KINETIC STUDY FOR BIOFILM MANGANESE REMOVAL

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ABSTRACT

Objective of this study is to determine the kinetic parameters of manganese (Mn) removal for the biofilm from two different sources; which were from drain and soil. There are four kinetic parameters needed to be determined which were yield coefficient (Y), decay rate constant (k_d), microbial growth rate (μ_{max}), and substrate concentration (Ks); the calculation was based on Monod kinetic model. The process for determining kinetic parameters of each biofilm included the acclimatization of biofilm, total suspended solids tests (TSS), Mn tests and chemical oxygen demand (COD) tests. Sequencing batch biofilm reactor (SBR) is used in this study and two parameters are tested in the experiment; Mn and COD concentration. Results showed that the kinetic value for drain biofilm of Mn concentration parameter were Y = 2.9912 mg TSS/mg Mn, k_d = 1.214 day^{-1}, μ_{max} = 1.374 day^{-1}, and Ks = 0.4262 mg/L Mn. The soil biofilm for Mn concentration parameter showed the value of kinetic as Y = 0.1317 mg TSS/mg Mn, k_d = 1.836 day^{-1}, μ_{max} = 1.846 day^{-1}, and Ks = 0.0433 mg/L Mn. For the research on the COD concentration parameter, the values of kinetic for drain biofilm are Y = 0.0078 mg TSS/mg COD, k_d = 1.0519 day^{-1}, μ_{max} = 1.136 day^{-1}, and Ks = 4.403 mg/L COD. Soil biofilm showed the kinetic values of Y = 0.0117 mg TSS/mg COD, k_d = 1.8348 day^{-1}, μ_{max} = 1.844 day^{-1}, and Ks = 0.0433 mg/L COD. By the evaluation of COD and Mn removal from wastewater, from the way of kinetic parameter estimation provide knowledge for future planning, designing and modelling of wastewater treatment plant.

ABSTRAK

Objektif untuk kajian ini adalah untuk mendapatkan parameter kinetik untuk penyingkiran mangan (Mn) oleh biofilem dari dua sumber berlainan; iaitu dari longkang dan tanah. Terdapat empat parameter kinetik yang perlu dicari iaitu hasil pekali (Y), pemalar kadar reput (k_d), kadar pertumbuhan mikrob (μ_{max}) dan kepekatan substrat (Ks); pengiraan adalah berdasarkan model kinetik Monod. Proses untuk mendapatkan parameter kinetik untuk setiap jenis biofilem adalah termasuk proses penyesuaian biofilem terhadap persekitaran baru, ujian pepejal terampai total (TSS), ujian Mn dan ujian keperluan oksigen kimia (COD). Reaktor biofilem sesekumpul berjujukan (SBR) digunakan untuk kajian ini dan dua parameter dikaji dalam eksperimen ini; iaitu kepekatan Mn dan COD. Keputusan eksperimen menunjukkan nilai kinetik untuk biofilem dari longkang bagi parameter kepekatan Mn adalah Y = 2.9912 mg TSS/mg Mn, k_d = 1.214 hari^{-1}, μ_{max} = 1.374 hari^{-1}, dan Ks = 0.4262 mg/L Mn. Biofilem dari tanah bagi parameter kepekatan Mn pula menunjukkan nilai kinetik seperti Y = 0.1317 mg TSS/mg Mn, k_d = 1.836 hari^{-1}, μ_{max} = 1.846 hari^{-1}, dan Ks = 0.0433 mg/L Mn. Untuk kajian terhadap parameter kepekatan COD, nilai kinetik untuk biofilem dari longkang adalah Y = 0.0078 mg TSS/mg COD, k_d = 1.0519 hari^{-1}, μ_{max} = 1.136 hari^{-1}, dan Ks = 4.403 mg/L COD. Biofilem dari tanah pula menunjukkan nilai kinetik Y=0.0117 mg TSS/mg COD, k_d = 1.8348 hari^{-1}, μ_{max} = 1.844 hari^{-1}, dan Ks = 0.0433 mg/L COD. Penilaian terhadap penyingkiran COD dan Mn daripada air buangan melalui pengiraan parameter kinetik dapat memberi pengetahuan mengenai perancangan masa depan, bentuk rekaan dan permodelan untuk loji rawatan air buangan.

