REMOVAL OF NICKEL BY USING MICROFLORA IN DRAIN

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ABSTRACT

The aims of this work was to study the influence of influent chemical oxygen demand (COD), the removal of initial concentration and the growth of biomass, effects from removal of nickel in wastewater by using microflora in drain. This experiment was using sequencing batch reactor (SBR) system with organic loading rate which range between 0.40mg.L.day to 4.00mg/L.day. The efficiency of COD reduction was found to be 51.5% at organic loading rate 1.54mg/l.day. 35.6% of nickel removal due to biodegradation was achieved at 4.00mg/l.day of organic loading rate. The growth of biomass was increased 310mg/l at 0.40mg/l.day organic loading rate. It is concluded that SBR system can effectively be used in the treatment of nickel wastewater for removal of ions nickel and reduction of COD.

ABSTRAK

Objektif kepada tugasan ini adalah menyelidik pengaruh kepada kemasukan keperluan kimia oksigen, pengurangan kepekatan nikel awal and juga peningkatan biojisim, kesan daripada pengurangan nikel di dalam bahan buangan dengan menggunakan bakteria iaitu mikroflora dari longkang. Eksperimen ini menggunakan sistem reaktor yang berturutan (SBR) dengan kadar bebanan organik dengan berkadaran antara 0.40mg/L.day sehingga 4.00mg/L.day. Kecekapan untuk pengurangan COD adalah sebanyak 51.5% pada kadar bebanan organik 1.54mg/l.hari. Manakala, 35.6% pengurangan nikel berlaku semasa biodegradasi telah dicapai pada kadar bebanan organic 4.00mg/l.hari. Pertumbuhan biojisism juga meningkat sehingga mencapai melebihi 310.9mg/l. Maka, disimpulkan bahawa sistem SBR boleh digunakan secara efektif dalam merawat bahan buangan nikel bagi mengurangkan kepekatan ions nikel dan juga pengurangan dalam COD.

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CHAPTER 1

INTRODUCTION

1.1 Background

Removal of heavy metals from wastewater and industrial wastes has become a very important environmental issue. Nickels are commonly used in silver refineries, electroplating, zinc base casting, storage battery industries, printing and in the production of some alloys and discharge significant amounts of nickel in various forms to the environment (Umesh, *et al*., 2007). High concentrations of heavy metals have been known to be toxic and carcinogenic to loving organisms.

At high concentrations, Ni^{2+} causes lungs, nose and bone cancer, headache, dizziness, nausea and vomiting, chest pain, tightness of the chest, dry cough and shortness of breath, rapid respiration, cyanosis and extreme weaknesses. Hence, it is essential to remove ions nickel from industrial wastewaters before discharges into natural's water sources. Several treatments methods for removal of metals ions from aqueous solutions have been reported mainly ion exchange, solvent extraction, evaporation, electrochemical reduction, chemical precipitation, reverse osmosis and adsorption. 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 (Pehlivan, 2007). This research is done to investigate the most economically way which is treating nickel waste by using microflora in drain.

This study is undertaken to evaluate the effectiveness of microflora in drain to remove the Ni^{2+}. Laboratory scale study will conduct to determine the effects of loading rate which is ranging between 0.4mg/l.day to 4.00mg/l.day in the degradation of nickel, reduction of chemical oxygen demand and also the effects to the biomass growth in terms of suspended solid that exist during the experiment.

1.2 Objective

The main objectives of this research are

- To study the effects of different loading rate on the nickel waste treatment.
- To study the effects of nickel concentration to the growth of microflora.
- To investigate the amount of suspended solids that will exist during the experiment.

1.3 Scope of Research

The scope of study in this experiment is to treat (degrade) nickel in wastewater that contains nickel was simulated with appropriate nutrients for microflora. The initial simulated wastewater was analyzed. Then, experiments were conducted separately in reactor with organic loading rate which range between 0.4mg/l.day to 4.00mg/l.day. The experiments are aerated. Each reactor has working volume of 5 liter. The efficiency of treatment for different nickel concentrations were evaluated in terms of water quality parameters (COD) and the removal of initial concentration. Besides, the effects of nickel concentration on biomass growth in terms of suspended solid that exist throughout the experiments.

