Kinetics, equilibrium isotherm and mechanisms of heavy metal biosorption by aerobic granules

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Kinetics, Equilibrium Isotherm and Mechanisms of Heavy Metal Biosorption by Aerobic Granules

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ABSTRACT

Biosorption is an effective technology for the removal of heavy metals from aqueous solution. Compared with conventional technologies for metal removal, such as chemical precipitation, evaporation, electroplating, adsorption and ion exchange, biosorption has advantages of high efficiency and low-cost when the heavy metals concentrations in wastewater are relatively low. To date, a vast array of biomaterials have been tested as biosorbents for heavy metals removal, such as algae, fungi, yeast, wasted activated sludge, digested sludge and so on. However, most biosorbents currently used are suspended biomass. The major problems-associated with this kind of biosorbents are separation of suspended biomass from the treated effluent and difficulty in maintenance of biosorbent stability. Consequently, these drawbacks limit application of biosorption in the removal of heavy metals from wastewater. Aerobic granules are microbial aggregates with strong structure and excellent settling ability. This study therefore investigated the feasibility of waste aerobic granules as a novel type of biosorbent to remove dissolved heavy metals from aqueous solution. For this purpose, cadmium, copper and nickel were used as model heavy metals to study the kinetics, equilibrium isotherm, and mechanisms of heavy metal biosorption by waste aerobic granules which were discharged from aerobic granular sludge sequencing batch reactor. For the purpose of easy understanding, the term aerobic granules instead of waste aerobic granules were used throughout the thesis.

To study the kinetics of heavy metal biosorption by aerobic granules, two series of batch experiments were conducted at different initial cadmium, copper concentrations (C_0) and initial concentrations of aerobic granules (X_0). Based on the experimental data obtained, a reversible first-order kinetic model was developed to describe the heavy metal removal by aerobic granules. Results showed that the biosorption kinetics of individual cadmium and copper by aerobic granules were closely related to C_0 and X_0 . It was further demonstrated that the real driving force of the cadmium and copper biosorption by aerobic granule could be described by the C_0/X_0 ratio rather than individual C_0 or X_0 . The C_0/X_0 ratio indeed provides a

Abstract

unified basis for interpretation of the biosorption data obtained under different initial conditions. It appears from this phase of study that aerobic granules can be used as effective biosorbent for efficient removal of cadmium and copper or other types of heavy metals from industrial wastewater.

To investigate the equilibrium isotherm of heavy metal uptake by aerobic granules, two series of batch experiments with Cd^{2+} and Cu^{2+} as model heavy metals were carried out. According to the thermodynamic principles of biosorption process, a general model was proposed to describe the biosorption equilibrium of individual Cd^{2+} and Cu^{2+} by aerobic granules. This model provides good insights into the thermodynamic mechanisms of biosorption of heavy metals. The model prediction was in good agreement with the experimental data obtained, indicated by a correlation coefficient was greater than 0.90. It was further shown that the Langmuir, Freundlich and Sips or Hill equations would be special cases of the proposed model.

The Ni^{2+} biosorption by aerobic granules was studied at various initial pH values of 2 to 7. Results showed that the initial pH would play an important role in the Ni^{2+} removal by aerobic granules, and also influenced the zeta potential of aerobic granules. The thermodynamic equilibrium isotherm developed can fit the experimental data very well at all studied pH values. The close relationship between the zeta potential and the Ni^{2+} biosorption capacity of aerobic granules showed the electrostatic attraction between the aerobic granules and Ni^{2+} ions. It was also found that some light metals, such as K^+ , Mg^{2+} and Ca^{2+} would be released into the bulk solution during the Ni^{2+} uptake by aerobic granules, which in turn indicated an ion exchange mechanism involved in the Ni^{2+} biosorption.

Equilibrium and thermodynamics of the Ni^{2+} biosorption by aerobic granules were studied at different temperatures, and it was found that the isotherm model developed fitted the experimental data very well. The Gibbs free energy change (ΔG^0), enthalpy change (ΔH^0) and entropy change (ΔS^0) during the biosorption were thus calculated. The positive ΔH implied that the Ni^{2+} biosorption was an

endothermic process in the temperature range of 25-55°C.

The biosorption mechanisms of Cd^{2+} , Cu^{2+} and Ni^{2+} by aerobic granules were investigated by a number of different methods, including powder X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS). The results revealed that ion exchange would be the main mechanism involved in the metal removal by aerobic granules. Light metal ions, such as calcium, magnesium and potassium in aerobic granules were found to be released into bulk solution during the biosorption of heavy metal. The main binding functional groups of aerobic granules involved in the biosorption were apparently ester, alcoholic and carboxylate groups. For Cd^{2+} and Cu^{2+} , metallic Cd and Cu precipitations were also contributed to the removal of Cd^{2+} and Cu^{2+} from aqueous solution.

Heavy metals like cadmium, copper and nickel could be successfully removed by aerobic granules from aqueous solution. The biosorption capacity of aerobic granules was in the order of $\text{Cd}^{2+} > \text{Cu}^{2+} > \text{Ni}^{2+}$. The operating parameters, such as initial metal and biomass concentrations, initial pH and temperature would affect the metal biosorption capacity of aerobic granules. The developed reversible first order kinetic model and the equilibrium isotherm based on thermodynamic approach could satisfactorily predict the experimental data. It seems that the multi-mechanisms, e.g. ion exchange, ECP binding and chemical precipitation, would be involved in the heavy metal biosorption by aerobic granules. The excellent settleability of aerobic granules can ensure a rapid biosolids separation from the treated effluent, which in turn leads to a simple process design. This study showed that aerobic granules could be used as an effective biosorbent for the heavy metal removal from aqueous solution.

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NOMENCLATURE

q_t	Biosorption capacity at time t (mg/g)
q_e	Biosorption capacity at equilibrium (mg/g)
C_0	Initial concentration (mg/l)
C_e	Equilibrium concentration (mg/l)
X_0	Initial biomass concentration (mg/l)
V	Volume of liquid in the reactor (l)
M	Mass of biosorbent (g)
K_F	Frendlich biosorption capacity factor
n_F	Frendlich intensity parameter
Q_L	Langmuir maximum biosorption capacity (mg/g)
K_L	Langmuir affinity constant (l/mg)
Q^0	BET biosorption capacity (mg/g)
B	BET constant
C_s	Saturation concentration of the solute (mg/l)
t	Contact time (min)
k_{s1}	Rate constant of first order sorption (l/min)
TS	Total solids (mg/l)
VS	Volatile solids (mg/l)
SVI	Sludge volume index (ml/g)
SV	Settling velocity (m/h)
Q	Biosorption capacity at time t in Eq. 3-8 (mg/g)
K_1	Overall biosorption rate in Eq. 3-8
Q_e	Equilibrium biosorption capacity in Eq. 3-8 and 4-8 (mg/g)
Q_{th}^e	Maximum biosorption capacity in Eq. 4-8 (mg/g)
K_{ads}	Constant of Eq. 4-8
n	Constant of Eq. 4-8
ΔG^0	Free energy change (kJ/mol)
ΔH^0	Enthalpy change (kJ/mol)

Nomenclature

ΔS^0	Entropy change (kJ/mol)
FTIR	Fourier transfer infrared
XPS	X-ray photoelectron spectroscopy
ECP	Extracellular polymer
PS	Polysaccharides
PN	Protein
XRD	X-ray powder diffraction
ICP	Inductively Coupled Plasma Emission
SEM	Scanning electron microscope

PUBLICATIONS

I: Papers related to the Ph.D study

Liu Y., **Xu H.**, Yang S.F. and Tay J.H. (2003) A general model for biosorption of Cd^{2+} , Cu^{2+} and Zn^{2+} by aerobic granules. *Journal of Biotechnology* 102, 233-239.

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Liu Y., **Xu H.**, Yang S.F. and Tay J.-H. (2004). A theoretical model for biosorption of cadmium, zinc and copper by aerobic granules based on initial conditions. *Journal of Chemical Technology and Biotechnology*, 79 (9), 982-986.

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Chapter 1

Introduction

1.1 Background

Heavy metals are often present in a wide variety of industrial wastewater. They are usually non-biodegradable and accumulative in the environment, and affect human health when they enter into the food chain. So far, stringent limits on metal concentration have been established due to the relatively high toxicity of heavy metals to environmental receptors. The toxicology of metals is concerned with a large number of elements and their compounds that range from comparatively simple ionic salts to complicated structures, such as complexes consisting of a metal atom and a set of ligands as well as organometallic compounds (Friberg et al., 1979).

