for the epigenome. Because the worldwide prevalence of zinc deficiency is at least 20%, fetal zinc deficiency is common. We suggest fetal zinc deficiency contributes to the pathogenesis of metabolic diseases in adults. In support of our thesis, research in experimental models and humans established the essentiality of zinc at all stages of intrauterine and infant life. Experiments in rodents and/or non-human primates found that fetal and/or suckling zinc deficiency impairs neuropsychological functions of progeny and that the effects persist in spite of nutritional rehabilitation. In addition, maternal zinc deficiency in mice is reported to impair immunity of progeny; effects persist in spite of nutritional rehabilitation into the next generation. We suspect that zinc deficiency is a far greater human health problem than is generally recognized (Barker, 2004).

Various techniques have been employed for the treatment of dye/metal bearing industrial effluents, which usually come under two broad divisions: abiotic and biotic methods. Abiotic methods include precipitation, adsorption, ion exchange, membrane and electrochemical technologies. Much has been discussed about their downside aspects in recent years (Atkinson et al., 1998 and Crini, 2006), which can be summarized as expensive, not environment friendly and usually dependent on the concentration of the waste. Therefore, the search for efficient, eco-friendly and cost effective remedies for wastewater treatment has been initiated.
1.3 **Objective of Study**

The mains objectives of this research are:

- To study the effect of different loading rate on the zinc in wastewater treatment.
- To study the effect of zinc concentration to the growth of bacterium.
- To study the degradation of zinc by mixed culture in biofilm reactor.

1.4 **Scope of Study**

The scope of the study in this experiment is to degrade zinc in wastewater. The initial simulated wastewater is analyzed. The experiments were conducted separately indifferent loading rate. In term of water quality parameters (COD) and the different concentration, the efficiency of treatment was evaluated. Each reactor has working volume of 5 liters. The effect of zinc concentration on biomass growth defined in terms of suspended solid.
CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Zinc is one of the most common elements in the Earth. Zinc can be found in the air, soil, water and in the foods. The word of zinc comes from German word which is zink. Before zinc discovered as a metallic form, the ancient people used zinc for healing wounds and sore eyes. Zinc ore used for making brass. By 1374, zinc was recognized in India as a new metal. In 16th century, zinc metal and zinc oxide were produced in India. In the 17th century, the technology moved to China and then in 1374, the separation zinc produced in Europe. In 1743, the first European zinc smelter was established at Bristol in the United Kingdom. In 1546, Georgius Agricola reported that he found a white metal in the Harz Mountains and called it zincum. Then, Paracelsus stated clearly that zincum was the new metal and it had properties distinct from other known metal. Finally, Andreas Marggraf created a method to extract the zinc from its true mineral in 1743 (Chinese-School, 2005).

Usually zinc ores are found in association with other minerals like lead, copper, gold and others. Usually, the ore can’t directly to be used by smelters. It needs to be concentrated first. Before concentrates, zinc ores contain 3% of zinc but after been concentrates, it contain 55% of zinc (Chinese-School, 2005).

Nowadays, there are zinc mines throughout the world with the largest producers are China, Australia and Peru. In 2005, China produced about ¼ of the
global zinc output. Some of used of zinc is to make white paints, producing rubber, ingredient in smoke bomb, deodorant and others (Chinese-School, 2005).

2.2 Heavy Metal

2.2.1 Properties of Zinc

The colour of zinc is bluish grey and when burn in air it turn to bright bluish green flame. It reacts with acids, alkalis and other nonmetals. If not completely pure, zinc reacts with acid and then released hydrogen. The one common oxidation state of zinc is +2. Zinc metal is malleable and can be easily made to various shapes in temperature between 100 °C to 210 °C. Above 210 °C, the metal becomes brittle and will be pulverized by beating. Zinc also is nonmagnetic (Thompson et al., 1983).

The symbol used for zinc is Zn and has atomic number of 30. In nonscientific context it is sometimes called spelter. The zinc has atomic number of 30. It is transition metal in group 12, period 4 and block d. The standard atomic weight is 65.409 g/mole (Thompson et al., 1983).

Zinc has two different main properties which are chemical properties (Table 2.2.1) and physical properties (Table 2.2.1) (Thompson et al., 1983).