1.4 Problem Statement

Nowadays, world has become aware about the seriousness of one of the major consequences of development, that is the quantity and diversity of hazardous waste generated by the industrial activities. A by-product of industrial operation involve heavy metals such as arsenic, cadmium, lead, nickel which is recognized as extremely toxic substances. Depending on the concentration of contaminants, their characteristics some of waste is extremely toxic and hazardous. (Theo, 1994).

So, this is need for controlling the heavy metals especially nickel emissions into the environment as the discharge of the heavy metals into ecosystem. Nickel may be derived from mining operation, electroplating industries, batteries and others. Nickel also can be danger to the human health. In the way to treat nickel wastewater, basically it need highly cost. In the same time, the methods to treat the nickel wastewater are economically unfavorable and technical complicated. Because of that, majority of the industries not really concern about the treatment. This research is proposed because the method that will be use is quite economical since it only uses microorganisms in drain which is readily available in the surroundings. These microorganisms are assumed can degrade the concentration of nickel in the wastewater.

CHAPTER 2

LITERATURE REVIEW

2.1 Heavy Metal

2.1.1 Terminology

The term heavy metal refers to any metallic chemical element that has a relatively high density and is toxic or poisonous at low concentrations. Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter our body via food, drinking water and air. As trace elements some heavy metals (e.g copper, selenium, and zinc) are essential to maintain the metabolism of the human body. However, at high concentrations they can lead poisoning. Heavy metal poisoning could result, for instance, from drinking water contamination (e.g lead pipes), high ambient air concentrations near emission sources or take via the food chain (Lentech, 1998). Heavy metals are major toxicants found in industrial wastewater. The source of heavy metals in wastewater treatment plants are mainly industrial discharges and urban storm water runoffs (Metcalt and Eddy, 1991).

2.1.2 Properties of Nickel

Nickel is a silvery white metal that takes on a high polish. It belongs to the transition metals, and is hard and ductile. It occurs most usually in combination with sulfur and iron in pentlandite, with sulfur in millerite, with arsenic in the mineral nickeline, and with arsenic and sulfur in nickel glance (Source: wikipedia, nickel,n.d). Nickel is hard, malleable, and ductile metal. It is of the iron group and it takes on a high polish. It is a fairly good conductor of heat and electricity. In its familiar compounds nickel is bivalent, although it assumes other valences. It also forms a number of complex compounds. Most nickel compounds are blue or green. Nickel dissolves slowly in dilute acids but, like iron, becomes passive when treated with nitric acid (Source: wikipedia, nickel, n.d)

Because of its permanence in air and its inertness to oxidation, it is used in coins, for plating iron, brass, etc., for chemical apparatus, and in certain alloys, such as German silver. It is magnetic, and is very frequently accompanied by cobalt, both being found in meteoric iron. It is chiefly valuable for the alloys it forms, especially many superalloys, and particularly stainless steel (Tchobanoglous, 2003)

Nickel is used in many industrial and consumer products, including stainless steel, magnets, coinage, and special alloys. It is also used for plating and as a green tint in glass. Nickel is pre-eminently an alloy metal, and its chief use is in the nickel steels and nickel cast irons, of which there are innumberable varieties. It is also widely used for many other alloys, such as nickel brasses and bronzes, and alloys with copper, chromium, aluminum, lead, cobalt, silver, and gold. (Pratt, 1996)

Nickel consumption can be summarized as: nickel steels (60%), nickel-copper alloys and nickel silver (14%), malleable nickel, nickel clad and Inconel (9%), plating (6%), nickel cast irons (3%), heat and electric resistance alloys (3%), nickel brasses and bronzes (2%), others (3%). In the laboratory, nickel is frequently used as a catalyst for hydrogenation, most often using Raney nickel, a finely divided form of the metal. Nickel may be found in slate, sandstone, clay minerals and basalt. (Gode, 2003)

The main nickel source is pentlandite. The element accumulates in sediments and is a part of various biological cycles. Nickel may end up in water from both point and non-point sources. Diffuse nickel emissions may stem from power plants, waste incinerators and metal industries. Nickel is directly emitted from various industries through discharge on surface waters. It is applied in alloys for treatment of heavy metal polluted surface water, in nickel-cadmium batteries, as a catalyzer and as a pigment. Pure nickel is often applied as a protective coating on steel and copper objects. Nickel-copper alloys have been applied in coins for a very long time. (Lentech, 1998)