The conventional methods for heavy metals removal from aqueous solution include precipitation with lime or other chemicals, chemical oxidation and reduction, coagulation, ion-exchange, filtration, electrodialysis, membrane and solvent extraction (Rosato et al., 1984; Shal and Devi, 1998; Yu and Admassu, 2000; Qdais and Moussa, 2004; Mohammadi et al., 2005). These conventional treatment technologies, like precipitation and coagulation, would become less effective and more expensive especially when dealing with large volume of matter which contains heavy metal ions in low concentration range of 1-100 mg/l (Kapoor and Viraraghavan, 1995; Dabrowski et al., 2004). Disadvantages of these processes also include consumption of considerable amounts of reagents for neutralization of acid and precipitation of metal, as well as generation of large amount valueless liquid waste (Zielinski et al., 1998). As for membrane process, its high costs, process

complexity and low removal efficiency have limited their use in heavy metals removal (Kapoor and Viraraghavan, 1995). Recently, adsorption of heavy metals at low concentration using activated carbon was reported (Kadirvelu et al., 2001; Mohan and Singh, 2002). Despite the versatility of activated carbon as adsorbent is obvious in water treatment, it might be very costly.

Biosorption is a process that uses inexpensive viable and nonviable biomass to sequester toxic heavy metal ions as well as their metallic compounds, and is particularly useful for the removal of contaminants from industrial effluents (Kratochvil and Volesky, 1998). Compared with conventional processes for removing toxic metals from industrial effluents, the biosorption process offers the advantages of low operating cost, minimization of the volume of chemical and/or biological sludge to be disposed of, high efficiency in detoxifying very dilute effluents, and no nutrient requirements (Kratochvil and Volesky, 1998). So far, the research for new technologies involving the removal of toxic metals from wastewaters has focused on biosorption, based on the metal binding capacities of various biological materials. Under this circumstance, a vast array of biomaterials had been tested as biosorbents for heavy metal removal, such as marine algae, fungi, wasted activated sludge, digested sludge and so on (Lodi et al., 1998; Zhou, 1999; Valdman and Leite, 2000; Taniguchi et al., 2000).

Most biosorbents currently used are suspended microorganisms in the form of bioflocs. One of the major operation problems associated with the suspended flocs is post-separation of biosorbent from the treated effluent. Contacting large volume of metal aqueous solutions with microbial biomass in conventional unit processing operation is not practical largely due to solid/liquid separation problem (Veglio and Beolchini, 1997). To overcome this drawback, cell immobilization technique has been deployed (Stoll and Duncan, 1997; Yan and Viraraghavan, 2001), but the

deployment of immobilization procedure is very expensive and complex. It should be realized that the disadvantages of conventional biosorbents in the form of bioflocs have seriously limited the application of biological process for the removal of metals from wastewater.

Aerobic granulation is an innovative technology developed recently (Beun et al., 1999; Tay et al., 2001a,b,c). Aerobic granules are microbial aggregates with strong and compact microbial structure, high porosity, and excellent settleability (Tay et al., 2001b). When selecting appropriate biosorbents for the removal of heavy metals from industrial wastewater, three criteria have to be seriously taken into account, i.e. effectiveness, robustness and reliability of biosorbents. It appears that the characteristics of aerobic granules may satisfy these requirements for biosorbents. To date, little information is available on biosorption of heavy metals by aerobic granules. Therefore, this study looked into the heavy metal biosorption using aerobic granules as a novel biosorbent.

1.2 Objectives and Scope

The objective of this study is to investigate the feasibility of aerobic granules as a novel type of biosorbents for the removal of heavy metals from wastewater. For this purpose, Cd^{2+} , Cu^{2+} and Ni^{2+} were used as model heavy metals. The scope of this research is

- ✧ To derive a kinetic model to describe the metal biosorption by aerobic granules and further investigate the kinetic behaviours of metal biosorption by aerobic granules.

- ✧ To develop a biosorption isotherm model from a thermodynamic approach.
- ✧ To investigate the effects of pH and temperature on the metal biosorption by aerobic granules.
- ✧ To study the mechanisms involved in the metal biosorption by aerobic granules.

1.3 Organization of the Thesis

This thesis consists of seven chapters. The organization of the thesis is described as follows.

- Chapter 1 was a brief introduction to the research background and the main research objectives.
- Chapter 2 presented a literature review on the heavy metal biosorption. The review covered various types of biosorbents, biosorption kinetics, equilibrium isotherms, mechanisms involved in the biosorption, operation factors affecting the biosorption performance as well as the characteristics of aerobic granules.
- Chapter 3 looked into the biosorption kinetics of metal biosorption by aerobic granules. It was shown that the proposed kinetic model could predict the heavy metals uptake very well. In this chapter, the effects of initial metal and biomass concentrations on the biosorption kinetics were also discussed.
- Chapter 4 explored the thermodynamic equilibrium of heavy metal biosorption by aerobic granules. A process thermodynamics-based isotherm model was developed and further applied to the metal biosorption by aerobic granules.

Chapter 1 Introduction

- Chapter 5 studied the effects of pH and temperature on the metal biosorption by aerobic granules.
- Chapter 6 showed the mechanisms involved in the metal biosorption by aerobic granules. It was found that ion exchange would be a main mechanism responsible for biosorption of metal ions by aerobic granules.
- Chapter 7 concluded the major findings of this study and some recommendations were then proposed for future study.

Chapter 2

Literature Review

2.1 Introduction

The development in industrial activities has intensified heavy metal pollution and many aquatic environments are facing metal concentration that exceeds water quality criteria designed to protect the environment, animals and human beings. Conventional processes for removing heavy metals from aqueous solution include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment and membrane separation. However, the applications of these processes have their limitations due to the low efficiency of metal removal at low metal concentration or high operating cost for large volume dilute wastewater. These disadvantages have created a strong incentive to develop the economic and effective methods for the removal of metals.

Biosorption refers to binding heavy metal from dilute solution to certain type of biological materials, such as algae, bacteria, yeasts and so on. In comparison with the conventional treatments, biosorption offers low cost and high metal removal efficiency for dilute solutions. Most biosorbents currently used are in the forms of suspended microbial biomass, which has low density, poor mechanical strength and little rigidity. The main drawback of the application of suspended biosorbent is the extremely poor solid-liquid separation. The immobilization of microbial biomaterials in solid surface has been employed to overcome the separation problem. However, the cell immobilization is a very expensive and complex process. These in turn limit the wide application of biological processes for heavy metal removal using conventional floc-like biomass.

Aerobic granulation is a novel environmental biotechnology recently developed for wastewater treatment. Comparing to conventional bioflocs, aerobic granules have

strong and compact microbial structure, high porosity and settling velocity (Tay et al., 2001b). Their high settling velocity of granules could overcome the solid-liquid separation problem involved in the floc-like biomaterials biosorption processes. In this chapter, a brief review on heavy metals sources, their toxic effects on environment, heavy metal biosorption is presented. In addition, the physical characteristics of aerobic granules are also discussed.

2.2 Toxicity of Heavy Metals

Generally, metals with specific gravity greater than 5g/cm^3 are defined as heavy metals. The heavy metals-containing wastewater is mainly generated from manufacturing industry, such as batteries, paints, mining processes, electroplating and metal-processing industries (Jang et al., 2001). Moreover, landfill leachate, agricultural runoff, domestic effluents and acid rain also contribute to heavy metals in wastewater (Yan, 2001). The heavy metals commonly found in wastewater include lead, copper, nickel, zinc, cadmium etc. (Bakkaloglu et al., 1998; Figueira et al., 2000).

Heavy metals are toxic to human beings and aquatic lives even at relatively low concentrations. Stringent regulations have been established to protect the health of human beings and aquatic lives. Cadmium can be accumulated in the human body, and it causes serious erythrocyte destruction nausea, salivation, diarrhea and muscular cramps, renal degradation, chronic pulmonary problems and skeletal deformity (Mohan and Singh, 2002). The toxicity of cadmium is best known for its association with itai-itai disease (Wase and Forster, 1997). Copper is a trace element for human beings and other livings. However, large and acute dose is harmful even fatal. Copper can cause damage to a variety of aquatic fauna including fish and invertebrates (Wase and Forster, 1997). Nickel is similar to copper. Although nickel does not have serious effects on human beings, it has an appreciable phytotoxicity. Hence, its concentration in sludge to be applied to agricultural land has to be restricted. On the other hand, nickel can also be injurious to some fish species, particularly to those in soft water.