Table 2.1: Chemical properties of zinc

<table>
<thead>
<tr>
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<tr>
<td>Oxidation states</td>
<td>+1,+2</td>
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<tr>
<td>Electronegativity</td>
<td>1.65 (Pauling scale)</td>
</tr>
<tr>
<td>Atomic radius</td>
<td>135 pm</td>
</tr>
<tr>
<td>Covalent radius</td>
<td>131 pm</td>
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</table>
### Table 2.2: Physical properties of zinc

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<tbody>
<tr>
<td>Phase</td>
<td>solid</td>
</tr>
<tr>
<td>Density (near r.t.)</td>
<td>7.14 g·cm⁻³</td>
</tr>
<tr>
<td>Liquid density at m.p</td>
<td>6.57 g·cm⁻³</td>
</tr>
<tr>
<td>Melting point</td>
<td>1519 K (1246 °C, 2275 °F)</td>
</tr>
<tr>
<td>Boiling point</td>
<td>692.68 K (419.53 °C, 787.15 °F)</td>
</tr>
<tr>
<td>Heat of fusion</td>
<td>7.32 kJ·mol⁻¹</td>
</tr>
</tbody>
</table>

#### 2.2.2 Disadvantage of Zinc

Even though zinc give lot of benefits to mankind, but there are still bad effect produced by the zinc. After doing research, the scientists realized that exceed of zinc in agricultural soils can give major worldwide problem. It can affect both crop yield and the quality. They said that severe soil zinc will reduce the yield of crops up to 30% (McGeer et al., 2003).

Even though zinc is essential requirement for a healthy body, too much of zinc can be harmful. The free zinc ion in our body can cause damage to our stomach. A research state that the free zinc ions have high solubility in the acidic stomach. The high solubility in acidic stomach would damage stomach lining. Stomach contains hydrochloric acid, and when metallic zinc dissolved in stomach, it becomes corrosive zinc chloride (Sharrett et al., 1982).

Metal fume fever is very common health effect when we deal with zinc especially zinc sulphate. Metal fume fever is characterized by chest pain, cough, reduce lung volumes and others. However, when we dealing with zinc chloride, the health result cause damage to the mucous membranes of the respiratory tract. Symptoms of high-concentration zinc chloride exposure include dyspnea, cough, pleuritic chest pain, bilateral diffuse infiltrations, pneumothorax, and acute
pneumonitis. Some cases, death resulted after acute exposure of zinc compounds. Two soldiers exposed without gas masks to zinc chloride smoke, died 25-32 days after the incident. According to authors, the fumes from the zinc chloride smoke cause the difficulty respiratory (Gerhardsson et al., 2002).

2.2.3 Level of Discharge for Zinc

The Environmental Protection Agency (EPA) makes some regulations and recommendation for zinc to minimize the health problem cause of the zinc. EPA has stated that drinking water should contain no more than 5 mg of zinc per liter of water (5 mg/L or 5 ppm). Furthermore, any release of more than 1,000 pounds (or in some cases 5,000 pounds) of zinc or its compounds into the environment (i.e., water, soil, or air) must be reported to EPA (Li et al., 2005).

The National Academy of Sciences (NAS) estimates an Recommended Dietary Allowances (RDA) according to some categories. The RDA of zinc for men is 11 mg/day. For women, the RDA of zinc is 8 mg/day, or 0.13 mg per kg of body. Lower zinc intake was recommended for infants (2–3 mg/day) and children (5–9 mg/day) because of their lower average body weights. For pregnant women, the RDA of zinc is 11–12 mg/day (Li et al., 2005).

To protect workers, the Occupational Safety and Health Administration (OSHA) has set an average legal limit of 1 mg/L for zinc chloride fumes and 5 mg/L for zinc oxide (dusts and fumes) in workplace air during an 8-hour workday, 40-hour work week. This regulation means that the workroom air should contain no more than an average of 1 mg/L of zinc chloride over an 8-hour working shift of a 40-hour work week (Li et al., 2005).

The National Institute for Occupational Safety and Health (NIOSH) similarly recommends that the level of zinc in workplace air should not exceed an average of 1 mg/L over a 10-hour period of a 40-hour work week (Li et al., 2005).
The Environmental Quality Act 1974 stated that the level of zinc in workplace air should not exceed an average of 2.0mg/L (Yayasan-Sabah, 1979).

2.3 Mixed Culture

2.3.1 Mixed Culture from Soil

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

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 can’t growth anaerobically, the structure of *Brevibacillus sp* was rod, can react with Aminopeptidase and can oxidase. 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 (Chen *et al.*, 2003).