Other alloys are applied for kitchen ware, jewelry and turbine production. Nickel may be applied as an anti-corrosive. Nickel acetate is applied as a mordant in textile printing, and nickel carbonate is applied as a catalyzer for fat hardening and for ceramic paint production, as is nickel chloride. Nickel tetra carbonyl is a by-product of nickel cleansing and is applied in various production processes. Nickel compounds are also applied in agriculture. Phosphate fertilizers contain traces of nickel. Nickel is often present in agricultural soils situated near fossil fuel industries. Organic matter often adsorbs nickel, causing coal and oil to contain traces of the element. Nickel compounds may also be found in sludge, and in slags and fly ashes from waste incinerators. Better waste separation would prove useful, because nickel is up to 60% recyclable. (Enghag, 2004)

2.1.3 Disadvantages of Nickel

Humans may be exposed to nickel by breathing air, drinking water, eating food or smoking cigarettes. Skin contact with nickel-contaminated soil or water may also result in nickel exposure. In small quantities nickel is essential, but when the uptake is toohigh it can be a danger to human health (Lentech, 1998)

An uptake of too large quantities of nickel has the following consequences such as higher chances of development of lung cancer, nose cancer, larynx cancer and prostate cancer, sickness and dizziness after exposure to nickel gas, lung embolism and respiratory failure. In the other hand, it will cause birth defects to the pregnancy woman. Asthma and chronic bronchitis, allergic reactions such as skin rashes, mainly from jewellery also the effects of nickel (Lentech, 1998)

Nickel fumes are respiratory irritants and may cause pneumonitis. Exposure to nickel and its compounds may result in the development of a dermatitis known as “nickel itch” in sensitized individuals. The first symptom is usually itching, which occurs up to 7 days before skin eruption occurs. The primary skin eruption is erythematous, or follicular, which may be followed by skin ulceration. Nickel sensitivity, once acquired, appears to persist indefinitely (Lentech, 1998)

2.1.4 Effects of Nickel to the Environment

Nickel is released into the air by power plants and trash incinerators. It will than settle to the ground or fall down after reactions with raindrops. It usually takes a long time for nickel to be removed from air. Nickel can also end up in surface water when it is a part of wastewater streams. The larger part of all nickel compounds that are released to the environment will adsorb to sediment or soil particles and become immobile as a result. In acidic ground however, nickel is bound to become more mobile and it will often rinse out to the groundwater. There is not much information available on the effects of nickel upon organisms other than humans. High nickel concentrations on sandy soils may damage plants and high nickel concentrations in surface waters can diminish the growth rates of algae. Microorganisms can also suffer from growth decline due to the presence of nickel, but they usually develop resistance to nickel after a while (Helmenstine, 2007)

For animals nickel is an essential foodstuff in small amounts. But nickel is not only favorable as an essential element; it can also be dangerous when the maximum tolerable amounts are exceeded. This can cause various kinds of cancer on different sites within the bodies of animals, mainly of those that live near refineries (Lentech, 1998)

Nickel is not known to accumulate in plants or animals. As a result nickel will not bio magnify up the food chain.

Standard Maximum Value for wastewater.

Standard A: For water disposal to the state reservoirs.

Standard B: for water disposal to the other reservoirs.

For Nickel, Standard A = 0.20mg/l

Standard B = 1.00 mg/l

(Source: Malaysia.1974, Department of Environment, Quality Act, 1974)

2.2 Mixed Culture

2.2.1 Terminology

Mixed culture of organisms is common in natural ecological systems. Microorganisms are involved in the natural cycles of most elements (for example, carbon, nitrogen, oxygen and sulfur). Organisms living in soil and aquatic environments actively participate in carbon and nitrogen cycles. For example certain organisms fix atmospheric CO_2 to form carbohydrates, while others degrade carbohydrates and release CO_2 into the atmosphere. Similarly, some organisms fix atmospheric nitrogen (N_2) to form ammonium and proteins while others convert ammonium into nitrate and nitrate (nitrification) and others reduce nitrate into asmospheric nitrogen (denitrification). In other ways, mixed culture microorganisms are used in biological treatment for wastewater in terms or anaerobic and aerobic treatment. (Shuler, *Bioprocess Eng*., 1992)

2.2.2 Microorganisms in Drain

Mixed culture in drain is containing both autotrophic and heterotrophic. An autotroph is an organism that produces complex organic compounds from simple molecules and an external source of energy such as light or chemical reactions of inorganic compounds. Bacteria that utilize the oxidation of inorganic compounds such as hydrogen sulfide, ammonium or ferrous as a energy source. They use carbon dioxide, bicarbonate or carbonate as their sole carbon source for the synthesis of cellular material, and obtain energy for metabolism by oxidation of reduced inorganic compounds (chemo-autotroph) or by photosynthesis (photo-autotroph) (Robertson, 1998).