2.3 Physico-Chemical Methods for Metal Removal

A variety of physico-chemical treatment technologies have been developed to remove heavy metals from water and wastewater. The conventional techniques applied for the removal of metal ions from industrial effluents include chemical precipitation, solvent extraction, oxidation, reduction, dialysis/electrodialysis, electrolytic extraction, reverse osmosis, ion-exchange, evaporation, dilution, adsorption, filtration, flotation, air stripping, steam stripping, flocculation, sedimentation and soil flushing/washing chelation etc (Mohan and Singh, 2002). For example, ferrous sulfide, lime and sodium carbonate are generally used in chemical precipitation. However, such treatment processes result in a large amount of metal-bearing sludge and show high efficiency in metal removal in high metal concentration solution. For low metal concentration wastewater in the range of 1-100 mg/l, the conventional methods may become inefficient or expensive (Say et al., 2001). Although activated carbon has a wide range of applications in organics removal, few studies had been investigated in heavy metals adsorption by activated carbon due to the high treatment cost (Mohan and Singh, 2002). Recently, studies have been turned to search for low cost but effective techniques for the removal of heavy metals from wastewater. Extensive research effort has been dedicated to the development of metal biosorption technology (Yang and Volesky, 1999; Khoo and Ting, 2001; Aksu, 2002; Gin et al., 2002). It is expected that biosorption would offer a potential alternative to existing technique for removal of heavy metals from industrial wastewater.

2.4 Biosorption of Heavy Metals

Biosorption can be defined as the removal of metals or metalloid species from solution using biological materials (Gadd, 1990). Biosorption includes adsorption reaction, ion exchange reactions with functional groups on the cell surface, and surface complexation reactions. It was found that carboxylic, hydroxylic, phosphate, and sulphonate groups of the lipids, proteins and polysaccharides

localized at the cell surface could provide binding sites for metal ions (Brown and Lester, 1982; Scott and Palmer, 1988).

2.4.1 Biosorbents

Biosorbents for metals removal can be living and dead biomass including algae, bacteria, yeast, fungi, aerobic and anaerobic sludges (Sağ and Kutsal, 2000; Taniguchi et al., 2000; Aksu and Gülen, 2002; Kim et al., 2002; Tang et al., 2002; Padmavathy et al., 2003).

2.4.1.1 Algae

The term algae refer to a large and diverse assemblage of organisms that contain chlorophyll and carry out oxygenic photosynthesis (Davis et al., 2003). A number of studies had been conducted to investigate heavy metals biosorption by algae (Aksu and Kutsal, 1998; Ahuja et al., 1999; Figueira et al., 2000; Aksu, 2002; Gin et al., 2003; Cruz et al., 2004; Raize et al., 2004; Sheng et al., 2004).

Aksu (2002) reported that the maximum Ni^{2+} uptake capacity 60.2 mg/g of green algae *Chlorella vulgaris* was obtained at 45°C and initial pH 4.5. The study by Gin (et al., 2003) found that both the removal efficiency of cadmium, copper, lead, zinc and their specific biosorption capacities by algae were considered to functions of the ratio of algal biomass concentration to the initial metal concentration for selected conditions, i.e. as at constant pH and temperature. In the study of Zn^{2+} adsorption by *Oscillatoria angustissima*, Ahuja et al. (1999) found that *Oscillatoria angustissima* could efficiently uptake Zn^{2+} from aqueous solution with a high uptake capacity 641 mg Zn^{2+} /g dry biomass. Sheng et al. (2004) reported that the metals affinity to different species of algae was not in the same order. The general affinity sequence for *Padina* sp. was $\text{Pb} > \text{Cu} > \text{Cd} > \text{Zn} > \text{Ni}$, while that for *Sargassum* sp. was $\text{Pb} > \text{Zn} > \text{Cd} > \text{Cu} > \text{Ni}$.

2.4.1.2 Fungus

Fungi could be used for the removal of metals from wastewater because of their great tolerance towards heavy metals and other adverse conditions, such as low pH, their high cell wall binding capacity and high intracellular metal uptake capacity (Gadd, 1987). Both living and dead fungal cells have capacity to uptake metal ions (Brady and Tobin, 1994). Many studies have been carried out to study the potential of using fungal biomass in the removal of heavy metals from aqueous solution or wastewater (Kapoor and Viraraghavan, 1997; Aksu and Gülen, 2002; Arica et al., 2004). In these studies, iron (III), iron(III)-cyanide complex (ferric-cyanide), lead, cadmium, copper, mercury and zinc could be removed by fungus biomaterials like *Rhizopus arrhizus*, *Aspergillus niger* and *Funalia trogii* immobilized in Ca-alginate gel beads. The reported metal biosorption capacities of the heat inactivated immobilized *F. trogii* for Hg^{2+} , Cd^{2+} and Zn^{2+} were 403.2, 191.6, and 54.0 mg/g, respectively, while Hg^{2+} , Cd^{2+} and Zn^{2+} biosorption capacities of the immobilized live form were 333.0, 164.8 and 42.1 mg/g, respectively (Arica et al., 2004).

2.4.1.3 Bacteria

The application of pure culture bacteria (Taniguchi et al., 2000; Esposito et al., 2001; Kim et al., 2002) and bacterial aggregate like activated sludge, digested sludge and biopolymers (Bux et al., 1999; Aksu et al., 2002; Arican et al., 2002; Sağ et al., 2003) has been recently incorporated into the concept of biosorption. These bacteria-based biosorbents have been used to remove a wide variety of heavy metals from solution, including zinc, copper, chromium, cadmium, nickel and so on. It was found that *Sphaerotilus natans* had the specific metal uptake of 1.81 meq/g (57.5 mg/g) for copper and 0.78 meq/g (43.8 mg/g) for cadmium at pH 6 (Kim et al., 2002). Sağ (et al., 2003) reported that the Cu^{2+} biosorption capacity of growing, resting and non-viable activated sludge at equilibrium reached 1.30, 1.87 and 2.24 mmol/g (82.6, 114.3 and 142.2 mg/g) dry cell while the initial Cu^{2+} concentration was 3.93, 4.60 and 4.94 mmol/l (249.5, 292.1 and 313.7 mg/l), respectively. The researchers reported that dried activated sludge achieved a higher adsorption

capacity for Cr^{6+} (294.1 mg/g) and Ni^{2+} (238.1 mg/g) at single-component situation at pH value of 1.0 and 4.5, respectively (Aksu et al., 2002).

2.4.1.4 Yeast

Yeasts have been used as the biosorbent because they are inexpensive and easily available sources of biomass (Vasudevan et al., 2003). Yeasts were reported to successfully remove copper, cadmium, lead and nickel from aqueous solutions (Marques et al., 1999; Padmavathy et al., 2003; Vasudevan et al., 2003). In the study of biosorption of Ni^{2+} by deactivated protonated yeast (Padmavathy et al., 2003), it was reported the Ni^{2+} biosorption capacity by yeast increased from 2.23 to 9.31 mg/g when the initial concentrations of Ni^{2+} varied from 10 to 200 mg/l at constant initial yeast concentration. While the initial yeast concentration increased from 0.5 to 8 g/l, the specific uptake capacity of Ni^{2+} reduced from 10.48 to 1.90 mg/g at the initial of Ni^{2+} concentration of 100 mg/l. Vasudevan et al. (2003) reported the highest Cd^{2+} adsorption capacity of Baker's yeast was 86.3 mg/g at a pH of 6.5.

2.4.1.5 Anaerobic Granules

Anaerobic granules are microbial aggregates with a strong, compact and porous structure and excellent settleability, and had been used as biosorbents to remove lead, cadmium, copper and nickel from aqueous solution (Hullebusch et al., 2005; Hawari and Mulligan, 2006a, b). The authors reported that the anaerobic granules' specific biosorption capacities for Pb^{2+} , Cd^{2+} , Cu^{2+} and Ni^{2+} were 255, 60, 55 and 26 mg/g, respectively (Hawari and Mulligan, 2006a).