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LIST OF ABBREVIATIONS/SYMBOLS

[S]	- substrate concentration
K	- maximum utilization rate for the substrate per unit mass of bacteria
[X]	- concentration of bacteria
[Ks]	- half-velocity coefficient for the substrate
Y	- reaction rate
v_{max}	- enzyme's maximum rate
K_M	- Michaelis-Menten constant
μ	- specific growth rate (h^{-1})
μ_m	- maximum specific growth rate without substrate inhibition (h^{-1})
K_s	- saturation constant (mg/L)
S	- substrate (ex: Mn) concentration (mg/L)
K_I	- substrate inhibition constant (mg/L)
X	- concentration of total suspended solids (TSS) (mg/L)
T	- time (day)
Y	- yield coefficient; mass of cells produced per unit mass of substrate utilized (mg TSS/mg COD or mg TSS/mg Mn)
Kd	- endogenous decay rate per unit of time (day^{-1})
U	- specific substrate utilization rate per unit time (day^{-1})
BRT	- biomass retention time (mg/day)
V	- volume of tank (L)
X_{in}	- amount of TSS in the tank (mg TSS)

X_{out} - amount of TSS out of the tank (mg TSS)

Q - flowrate of substrate in and out of tank (mg/L.day)

HRT - hydraulic retention time (day)

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CHAPTER 1

INTRODUCTION

1.1 Background

Naturally, manganese (Mn) is found as a free element (often in combination with iron) and in many minerals. As a free element, manganese is a metal with important industrial metal alloy uses especially for stainless steel. There are many application of manganese in several industries such as steelmaking which Mn essential to iron and steel production by virtue of its sulfur-fixing, deoxidizing and alloying properties.

Mn also used as alloying agents for aluminium with 1.5% content of Mn can increase resistance against corrosion due formation grains absorbing impurities that lead to galvanic corrosion. Besides, Mn compound is used as pigments and for coloring of ceramics and glass. The human body contains about 10 mg of Mn, stored mainly in liver and kidneys. Mn is important in photosynthetic oxygen evolution in chloroplasts in plants (Katsoyiannis and Zouboulis, 2004).

However the present of Mn in drinking water is undesirable. Therefore the removal of Mn compound from water is crucial and biological treatment is a great way to manage water treatment problems. Most of the work related to bacterial manganese oxidation has been focused on investigation during batch experiments or in natural environments. Detailed information about the biological oxidation of manganese and the products produced has been also reported for the case of in situ groundwater treatment plants. Recently, several studies have been carried out for the case of continuous groundwater treatment. A filter able to remove Mn from groundwater by biological oxidation and accumulation (bioaccumulation) without the addition of chemicals appears to be the most common method (Tekerlekopoulou *et al*., 2007).

Biofilm is a substance that forms readily in water distribution lines, water storage tanks, and any other aqueous environment. A biofilm forms when bacteria begins to excrete a slimy, sticky substance that allows them to adhere to surfaces. Biofilm is resistant to chlorine and is difficult to remove once initial adhesion occurs (MIOX, 2009).

1.2 Problem Statement

The research will include the kinetic consideration for the removal of biofilm manganese. Bacterial growth kinetics is slightly more complex and follows the classical "Monod-type" kinetics. In this case, the rate of substrate utilization is proportional to the concentration of the microorganisms present [X] and is a function of the substrate concentration (Roth, 2004).