Chemo-autotroph bacteria are able to oxidize a range of elements that are present in a reduced form (i.e low oxidation state). However, the condition required for the active growth of these bacteria are critical and in some ways conflicting. The group requires a plentiful supply of the reduced inorganic compound (e.g NH_3, NO_2^-, H_2S), oxidizing agent (e.g O_2 or the some species NO_3^-) which must have a more positive redox potential than the reducing agent, and carbon dioxide as the carbon source. Chemo-autotrophs are aerobic organisms although they can grow at low partial pressures of oxygen and some species are able to utilize other oxidizing agent. Unlike the photo-autotroph, they do not require light and do not contain pigments. However, they restricted in wastewater treatment units to the areas where there are plentiful supplies of reduced inorganic inorganic compounds, carbon dioxide and some oxygen. As a group, they can oxidize a wide range of reduced inorganic compounds, although individual species are highly specific in the reaction they catalyse and in the environmental conditions they require (Pisman, ***et.al***., 2003)

Other organisms, called heterotrophs, utilize autotrophs as food to carry out these same functions. Heterotroph can be defined as anaerobic heterotrophic and aerobic heterotrophic. Anaerobic heterotrophic bacteria are either obligate or unable to grow in the presence oxygen, or facultative and can adapt to environments either with or without oxygen. The major role of anaerobic heterotrophs is in sludge digestion converting unstable sewage into a more stabilized form. Digestion normally takes place in specially constructed reactors, although anaerobic digestion also occurs in the sludge blanket of waste stabilization ponds. Anaerobic treatment is an effective method for the complete treatment of many organic wastes, especially animal wastes and inorganic effluents from the food processing industries (Artin, ***et.al***., 2006)

Many researchers reported that several microorganisms (fungus, bacteria and algae) and bio-sludge of activated sludge system adsorbed both organic and inorganic matter from wastewater (Al-Asheh and Duvnjuk, 1995, Gulnaz, *et al.*, 2005, Warget, *et al*., 2006, Al-Qodah, 2006)

2.2.2. Nutrients for Mixed Culture

Nutrients can be divided into two categories, complementary and substitutable. Complementary nutrients are those that meet entirely different needs by growing microorganisms. For example, ammonia provides the nitrogen needed for protein synthesis while glucose provides carbon and energy. If either was missing from the growth medium and no substitute was provided, no growth would occur. Substitute nutrients, on the other hand, are those that meet the same need. For example, ammonia and nitrate can both provide carbon and energy. Thus, ammonia and nitrate are substitutable for each other, as are glucose and phenol. (Cateryna, *et.al*., 2006)

Like all other biochemical operations, nutrients are required are required by anaerobic processes because they are essential components of the biomass produced. However, biomasss yields are much lower in anaerobic processes than in aerobic ones, and this result in reduced nutrients requirements. Consequently, adequate nutrients will generally be available when complex wastes are being treated. However, nutrients addition may be required when carbon rich industrial wastes are being treated. Such wastewater may be deficient in the macronutrients nitrogen, and phosphorus. The concentration of micronutrients such as iron, nickel, cobalt, sulfur, and calcium may also be limiting. Nickel and cobalt are particularly important for growth of methanogens (Roussos, *New Horizons in Biotechnology*, 2004)

A range of nutrients combinations have been used to isolate bacterial cultures and for use in commercial operations. The basic nutrient needs are nitrogen, potassium and phosphorus with a variety of trace elements depending on the particular species (Ghulam, *et.al.*, 2008)