2.4.1.6 Other Biosorbents

Chitin is a complex molecule, which is a natural polymer of acetylated and non-acetylated glucosamine. Chitin can be found in crustaceans, such as crabs, lobsters and shrimp. It can also be found in insects, worms, fungus and mushrooms.

Benguella and Benasissa (2002) reported that cadmium could be removed from aqueous solutions by chitin, while Annadurai et al. (2002) used banana and orange peels to adsorb copper, cobalt, nickel, zinc and lead ions from water. The adsorption capacities of banana peel for lead, nickel, zinc, copper and cobalt were 7.97, 6.88, 5.80, 4.75 and 2.55 mg metal/g biomass, respectively (Annadurai et al., 2002). In the case of orange peel, the adsorption capacities for lead, nickel, zinc, copper and cobalt were 7.75, 6.01, 5.25, 3.65 and 1.82 metal/g biomass, respectively (Annadurai et al., 2002).

2.4.2 Biosorption Isotherms

A biosorption isotherm represents the relationship between the amount of material adsorbed and the composition of the bulk phase under equilibrium conditions at constant temperature (Metcalf and Eddy, 2003). To describe and analyze biosorption equilibrium, a number of biosorption isotherm models have been developed, such as the Langmuir, Freundlich and Brunauer, Emmett and Teller isotherms (BET) and so on.

2.4.2.1 Freundlich Isotherm

Freundlich isotherm is an empirical model as shown in Eq. 2-1 and it is generally applicable to a heterogeneous surface energy distribution.

$$q_e = K_F (C_e)^{n_F} \quad (2-1)$$

where q_e is biosorption capacity at equilibrium (mg adsorbate/g adsorbent); K_F is the Freundlich capacity factor; C_e is the equilibrium concentration of adsorbate in solution after adsorption (mg/l); n_F is the Freundlich intensity constant.

2.4.2.2 Langmuir Isotherm

The Langmuir biosorption isotherm is defined as:

$$q_e = \frac{Q_L K_L C_e}{1 + K_L C_e} \quad (2-2)$$

where Q_L is the maximum biosorption capacity (mg/g); K_L is an affinity constant.

The Langmuir biosorption isotherm was developed with the following assumptions:

1. There is a finite number of binding sites which are homogeneously distributed over the adsorbent
2. These binding sites have the same affinity for adsorption of a single molecular layer
3. There is no interaction between adsorbed molecules
4. Adsorption is reversible

When the rate of adsorption of molecules onto the surface is the same as the rate of desorption of molecules from the surface, the equilibrium is reached. The rate at which adsorption proceeds is proportional to the driving force, which is the difference between the amount adsorbed at a particular concentration and the amount that can be adsorbed at that concentration. At the equilibrium concentration, this difference is zero. (Metcalf and Eddy, 2003)

2.4.2.3 BET Isotherm

Brunauer, Emmett and Teller developed an equation by applying Langmuir's idea to multimolecular adsorption, which is known as the BET equation (Eq. 2-3).

$$q_e = \frac{BC_e Q^0}{(C_s - C_e)[1 + (B - 1)C_e / C_s]} \quad (2-3)$$

where Q^0 is the amount of solute adsorbed per unit weight of adsorbent in forming a complete monolayer on the surface (mg/g); B is the constant expressive of the energy of interaction with the surface; C_s is the saturation concentration of the solute (mg/l).

The BET model assumes that a number of layers of adsorbate molecules form at the surface. A further assumption of the BET model is that a given layer need not complete formation prior to initiation of subsequent layers; the equilibrium condition will therefore involve several types of surfaces in the sense of number of layers of molecules on each surface site. For adsorption from solution with the additional assumption that layers beyond the first have equal energies of adsorption.

2.4.3 Biosorption Kinetics

The kinetic of biosorption is to establish the time course of heavy metal uptake on the aerobic granules. Some models such as pseudo-first order equation, pseudo-second order equation and ion-exchange based equation are suggested to analyze the kinetics of biosorption process. However, these models were derived and used in the literature without any assumption or specialization of process conditions.

2.4.3.1 Pseudo-First Order Model

The pseudo-first order is expressed as in the following form (Lagergren, 1898):

$$\frac{dq_t}{dt} = k_{s1}(q_e - q_t) \quad (2-4)$$

where k_{s1} is the rate constant of first order adsorption (l/min); q_e is the biosorption capacity at equilibrium (mg/g); q_t is the biosorption capacity at any time t (mg/g).

Integrating Eq. 2-4 gives:

$$\log(q_e - q_t) = \log(q_e) - \frac{k_{s1}}{2.303} t \quad (2-5)$$

2.4.3.2 Pseudo-Second Order Model

Ho and Makay (1998) assumed that the sorption process of lead on sphagnum moss peat might be pseudo-second order and the rate-limiting step might be chemical sorption or chemisorption involving valence forces through sharing or the exchange of electrons between sorbent and sorbate as covalent forces, i.e.

$$\frac{dq_t}{dt} = k_{s2}(q_e - q_t)^2 \quad (2-6)$$

where k_{s2} is the rate constant of second order sorption (g/mg min); q_e is the biosorption capacity at equilibrium (mg/g); q_t is the biosorption capacity at any time t (mg/g).

After integrating at the boundary conditions $t=0$ to $t=t$ and $q_t = 0$ to $q_t = q_t$, Eq. 2-6 can be expressed as:

$$\frac{1}{(q_e - q_t)} = \frac{1}{q_e} + k_{s2}t \quad (2-7)$$

It should be pointed out that both the pseudo-first and second order models are empirical with no any theoretical basis.

2.4.3.3 Ion Exchange Model

Boyd et al. (1947) developed a rate equation, which was based on the exchange sorption of ions from aqueous solution by organic zeolites. For the case of two monovalent ions, the mass action law applied to the exchange reaction can be expressed as follows:

where A and B are free metal ion, and BR and AR represent metal ion-adsorbent complex.

If m_{A^+} and m_{B^+} denote the concentrations of the ions A^+ and B^+ in solution, and n_{AR} and n_{BR} are the moles of A^+ and B^+ in the adsorbent, then the reaction rate can be written as

$$\begin{aligned}\frac{dn_{AR}}{dt} &= k_{E1}m_{A^+}n_{BR} - k_{E2}m_{B^+}n_{AR} \\ &= -n_{AR}(k_{E1}m_{A^+} + k_{E2}m_{B^+}) + k_{E1}m_{A^+}E\end{aligned}\quad (2-9)$$

where k_{E1} and k_{E2} are the forward and reverse rate constants and E is a constant defined by

$$E = n_{AR} + n_{BR} \quad (2-10)$$

When the concentrations of A^+ and B^+ in solution are constant, integration of Eq. 2-10 gives

$$n_{AR} = \frac{k_{E1}m_{A^+}E}{k_{E1}m_{A^+} + k_{E2}m_{B^+}}(1 - e^{-St}) = q_t \quad (2-11)$$

where q_t is the adsorption capacity at time t , $S = k_{E1}m_{A^+} + k_{E2}m_{B^+}$, and Eq. 2-11 can be rearranged to

$$q_e - q_t = q_e e^{-St} \quad (2-12)$$

where q_e is the biosorption capacity at equilibrium (mg/g).

2.5 Factors Affecting Metal Biosorption

The biosorption of metals can be affected by a number of factors, e.g. solution pH, temperature, cation and anion concentrations of external media, metal speciation, biomass concentration and cell size and morphology etc.

2.5.1 pH

The biosorption of metals is usually pH dependent (Harris and Ramelow, 1990; Matheickal et al., 1999; Kaewsarn, 2002; Tang et al., 2003). Generally, metals

biosorption capacity increases with the increase of pH value in a certain pH range which varied with studied metal ions and biomaterials (Matheickal et al., 1999; Nuhoglu and Oguz, 2003). At a lower pH value, sorption of cationic lead should be less due to the higher dissociation of lead, as well as the positively charged tree fern surface (Ho, 2005). The pH dependence of metal uptake could be largely attributed to surface functional groups of cell wall of biomass and also on the metal chemistry in solution (Mohan and Singh, 2002).