The Monod bacterial growth kinetics is traditionally written as:

$$\frac{dS}{dt} = \frac{k\,[x][S]}{y(Ks) + [S]}$$

Where:

[S] = substrate concentration

k = maximum utilization rate for the substrate per unit mass of bacteria

[X] = concentration of bacteria

[Ks] = half-velocity coefficient for the substrate

y = yield coefficient = d[X]/d[S]

There are several good reasons for adding kinetic modeling to the skills for biological investigation. First, experience has shown, that there is much more information contained in dynamic biological data than can be extracted by simple inspection.

Second, building successful dynamic or kinetic models is heavily dependent on knowing or discovering the underlying biological mechanisms (Russel, 2006). Biological removal is preferred as the treatment takes minimum cost, no addition of chemical and smaller reactor to be constructed (Gage *et al*., 2001; Tekerlekopoulou *et al*., 2008).

1.3 The Objective of Study

To study the kinetic parameter of biofilm manganese removal

1.4 Scope of Study

In order to achieve the objective of this study, this research will use Monod growth model for the calculation of the kinetic study for the biofilm manganese removal. The kinetic parameters which are important for wastewater treatment are identified and will be determined from the experiment. The kinetic parameters are K, k_d, μ_m, and Y. Therefore, several tests such as total suspended solids (TSS) test, chemical oxygen demand (COD) test, and manganese (Mn) test by HACH spectrophotometer will be run to determine the kinetic parameters of the biofilm. The biofilm sources are from soil and drain microflora which the biofilm will be acclimatized by using the sequencing batch biofilm reactor of 8 L each. The biofilm will be monitored everyday by running TSS test until the value of TSS equal to 3000 mg/L per microflora. After the acclimatization process, kinetic parameters determination process is run everyday and MnO_2 is introduced to the biofilm. The experiment will be run until the kinetic parameters have been determined and stabilized.

CHAPTER 2

LITERATURE REVIEW

2.1 Manganese

2.1.1 Manganese sources in industrial wastewater

The presence of manganese (Mn) in drinking water or water supply is unwanted as it gives water an unpleasant taste and color. Manganese oxide forms a dark, brown-black precipitate that clogs pipes and discolors fabric (Vandenabelee *et al*., 1995).

Tekerpoulou (2007) found that Mn exist in several different oxidation states ranging from 0 to +7, although it is almost always found in nature in its +2, +3 and +4 state. Mn (II) is readily soluble in water while Mn (III) is more unstable and has a tendency to precipitate or dissociate to Mn (II) and 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 solution.

In addition, Mn is a common contaminant in many mine waters and though not as other metals found in such waters (such as Fe, Al, and Zn), it nevertheless has various undesirable properties, including a propensity for precipitating in water distribution pipe network that eventually causing blockage of supply pipes (Johnson and Younger, 2005).

2.1.2 Effect of manganese to the health and environment

Manganese is a naturally occurring mineral that is an essential nutrient for plants and animals alike. As with any mineral though, excessive exposure may become harmful to one's health. Manganese is no exception. Manganese toxicity is a serious threat to human health and can cause the physically and mentally debilitating disease called Manganism**.**

Manganism, also called welder's disease, is classified as a Parkinson's disease. It is characterized by diminished motor skills and psychological problems similar to dementia. Symptoms of Manganism include hand and body tremors, impaired hand-eye coordination, weakness, speech difficulties, respiratory difficulties, decreased sexual function, and damage to developing fetus. Although the cause of Parkinson's disease is a mystery, Manganism is known to be caused by excessive exposure to manganese fumes or manganese particulates. Manganese cases are usually diagnosed in people who work with manganese on a daily basis (Jiang *et al*., 2006).

Manganese compounds exist naturally in the environment as solids in the soils and small particles in the water. Manganese particles in air are present in dust particles. These usually settle to earth within a few days. Humans enhance manganese concentrations in the air by industrial activities and through burning fossil fuels. Manganese that derives from human sources can also enter surface water, groundwater and sewage water. Through the application of manganese pesticides, manganese will enters oils.