2.3 Biological Treatment for Nickel

2.3.1 Biosorption of nickel by heavy metal resistant fungal and bacteria isolates.

Recently, microbial systems like fungus, bacteria and algae have been successfully used as adsorbing agents for removal of heavy metals (Terres, 1998) Microbial populations in metal polluted environment adapt to toxic concentrations of heavy metals and become metal resistant. Different species of *Aspergillus, Pseudomonas, Sporophyticus, Bacillus, Phanerochaete*, etc., have been reported as efficient nickel reducers (Gopalan, *et.al.*, 1994). Nickel resistant fungal and bacteria strain were isolated form the soil sample using fungal and bacteria medium [potato dextrose (PDB) and nutrient broth (NB)]. Potato dextrose broth and agar were prepared using 250g of potato boiled in 100mL of distilled water for 30min and the filtrate is mixed with 2g of dextrose and for agar plates 1.5g of agar was added with this mixture. Nutrient broth and agar plates were prepared using peptic digest of animal tissue (5 gL^{-1}), beef extract (3 gL^{-1}), NaCl (5 gL^{-1}), and 1.5

g agar for 100 ml medium. To isolate metal resistant fungal and bacterial strains these medium were amended with 100 mg L^{-1} Cr(VI) and 50 mgL^{-1} Ni(II) and standard spread plate method was performed. The inoculated plates were incubated at room temperature (30–35 ◦C) for 48 h. After 48 h incubation larger identical colonies from each plate were isolated. These isolates were characterization and further employed for heavy metal removal and tolerance studies. Morphological, physiological, and biochemical characteristics of the isolated fungal and bacterial species is given in Tables 1 and 2, respectively (Congeevaram, *et al.,* 2007)

Table 2.1: morphological, physiological and biochemical characteristics of the isolated fungal species.

Morphological, physiological and biochemical characteristics	Isolated fungal strain
Colony diameter	28mm
Conidial color	Dark brown-black
Conidial shape	Globose
Vesicle shape	Globose
Conidiophore color	Brown
Mycelial color	Whitish
Colonial reverse	Whitish grey
Sterigmata color	Brown
No of sterigmata	Present in two series.

Table 2.2: morphological, physiological, biochemical characteristics of the isolated bacteria species.

Morphological, physiological and biochemical characteristics	Isolated bacteria strain
Cell shape	Soft, smooth, yellow growth
Growth at temperature (^{o}C)	37
Growth at pH	5-11
Urease activity	+
Catalese activity	+
Gelatin liquefaction	+
Type strain	Micrococcus
Starch hydrolysis	-
Lipid hydrolysis	-

For the result, maximum removal of Ni(II) (90%) was observed around pH 5 in the case of *aspergillus* sp. While, *micrococcus* sp. reported a maximum removal for Ni(II) is 55% at pH 7.0. The study demonstrated that newly isolated *Micrococcus* sp. and *Aspergillus* sp. strains have potential application for removal of nickel from industrial wastewater. (Congeevaram, *et al.,* 2007)

2.3.2 Nickel Biosorption by two *Chlorella* species, *C.Vulgaris* (a commercial species) and *C.Miniata* (a local isolate)

The nickel ion, compared with other heavy metal ions, was a more recalcitrant pollutant and many metal tolerant microalgae had a relatively low Ni-binding capacity. The nickel ion, compared with other heavy metal ions, was a more recalcitrant pollutant and many metal tolerant microalgae had a relatively low Ni-binding capacity. Chan *et al*, (1991) found that *Chlorella* biomass removed 90% copper but the removal of nickel ions from electroplating effuent was far from satisfactory. Tsezos *et al*. (1996) also found that the biosorption of nickel by di.erent strains of microorganisms was less than that of other metal ions. This was probably due to the intrinsic chemical properties of nickel ions leading to steric hindrance of biosorption (Tsezos, *et al*., 1996). Studies focusing specifically on nickel removal are rare. To date, there is still no satisfactory precedent of employing *C. vulgaris* in the removal of nickel ions and the feasibility of employing microalgae to remove Ni from electroplating wastewater is uncertain.

Microalgal species growing in polluted environment were usually more resistant to metals and the tolerant species had a higher capability of accumulating heavy metals (Trollope and Evans, 1976; Wong and Pak, 1992). An indigenous microalga, WW1 (tentatively identified as *Chlorella miniata*) was isolated from domestic wastewater collected from secondary sewage treatment works in Hong Kong. The morphological appearance of this unicellular green alga was very similar to *C. vulgaris,* except for a smaller cell size of about 2 lm in diameter (approximate mean cell diameter of *C. vulgaris* is 4.5 lm). This local isolate might have been a more tolerant and capable species in removing heavy metals. (Wong *et.al*., 2000)