2.5.2 Initial Metal Concentration and Biosorbent Concentration

The specific biosorption capacity of the biomass was found to increase with the increase of initial metal concentration (Say et al., 2001; Aksu and Isoglu, 2005; Tewari et al., 2005). However, the increase of metal uptake became slower at higher metal concentrations and equilibrium uptake showed a saturation trend beyond a certain metal concentration due to a finite number of surface binding sites (Salehizadeh and Shojaosadati, 2003; Aksu and Isoglu, 2005). In the case of lower concentrations, the ratio of initial number of metal ions to the available sorption is low and subsequently the fractional biosorption becomes independent of initial concentration (Aksu and Isoglu, 2005). On the other hand, at higher concentrations, the available sites of biosorption become fewer and subsequently the removal of metals depends on the initial concentration (Aksu and Isoglu, 2005).

2.5.3 Temperature

In a biosorption process, temperature may affect (i) the stability of metal ion species initially placed in solution; (ii) the stability of the microorganism-metal complex which depends on the biosorption sites; (iii) the effect of temperature on the microorganism cell wall configuration; and (iv) the ionization of chemical moieties on the cell wall. An increase in temperature will be followed a decrease in sorption capacity when the sorption process is governed only by physical phenomena. The decrease of biosorption capacity at higher temperature might due to the damage of active binding sites in the biomass (Ozer and Ozer, 2003). Temperature could

influence the desorption step and consequently the reversibility of the sorption equilibrium. Generally, an increase in temperature would result in an increase in the diffusivity of the ion, and consequently an increase in the sorption rate if diffusion is the rate controlling step. The diffusivity of metal ions through biomaterials could not be affected by temperature under some experimental conditions.

2.6 Possible Biosorption Mechanisms

The complexity of the microorganism structure implies that there are many ways for the metal to be captured by the cell. Biosorption mechanisms are therefore various and in some cases they are not very well understood. Biosorption mechanism might be classified according to metabolism dependent and no-metabolism dependent, while biosorption may also be classified as extracellular accumulation/precipitation, cell surface sorption/precipitation and intracellular accumulation according to the location where the removed metal is found. Fig. 2.1 schematically shows the various biosorption mechanisms.

The intracellular accumulation or metabolic processes may result in the accumulation of relatively large amounts of metals, but these processes are slow and mostly dependent on nutrient and environmental conditions (Brierley et al., 1985). Surface and wall binding is a passive process and takes place with both living and dead biomass. This non-metabolic surface binding is very rapid, and this kind of metal uptake occurs by ion exchange process involving specific chemical sites on the cell wall.

Figure 2.1 Possible biosorption mechanisms. (a) According to the dependence on the cell's metabolism; (b) according to the location where the removed metal is found. (Veglio and Beilchini, 1997)

Main Functional Groups

In general, the mechanism of algae biosorption is based on a number of metal-binding processes taking place with components of the algal cell wall (Volesky, 1990; Wase and Forster, 1997). Algal cell walls can be made up with polysaccharides: mannan, xylan, alginic acid, chitin, etc. These components, along with the proteins present, can provide acid binding sites, such as amino, amine, hydroxyl, phosphate and sulphate groups (Crist et al., 1981). Figure 2.2 illustrates functional groups for binding provided by the proteins in the cell wall (Crist et al., 1981). Greene and Darnall (1990) reported that ionic charge and bonding were involved in the metal biosorption process and van der Waals' forces at the cellulose network of the cell wall was not involved in the biosorption mechanism. It is thought that the proteins and polysaccharides are the major components responsible for the biosorption. Covalent bonding could be expected with amino and carboxyl groups and ionic charge bonding with carboxyl and sulphate groups associated with these components. The role of carboxylic groups in the adsorption process has been clearly demonstrated by a reduction in cadmium and lead uptake by dried *Sargassum* biomass following partial or complete esterification of the carboxylic sites (Fourest and Volesky, 1996). Raize et al. (2004) reported that the main chemical groups involved in the metallic cation like Cd^{2+} , Ni^{2+} and Pb^{2+} biosorption were apparently carboxyl, amino, sulphhydryl and sulphonate, and these groups were part of the algal cell wall structural polymers, namely, polysaccharide, protein and peptidoglycans.

Figure 2.2 Protein functional groups involved in biosorption of metals by algae (Crist et al., 1981)

Ion Exchange

Ion exchange would be one of the mechanisms involved in the removal of heavy metal by biomass because it can explain many the observations made during heavy metal uptake experiments. The multi-elemental analysis of solution after biosorption revealed the release of some light metal ions (Na^+ , Mg^{2+} , Ca^{2+} , K^+), which were not present in the solution before the process (Chojnachka et al., 2005). This may confirm that ion exchange occurred during the biosorption process. The simultaneous release of Ca ions with the uptake of lanthanides indicated an ion exchange mechanism was involved in biosorption of La by *Sargassum* biomass (Diniz and Volesky, 2005). Biosorption of lead and cadmium on raw biomass accompanied with Ca^{2+} , Mg^{2+} and K^+ release into the reaction solution, suggesting that biosorption was similar to metal removal by ion-exchange resins (Kapoor and Viraraghavan, 1997). The release of light metals (K^+ , Na^+ , Ca^{2+} and Mg^{2+}) is available during the biosorption because untreated biomass generally contains these metals (Davis et al., 2003).

Complexation

The metal removal from solution may also take place through complex formation on the cell surface after interaction between the metal and active groups. Yee and Fein (2001) reported the Cd^{2+} adsorption onto bacterial surface would be attributed to Cd-phosphato surface complexes along with Cd-carboxyl complexation. While metal carboxyl groups complexation plays a more important role in the sorption of zinc than in the sorption copper and nickel (Chubar et al., 2004).

Precipitation

Precipitation could occur in both cellular metabolism dependent and independent processes (Veglio and Beochini, 1997). Micro-precipitation is the deposition of electrically neutral material at the surface of the biomass and does not necessarily involve a bond between the biomass and the deposited layer. Micro-precipitation

may, however, be facilitated by initial binding of metal ions to reactive sites of the biomass, which serve as nucleation sites for further precipitation (Mayers and Beveridge, 1989). And this process is not limited to a mono-layer (or saturation of sites). Schneider et al. (2001) reported that heavy metals could be adsorbed onto the dead biomass of many macrophytes through two mechanisms: specific ion exchange and simple surface precipitation, although it is not possible to differentiate between them based solely on sorption data. For cellular metabolism independent precipitation, it might be a consequence of the chemical interaction between the metal and the cell surface. For the cellular metabolism dependent precipitation, the active defense system microorganism would react in the presence of a toxic metal, producing compounds which favour the precipitation (Veglio and Beochini, 1997).

Physical Adsorption

Physical adsorption occurs as a result of van der Waals forces, and the adsorbed molecule is not affixed to specific site (Arıcan et al., 2002). Kuyucak and Volesky (1988) hypothesized that uranium, cadmium, zinc, copper and cobalt biosorption by dead biomass of algae, fungi and yeasts, would take place through electrostatic interactions between ion in solution and cell walls. The heats of Cr(VI) and Pb(II) adsorption by *Z. ramigera* were found to be of the same order of magnitude as the heat of physical adsorption, and equilibrium between the cell surface and the metal ions was usually rapidly attained and easily reversible (Sag and Kutsal, 2000).

2.7 Characteristics of Aerobic Granules

Aerobic granulation technology is a novel biotechnology developed for wastewater treatment (Beun et al., 1999; Tay et al., 2001a; Lin et al., 2003; Qin et al., 2004b; Yang et al., 2004). Mature aerobic granules have excellent physical characteristics as compared with fluffy, irregular and loose-structured activated sludge flocs, such as

- Round and regular shape with a clear and smooth outer surface;
- Dense and compact microbial structure;

- Large enough to be visible as separate entities in the mixed liquor during mixing and settling phase;
- Excellent settleability to ensure a fast and easy liquid-solid separation.
- High porosity and large surface area;

Morphology and Size

Microscopic examination showed that the morphology of aerobic granules was completely different from the floc-like sludge. The shape of the granules was nearly spherical with a very clear outline (Tay et al., 2001a). In general, the mean size of aerobic granules would vary in the range of 0.2 to 5 mm and the roundness in terms of aspect ratio is above 0.6.