For animals manganese is an essential component of over thirty-six enzymes that are used for the carbohydrate, protein and fat metabolism. With animals that eat too little manganese interference of normal growth, bone formation and reproduction will occur. For some animals the lethal dose is quite low, which means they have little chance to survive even smaller doses of manganese when these exceed the essential dose. Manganese substances can cause lung, liver and vascular disturbances, declines in blood pressure, failure in development of animal fetuses and brain damage.

When manganese uptake takes place through the skin it can cause tremors and coordination failures. Finally, laboratory tests with test animals have shown that severe manganese poisoning should even be able to cause tumor development with animals.

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. Manganese can cause both toxicity and deficiency symptoms in plants. When the pH of the soil is low manganese deficiencies are more common (Lenntech, 1998).

2.1.3 Manganese level of discharge

Manganese is an essential trace element with an estimated daily nutritional requirement of 30-50 µg/kg of body weight. Its absorption rate can vary considerably according to actual intake, chemical form, and presence of other metals, such as iron and copper, in the diet.

The current level of Mn allowed in private water supplies according to the Private Water Regulations 1991 is 50 µg/L. However the World Health Organization advocates a guideline value of 500 µg/L to protect public health although it should be noted that the discoloration starts to affect water after 150 to 200 µg/L (Gage *et al*., 2001).

2.2 Biological Manganese Removal

2.2.1 Biofilm

Biofilm is a substance that forms readily in water distribution lines, water storage tanks, and any other aqueous environment. A biofilm forms when bacteria begins to excrete a slimy, sticky substance that allows them to adhere to surfaces. Biofilm is resistant to chlorine and is difficult to remove once initial adhesion occurs (MIOX, 2004).

In a biofilm system for Mn removal, for a certain influent COD concentration not all COD supplied in the influent can be taken up during the anaerobic period. Other heterotrophic bacteria will then dominate the biofilm resulting in an increase of the effluent phosphorus concentration. A larger biofilm thickness will result in an increase of the total mass of manganese-accumulating organisms in the system. However, it is shown that a larger biofilm thickness results in higher effluent phosphorus concentrations. (Morgenroth and Wilderer, 1998)

2.2.2 Trickling Filter

Based on the research by Tekerloupou *et.al* (2007), they run two series of experiments were carried out in order to investigate the effect of feed manganese concentration and volumetric flow rate, as well as the effect of the size of the support material, on filter performance. Findings showed that monolayer filter has the highest removal efficiency (100% removal, up to 2850 mg Mn/day). Multilayer filter is less effective for high concentration of Mn but could remove up to 3250 mg Mn/day.

2.2.3 Rapid Sand Filter

Rapid sand filter can be used to remove Mn. According to Vandenabelee *et al.* (1995) which utilize the microorganisms present on the sand filter in drinking water plants as an alternative for removal of Mn. A pilot-scale rapid sand filter reactor was

constructed at the groundwater plants by Drinking Water Company. The bacteria used are *Nitrosomonas europaea* strain and *Nitrobacter winogradskyi*. From the results, within 12-week period; Mn oxidizing organisms were present at concentration of 1.10 mg/L of moist sand at the end of experiment.

The experiment also investigates the influence of nitrite in media culture for Mn removal. As a results, it is showed that the absence of nitrite in media contribute to highest removal of Mn with only 15 mg/L left in the end of experiment. Compared to medium with nitrite presence which is less effective to remove Mn with the Mn concentration was 77 mg/L at the end of experiment.

2.2.4 Aerated Packed-Bed Bioreactor

Aerated packed-bed bioreactor was used to remove Mn from mine waters which the research has been done by Johnson and Younger (2005). The research was done to understand the role of aeration in enhancing Mn oxidation at various stages of biofilm development and under different environmental conditions. There are two bioreactors used with different support media; first is dolomite and the second is quartziter. From the results, it is showed that quartziter reactor recovers to approximately 67% Mn removal and the dolomite reactor recovers completely to approximately 97% Mn removal. Therefore, it is proven that dolomite substrate provides a better surface for the attachment of Mn oxyhydroxide precipitates than the quartziter gravel.