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°C but poor growth at 37°C (Little, 1969).
2.3.2 Mixed Culture from Drain

*Trichoderma* were the most prevalent culturable fungi. Cultures were typically fast growing at 25-30° C, typically not growing at 35° C. One of it sub-species which was *Trichoderma koningii* was used in degradation of hydroxyaspergillic acid. A microorganism, strain M 102, capable of degrading aspergillic acid (AA), was first isolated from a soil sample in a drainage ditch and was identified as *Trichoderma koningii Oudemans*. This fungus degraded AA, but not hydroxyaspergillic acid (HAA) or deoxyaspergillic acid (DAA). The AA-degrading ability of M 102 was induced by incubation with AA but not with HAA or DAA. AA-degradation activity was found in a crude enzyme prepared from the mycelia induced by AA; this AA degradation reaction required NAD (P) H and oxygen (Harman *et al.*, 1991).

*White rot fungus* is a bioremediation technology. Wood-rotting enzymes in *white rot fungus* degrade a variety of pollutants. Treatment involves mixing soil with fungus and a suitable substrate such as wood chips. *White rot fungus* has been tested in situ (i.e., in place) and in an above ground bio-reactor. Moisturized air on wood chips is used in a reactor for biodegradation. This system is similar to composting, except that *white rot fungus* works best in a nitrogen-limited environment. *White rot fungus* was found to be able to colonise and bleach the wood tissue and form large holes within the already heavily degraded wood. Concentrations of cellulose and hemicelluloses in the Nydam ash were obviously high enough for supporting degradation of the lignin network, leading to a complete destruction of the wood. Bacterial slime and degradation products present in the heavily degraded fibres may also represent an important source of nutrients for white rot fungi during decay (Björdal and Nilsson, 2002).

2.3.3 Mixed Culture from River

*Bacillus spp* was on type bacteria found in river. There are six main types of *Bacillus spp* but the most famous was *Bacillus cereus*. Although *Bacillus spp* used in
degradation of petroleum pollutant but it have bad effect in our health (Okerentugba et al., 2003). *Bacillus anthracis* can causes anthrax.*Bacillus subtilis, Bacillus licheniforms, Bacillus amyloliquefaciens and Bacillus pumilis* all cause food borne illness. There are symptoms causes by *Bacillus spp* which were nausea, vomiting, stomach pains, diarrhea, headache and flushing (Lancaster, 2005).

*Aspergillus* was one of fungi found in river. It also used in degradation of petroleum pollutant. *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. (Bozkurt et al., 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. It categorized in *gram-positive cocci*. Most strains are saprophytic and non-pathogenic found in soil, water, dust, frequently found on the skin of man and other animals. Hazard of infection is low and there weren’t any report about the infection by this bacterium (Bozkurt et al., 2008).

**2.3.4 Selection Mixed Culture of Drain**

For this study, microorganism from the drain is selected to be used in removal of zinc. This is supported by fact which is drainage followed by a sinking water table can disrupt the balance of the long-term stability of wood in waterlogged
environments. During drainage, the oxygen concentration increases slowly and a more aerobic environment is created around the wooden material. Earlier, the most obvious/discussed problem concerning drainage of wetlands was the physical distortion of the archaeological wooden objects when drying out. Bacterium from drain also less danger compare to the bacterium from river.

2.4 Zinc Removal Method

2.4.1 Biosorption Process

Biosorption is a technique that can be used for the removal of pollutants from waters, especially those that are not easily biodegradable such as metals and dyes. A variety of biomaterials are known to bind these pollutants, including bacteria, fungi, algae, and industrial and agricultural wastes (Vijayaraghavan and Yun, 2008). Biosorption possesses certain inherent advantages over bioaccumulation processes. The use of living organisms may not be an option for the continuous treatment of highly toxic organic/inorganic contaminants. Once the toxicant concentration becomes too high or the process operated for a long time, the amount of toxicant accumulated will reach saturation (Eccles, 1995). It has been reported that extreme characteristics (pH, conductivity and total hardness) may affect the binding abilities of a biosorbent (Vijayaraghavan et al., 2006).

Biosorption is a proven technique potentially for the removal of metals/dyes from aqueous solutions. There have been few investigations examining the compatibility of the biosorbent for real industrial effluents. Kratochvil and Volesky (1998) explained the necessity of extended testing of a biosorption process before its commercial application. In general, industrial effluents can be classified into two broad categories: those bearing low contaminant concentrations in large volumes, i.e. mining wastewaters, and those characterized by high TDS values in small volumes, i.e. electroplating and textile dye bath effluents. For the first case, a biosorbent with