Settleability

Aerobic granules have excellent settleability compared to conventional bioflocs, which would determine the solid-liquid separation efficiency. Both sludge volume index (SVI) and settling velocity are used to characterize the sludge settleability. The SVI of aerobic granules was found to be below 50 ml/g, which is much smaller than that of conventional bioflocs (Toh et al., 2003). The settling velocity of aerobic granules fell in the range of 30–80.8 m/h (Tay et al., 2001b; Toh et al., 2003), which is comparable with that of anaerobic granules in UASB, and is several times higher than that of activated sludge flocs with a typical settling velocity of around 5 to 10 m h⁻¹ (Shin et al., 1992; Etterer and Wilderer, 2001; Tay et al., 2002b; Toh et al., 2003).

Density and Strength

The average density of aerobic granule ranged from 1.004 to 1.065 g/ml (Etterer and Wilderer, 2001; Tay et al., 2001b; Tsuneda et al., 2003), which were significantly denser than activated sludge flocs. The granule strength can be expressed as an integrity coefficient, which is the percentage ratio of residual

granules to the total weight of the granular sludge after 5 min of shaking at 200 rpm on a platform shaker (Toh et al., 2003). The strength of aerobic granules grown on glucose and acetate was higher than 95% (Tay et al., 2002e), which is comparable with that of anaerobic granules. The high-strength aerobic granules would have a strong ability to withstand high abrasion and shear.

Microbial Structure

Confocal laser-scanning microscopy (CLSM) has been employed with different oligonucleotide probes, specific fluorochromes, and fluorescent microspheres to study the microstructure of aerobic granules (Tay et al., 2002d, 2003a; Toh et al., 2003; Jang et al., 2003; Meyer et al., 2003). It was found that aerobic granules contained many channels and pores that penetrated to a depth of 900 μm below the granule surface, and the porosity peaked at depths of 300 to 500 μm from the granule surface (Tay et al., 2002d, 2003a). These channels and pores would facilitate the transport of oxygen and nutrients into and metabolites out of the granules.

2.8 Summary

Heavy metal pollution represents serious environmental problem. The conventional processes for removal of heavy metals from wastewater have their limitations, such as low efficiency of metal removal at low metal concentrations and relatively high operating cost and so on. As an alternative, biosorption would be a potential efficient process for the treatment of high volume and low concentration heavy metal-containing wastewater.

Aerobic granules have distinguished characteristics including excellent settling ability, strong and compact microbial structure, high porosity and large surface area. Unlike most of other biomaterial, immobilization or stiffening is not necessary prior to using aerobic granules. High density and large settling velocities of aerobic granules make them to separate from the aqueous solution in a very short time. The

aerobic granules were also found to possess high mechanical strength. When selecting appropriate biosorbents for the removal of heavy metal from industrial wastewater, three criteria should be seriously taken into account, i.e. effectiveness, robustness and reliability of biosorbents. It appears that the characteristics of aerobic granules could satisfy these requirements for biosorbents. However, little information is currently available on biosorption of heavy metals by aerobic granules.

Chapter 3

Development of a Kinetic Model for Heavy Metal Biosorption by Aerobic Granules

3.1 Introduction

Extensive research has been conducted to develop cost-effective heavy metals removal techniques. Physicochemical methods, such as chemical precipitation, solvent extraction and ion-exchange processes, have been traditionally employed for heavy metal removal from industrial wastewater. However, those techniques are rather expensive and are not selective enough to allow recovery of heavy metals present in the effluent (Valdman et al., 2000). Therefore, research efforts have been directed towards bioremediation of heavy metal pollution. For this purpose, a vast array of biomaterials, e.g. digested sludge, activated sludge, and biomass waste from commercial biotechnological processes had been tested as metal biosorbents (Lodi et al., 1998; Marques et al., 2000; Valdman et al., 2000). Bacterial biomass because of its nature and membrane composition is a natural adsorbent of metals. The bacterial surfaces contain polarizable groups (sites), such as phosphate, carboxyl, hydroxyl and amino-group capable of interaction with cations, may contribute to the reversible metal binding capacity of the biomass.

To date, most biosorbents used are suspended microorganisms in the forms of bioflocs. One of the major problems associated with the suspended flocs is post-separation of biosorbent from the treated effluent. To overcome this drawback, cell immobilization technique has been developed, but the employment of immobilization procedure is expensive and complex. Recent research showed that aerobic granules would have compact, porous structure and excellent settling ability (Tay et al., 2001a,b; 2002c). It seems that aerobic granules would be a good candidate of biosorbent for metals removal from wastewater, but none, within the scope of the literature review, has attempted to study the biosorption characteristics

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of aerobic granules. Therefore, this chapter aimed at developing a kinetic model to describe the biosorption behaviors of soluble heavy metals by aerobic granules.

3.2 Materials and Methods

3.2.1 Cultivation of Aerobic Granules

Aerobic granules were cultivated in a column (80 cm in height and 6 cm in diameter) sequencing batch reactor (SBR) with a working volume of 2.4 l. The SBR was operated sequentially with a cycle time of 4 h including the sequence of 4 min of feeding, 230 min of aeration, 2 min of settling and 4 min of effluent withdrawal. Effluent was discharged from the middle port of the column SBR. In the experiment, the reactor was supplied with an air velocity of 4.0 l/min, which resulted in a superficial upflow air velocity of 2.4 cm/s. Synthetic wastewater used mainly consisted of sodium acetate as sole carbon source, while its detailed composition can be found elsewhere (Tay et al., 2001a). The influent chemical oxygen demand (COD) concentration was fixed at 500 mg/l. The SBR was operated at an organic loading rate of 3.0 kg COD/m³ day.

3.2.2 Preparation of Stock Metal Solution

In this study, cadmium and copper were used as model heavy metals. A stock solution of Cd²⁺ and Cu²⁺ was prepared by dissolving 2.103 and 2.119 grams of cadmium nitrate (Cd(NO₃)₂) and copper chloride (CuCl₂) in one liter de-ionized water, respectively. These gave a Cd²⁺ concentration of 1 g/l and 1 g/l for Cu²⁺.

3.2.3 Batch Biosorption Experiments

Mature aerobic granules harvested from the SBR were gently washed three times by de-ionized water before adding in heavy metal solutions. Biosorption experiments were carried out in a series of one liter beakers with gentle agitation at temperature of 25 °C.

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The experiments were divided into two phases:

- Phase 1: This phase investigated the biosorption kinetics of Cd^{2+} and Cu^{2+} by aerobic granules at various initial metal concentrations (C_0), while the initial aerobic granules concentration (X_0) was kept at 150 mg dry weight/l. The initial Cd^{2+} and Cu^{2+} concentrations varied in the range of 2 to 200 mg/l.
- Phase 2: The 2nd phase was designed to study the biosorption kinetics of Cd^{2+} and Cu^{2+} by aerobic granules at different X_0 in the range of 50 to 300 mg dry weight/l. For this purpose, C_0 of Cd^{2+} was fixed at 150 mg/l and C_0 of Cu^{2+} was kept at 100 mg/l.

In these two phases of study, the initial solution pH was adjusted to 7.0 ± 0.1 by 0.1M HNO_3 or NaOH .

3.2.4 Analytical Methods

Soluble Cd^{2+} and Cu^{2+} concentrations were analyzed by Inductively Coupled Plasma Emission Spectrometry (ICP) (Perkin-Elmer, Plasma 2000, Perkin-Elmer Corporation, Norwalk, USA). Total solids (TS), volatile solids (VS) and sludge volume index (SVI) were measured by standard methods (APHA, 1998). Granule settling velocity was determined by the method described by Liu et al. (2005). The mean size of aerobic granules was determined by image analyzer (Quantimnet 500 Image Analyzer, Leica Cambridge Instruments). In this study, a scanning electron microscope (SEM, Stereoscan 420, Leica Cambridge Instruments) was employed for the observation of granule structure.

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3.3 Results

3.3.1 Characteristics and Microstructure of Aerobic Granules

The physical characteristics of aerobic granules used in the biosorption experiments were summarized in Table 3.1. The ratio of VS to TS of aerobic granules was about 0.68. The low SVI and high settling velocity indicate the excellent settleability of aerobic granules, i.e. aerobic granules can be easily separated from liquid by simple gravity sedimentation. Figures 3.1 and 3.2 show the morphology of aerobic granules with a mean size of 1.0 mm.

Table 3.1 Characteristics of aerobic granules.