2.2.5 Selection of biofilm process as treatment method

For this research, biofilm process will be used as the treatment method because the utilization of microflora that is readily in the environment to entrap or consume manganese seems to be promising alternative way for biological removal process. The optimum condition for biological removal of biofilm and types of bacteria that will consume the biofilm have been determined by Yeop (2008). Therefore, this research is a further study on the removal of biofilm contains manganese in it by applying the kinetic study.

2.3 Kinetic Model for Manganese Removal

2.3.1 Monod Model

The Monod model was suggested by Monod in 1942 and for more than 60 years has been one of the most frequently used models in microbiology. Most models of chemostat growth are based on the Monod equations and numerous models of microbial ecology incorporate Monod's growth kinetics. One of the very important practical applications of this model is the evaluation of the biodegradation kinetics of organic pollutants in environmental systems. The Monod model describes microbial growth with three parameters:

a) Maximal specific growth rate, μ_{max};
b) A saturation constant, Ks;
c) A yield coefficient, Y.

In the case of biodegradation kinetics, these parameters can be used as criteria for the biodegradability of organic pollutants. One of the most important problems in practical applications of the Monod model is evaluating the parameters' values from experimental data. It was shown that in many cases, reasonable estimates of the parameters cannot be obtained by a simple application of non-linear least squares estimators. A possible way to circumvent this problem is the application of optimal experimental design techniques. In general, the application of optimal experimental designs can be useful in

a) reducing the number of necessary experimental measurements;
b) improving the precision of model parameter value determinations

Optimal design theory is well developed for linear regression models, and for several simple non-linear regression models. Recently, a complete theoretical examination of optimal designs for the Monod model was presented, where two different approaches to the problem of designing experiments for the Monod model were investigated, namely local optimal experimental designs; and maximum optimal experimental designs (Johnson and Younger, 2005).

2.3.2 Michealis-Menten Model

Michaelis–Menten kinetics (occasionally also referred to as Michaelis–Menten–Henri kinetics) approximately describes the kinetics of many enzymes. It is named after Leonor Michaelis and Maud Menten. This kinetic model is relevant to situations where very simple kinetics can be assumed, (i.e. there is no intermediate or product inhibition, and there is no allostericity or cooperativity). More complex models exist for the cases where the assumptions of Michaelis–Menten kinetics are no longer appropriate.

The Michaelis–Menten equation relates the initial reaction rate v_0 to the substrate concentration [S]. The corresponding graph is a hyperbolic function; the maximum rate is described as v_{max}.

$$v_0 = \frac{v_{max}\,[S]}{K_m + [S]} \qquad (2.1)$$

Where,

v = reaction rate

[S] = substrate concentration

v_{max} = enzyme's maximum rate

K_M = Michaelis-Menten constant

The Michaelis–Menten equation describes the rates of irreversible reactions. A steady state solution for a chemical equilibrium modeled with Michaelis–Menten kinetics can be obtained with the Goldbeter–Koshland equation.

As enzyme-catalysed reactions are saturable, their rate of catalysis does not show a linear response to increasing substrate. If the initial rate of the reaction is measured over a range of substrate concentrations (denoted as [S]), the reaction rate (v) increases as [S] increases, as shown on the right. However, as [S] gets higher, the enzyme becomes saturated with substrate and the rate reaches V_{max}, the enzyme's maximum rate (Wrighton *et al*., 1993).

2.3.3 Haldane Model

The Haldane equation has been used extensively to model bacterial growth kinetics on of Phenol degradation by a psychrotropic strain of *Pseudomonas putida*. The Haldane equation is:

$$\mu = \frac{\mu_m \, . \, S}{K_s + S + \left(\frac{S^2}{K_I}\right)} \qquad (2.2)$$

Where:

μ = specific growth rate (h^{-1})

μm = maximum specific growth rate without substrate inhibition (h^{-1})

K_s = saturation constant (mg/L)