Characteristics	Values
VS/TS	0.68±0.02
Mean size	1.01±0.25 mm
Sludge volume index (SVI)	21.8±1.2 ml/g
Settling velocity (SV)	36.4±9.0 m/h

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Figure 3.1 Mature granules used in the biosorption experiments.

It appears from Fig. 3.2 that aerobic granule had a porous, regular and relative compact structure. High porosity and channel linkage can be clearly seen in Fig. 3.2b, which would favour the diffusion of heavy metals into the interior of aerobic granule. Different microbial species can also be found in a single aerobic granule (Fig. 3.2b), i.e. these species might provide different binding sites to heavy metals and exhibit various affinities to heavy metals. Moreover, extracellular polymeric substances (EPS) could be observed in Fig. 3.2b, which contain different types of functional groups that could serve as the metal binding sites.

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(a)

(b)

Figure 3.2 SEM photos of aerobic granules used in the biosorption experiments (a) macrostructure (b) microstructure.

3.3.2 Kinetics of Metal Biosorption by Aerobic Granules

3.3.2.1 Model Development

Figures 3.3 and 3.4 show the biosorption profiles of Cd^{2+} and Cu^{2+} at different initial Cd^{2+} and Cu^{2+} concentrations. The cadmium and copper adsorbed on aerobic granules gradually increased as a function of contact time until a stable value at each initial metal concentration. As shown in Appendix, no Cd and Cu precipitation would form in the studied pH range. It has been assumed that functional groups or biopolymers of cells would contribute to the binding of metallic cations and heavy metal biosorption could be characterized as a physicochemical process (Guibaud et al., 1999; Jeon et al., 2001; Pethkar et al., 2001) in a way such that

where S is the available site for metal binding on aerobic granules, M is the free metal ion, and SM represents metal ion-granule complex, while k_1 and k_2 are the rate constants for the biosorption and desorption processes, respectively.

Figure 3.3 Biosorption profiles of Cd^{2+} at different initial Cd^{2+} concentrations. $X_0=150 \text{ mg/l}$ and $\text{pH}=7.0$. The model prediction is shown by solid curve.

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Figure 3.4 Biosorption profiles of Cu^{2+} at different initial Cu^{2+} concentrations. $X_0 = 150 \text{ mg/l}$ and initial pH value = 7.0. The model prediction is shown by solid curve. It appears from Figs. 3.3 and 3.4 that the Cd^{2+} and Cu^{2+} adsorption by aerobic granules follow a first-order reversible kinetics. Hence, the overall biosorption rate can be written as:

$$-\frac{dC}{dt} = k_1 C - k_2 C_b \quad (3-2)$$

where C is the concentration of the soluble metal ion at time t , C_b is the apparent concentration of the bind metal ions at time t , i.e., the metal ions adsorbed by aerobic granule per unit volume of the solution. A mass balance on metal ions gives

$$C_b = C_0 - C \quad (3-3)$$

where C_0 is initial concentration of metal ions. When the biosorption process reaches its equilibrium, Eq. 3-3 becomes

$$C_{be} = C_0 - C_e \quad (3-4)$$

and Eq. 3-2 reduces to

$$\frac{C_{be}}{C_e} = \frac{k_1}{k_2} \quad (3-5)$$

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where C_{be} and C_e is the apparent concentration of the bind metal ions and concentration of free metal ions at biosorption equilibrium, respectively.

By combining Eqs. 3-2 to 3-5, the following expression is obtained:

$$-\frac{dC}{dt} = (k_1 + k_2)(C - C_e) \quad (3-6)$$

Integration of Eq. 3-6 results in

$$\frac{C_0 - C_e}{C - C_e} = e^{K_1 t} \quad (3-7)$$

where $K_1 = k_1 + k_2$ is termed the overall biosorption rate of the metal to aerobic granule. Substituting Eqs. 3-3 and 3-4 into Eq. 3-7 yields

$$C_b = C_{be} \frac{e^{K_1 t} - 1}{e^{K_1 t}} \quad (3-8)$$

Dividing Eq. 3-8 by aerobic granule concentration yields a general model that describes the metal biosorption on the surfaces of aerobic granules:

$$Q = Q_e (1 - e^{-K_1 t}) \quad (3-9)$$

where Q is the amount of metal biosorbed on aerobic granule in terms of mg metal ions per g granules at time t , and Q_e is the biosorption capacity at the equilibrium.

3.3.2.2 Kinetics of Cd^{2+} Biosorption

Figures 3.3 and 3.5 show the Cd^{2+} biosorption by aerobic granules at different C_0 and X_0 , respectively. It is obvious that Eq. 3-9 can provide a satisfactory description for the cadmium biosorption data obtained at various initial cadmium and aerobic granules concentrations, indicated by a coefficient of correlation greater than 0.91. It was found that about 50% of the cadmium was removed in the first 1 hour of the test, and the biosorption equilibrium was gradually achieved in 3 hours. At the constant initial granules concentration, increased Cd^{2+} concentration led to an increase in the Cd^{2+} uptake capacity from 40.7 mg Cd^{2+} /g granules at 10 mg/l Cd^{2+} to 193.0 mg Cd^{2+} /g granules at 100 mg/l Cd^{2+} . These seem to imply that the initial

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Cd^{2+} concentration would have a significant effect on its biosorption by aerobic granules, i.e., biosorption process would be slow at low initial metal concentrations. These are in agreement with that reported by Scott and Karanijkar (1992) showing that when the cadmium concentration increased from 25 to 500 mg/l, the corresponding time required to reach the equilibrium of biosorption by *Enterobacter aerogens* was shortened accordingly.

Figure 3.5 shows the biosorption profiles of cadmium at different initial aerobic granules concentrations (X_0), while the initial cadmium concentration was fixed at 150 mg/l. The cadmium biosorption could reach equilibrium within 4 hours at all X_0 studied, however the cadmium uptake capacity by aerobic granules tend to decrease with the increase of the initial aerobic granules concentration.

Figure 3.5 Biosorption profiles of Cd^{2+} at different X_0 . $C_0 = 150$ mg/l and initial pH=7.0. The model prediction is shown by solid curve.

3.3.2.3 Kinetics of Cu^{2+} Biosorption

Figure 3.4 shows the profiles of copper biosorption at different initial copper concentrations and a fixed granules concentration of 150 mg/l, while Fig. 3.6

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displays the biosorption of copper at different initial granules concentrations and a constant initial copper concentration of 100 mg/l. It can be seen that the predictions by Eq. 3-9 are in good agreement with the experimental data, indicated by a correlation coefficient greater than 0.93 for both cases.

Figure 3.4 and 3.6 show that the patterns of copper biosorption by aerobic granules are similar to those of cadmium biosorption at various initial metal and aerobic granules concentrations. At the constant initial aerobic granules concentration, the increase in the Cu^{2+} concentration led to an increase in the Cu^{2+} biosorption capacity from 13.4 mg Cu^{2+} /g granules at 2.0 mg/l Cu^{2+} to 60.1 mg Cu^{2+} /g granules at 100.0 mg/l Cu^{2+} . It seems that the Cu^{2+} biosorption capacity by aerobic granules is related to initial Cu^{2+} concentration, while at a fixed initial Cu^{2+} concentration, an increase of initial aerobic granules concentration would result in a decline in the Cu^{2+} uptake capacity by aerobic granules.

Compared with the cadmium biosorption by aerobic granules, the copper removal by aerobic granules is a fast process, e.g. about 50% of the copper at equilibrium was removed in the first half hour of contact, and the equilibrium was attained after two hours of contact. Moreover, Fig. 3.4 reveals that the uptake of copper at higher initial copper concentrations was faster than that observed at the lower initial copper concentrations, e.g. at an initial copper concentration of 2 mg/l, the time required to achieve the biosorption equilibrium was about two hours, however the copper biosorption can reach the equilibrium within one hour at an initial copper concentration of 100 mg/l.

Figure 3.6 Biosorption profiles of Cu^{2+} at different X_0 . $C_0 = 100 \text{ mg/l}$ and initial pH value = 7.0. The model prediction is shown by solid curve.

3.3.3 Effect of Initial Metal Concentration (C_0) on Cd^{2+} and Cu^{2+} Biosorption

This section looked into the effect of initial metal concentration (C_0) on the biosorption capacity and the rate of biosorption determined from Eq. 3-9.

3.3.3.1 Effect of C_0 on Biosorption Capacity at Equilibrium

Figures 3.7 and 3.8 exhibit the relationships between biosorption capacity (Q_e) at equilibrium and C_0 for Cd^{2+} and Cu^{2+} at the fixed X_0 , respectively. Q_e was found to be positively related to C_0 , i.e. a relatively high C_0 would enhance the biosorption capacities. Puranik et al. (1999) also reported that the amounts of zinc and lead adsorbed onto *S. cinnamomeum*, *P. chrysogenum* and *Citrobacter sp.* were increased with the increase of the initial metal concentration.

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Figure 3.7 Effect of initial Cd^{2+} concentration on Q_e .

Figure 3.8 Effect of initial Cu^{2+} concentration on Q_e .

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3.3.3.2 Effect of C_0 on Overall Biosorption Rate Constant

The relationships between the overall biosorption rate constant (K_1) and C_0 were shown in Figs. 3.9 and 3.10 for Cd^{2+} and Cu^{2+} , respectively. These results suggest that the increased C_0 would favour the biosorption of metal ions, i.e. a faster metal biosorption would be expected at higher initial metal concentration. For Cd^{2+} , the K_1 estimated at C_0 of 100 mg/l was 0.032 min^{-1} , while the K_1 at C_0 of 10 mg/l was 0.021 min^{-1} . For Cu^{2+} biosorption at fixed X_0 of 150 mg/l, K_1 increased from 0.032 to 0.092 min^{-1} when C_0 was increased from 2 to 100 mg/l. The K_1 value of Cu^{2+} is higher than that of Cd^{2+} at the same C_0 , i.e. the Cu^{2+} biosorption would be a faster process than the biosorption of Cd^{2+} .

Figure 3.9 Effect of initial Cd^{2+} concentration on K_1 .

Figure 3.10 Effect of initial Cu^{2+} concentration on K_1 .

3.3.4 Effect of Initial Granule Concentration on Cd^{2+} and Cu^{2+} Biosorption

3.3.4.1 Effect of X_0 on Biosorption Capacity at Equilibrium

The effect of X_0 on the biosorption capacity (Q_e) of Cd^{2+} and Cu^{2+} at equilibrium was shown in Figs. 3.11 and 3.12, respectively. It appears that an increased X_0 would result in a decreased Q_e for both kinds of metal ions.

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Figure 3.11 Effect of X_0 on Q_e of Cd^{2+} .

Figure 3.12 Effect of X_0 on Q_e of Cu^{2+} .

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3.3.4.2 Effect of X_0 on Overall Biosorption Rate Constant

Figure 3.13 showed the relationship between the overall biosorption rate constant (K_1) of Cd^{2+} and initial aerobic granules concentration. The K_1 of Cd^{2+} decreased with the increase of X_0 , e.g. K_1 decreased from 0.01 min^{-1} at X_0 of 50 mg/l to 0.0083 min^{-1} at X_0 of 300 mg/l. A Similar phenomenon was also observed in the Cu^{2+} biosorption by aerobic granules (Fig. 3.14).

Figure 3.13 Effect of X_0 on K_1 for Cd^{2+} .

Figure 3.14 Effect of X_0 on K_1 for Cu^{2+} .

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3.4 Discussion

As shown in Figs. 3.3 to 3.6, the proposed model can predict the Cd^{2+} and Cu^{2+} biosorption data very well at various initial cadmium, copper and granule concentrations. The positive correlation of Q_e and K_1 to the respective Cd^{2+} and Cu^{2+} concentration may imply that the concentration gradient-driven mass transfer would play an important role in the overall Cd^{2+} and Cu^{2+} biosorption by aerobic granules. Microscopic observation clearly shows that the aerobic granules had high porosity and channel structure, i.e. aerobic granules may have a similar internal structure to granular activated carbon. Thus, the Cd^{2+} and Cu^{2+} biosorption by aerobic granules would be determined by external and internal mass transfer efficiency. Obviously, the net rate of transport of Cd^{2+} and Cu^{2+} should be proportional to its concentration in bulk solution. As a result, both Q_e and K_1 tend to increase with the increase of initial Cd^{2+} and Cu^{2+} concentrations (Figs. 3.9 and 3.10). The proportional relationship between Q_e and C_0 (Figs. 3.7 and 3.8) indicates that the cadmium and copper biosorption by aerobic granule could be driven by the concentration gradient of metal at a constant granule concentration.

It is a reasonable consideration that the number of binding sites to Cd^{2+} and Cu^{2+} carried by aerobic granules is proportional to the amount of aerobic granules added, i.e. high granule concentration could result in a lower relative Cd^{2+} and Cu^{2+} concentration on the basis of unit mass of aerobic granules (Figs. 3.11 and 3.12). This in turn provides a plausible explanation for the observed decline of the biosorption rate constant at high granule concentrations (Figs. 3.13 and 3.14). In fact, similar phenomenon has also been reported in metal biosorption study with marine algae as biosorbents (Aksu and Gülen, 2002).

As discussed above, both the initial metal (C_0) and granule concentrations (X_0) would influence the biosorption capacity of metal at equilibrium (Q_e). It might be expected that the metal biosorption capacity of aerobic granules could be described by combined effect of C_0 and X_0 . It is a reasonable consideration that at a given initial metal concentration, a high biomass concentration could lower real metal

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concentration on the basis of unit biomass. To interpret the combined effect of X_0 and C_0 on the biosorption of metal ions by aerobic granules, a concept of relative metal concentration was proposed and defined as the ratio of initial metal concentration to initial granules concentration, namely C_0/X_0 . The relationship between Q_e and C_0/X_0 ratio obtained at various C_0 or X_0 values for cadmium and copper are presented in Figs. 3.15 and 3.16, respectively. It can be seen that Q_e increases with the increase of C_0/X_0 ratio. These results indicate that the individual effect of C_0 and X_0 on the metal biosorption capacity of aerobic granules can be unified by the C_0/X_0 ratio for biosorption batch experiments initiated at different C_0 and X_0 . An important implication of Figs. 3.15 and 3.16 is that if C_0 or X_0 is not strictly controlled in batch experiments, the C_0/X_0 ratio could better reflect the real driving force for the metal biosorption by microorganisms, and this concept provides a unified basis for interpretation of the biosorption data obtained at different initial metal and biomass concentrations.

Figure 3.15 Profile of relationship between Q_e and C_0/X_0 ratio obtained in the Cd^{2+} biosorption experiments.

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Figure 3.16 Profile of relationship between Q_e and C_0/X_0 ratio obtained in the Cu^{2+} biosorption experiments.

Biosorbents currently used are in the form of microbial flocs or dispersed bacteria. One serious technical problem-associated with bioflocs is post-separation of biosorbents from the treated effluent. As compared with conventional bioflocs, aerobic granules have the advantages of high microbial density and excellent settling ability. In fact, the settling velocity of the aerobic granules used in this study was about 36 m/h, which was much higher than that of bioflocs with a typical value of less than 5-10 m/h (Campos et al., 1999). Such a unique feature of aerobic granules can ensure a fast and good separation of used aerobic granules from the treated effluent in 1 min after the biosorption tests. It is expected that the aerobic granules-based biosorption process is an efficient and cost effective technology for the treatment of industrial wastewater containing cadmium, copper or other kinds of heavy metals.

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3.5 Conclusion

A kinetic model based on the first-order reversible reaction was developed and verified using the data obtained from the Cd^{2+} and Cu^{2+} biosorption experiments by aerobic granules. The proposed model can provide a satisfactory prediction for the data obtained at different initial metal and granules concentrations. The respective maximum uptake capacity of cadmium, copper by aerobic granules was found to be 573.3 mg Cd^{2+} /g granules and 69.3 mg Cu^{2+} /g granules. The contact time required to achieve the equilibrium of the Cd^{2+} and Cu^{2+} biosorption was 4 and 2 h, respectively.

The heavy metal biosorption capacity at equilibrium and the overall biosorption rate constant calculated from the proposed model were closely related to the initial metal and aerobic granules concentrations. At a fixed initial aerobic granules concentration, the biosorption capacity and overall rate constant of cadmium and copper tended to increase with the increase of initial cadmium and copper concentrations. While the initial cadmium and copper concentration keep as a constant, the respective uptake capacity of cadmium and copper by aerobic granules decreased with the increase of initial aerobic granules concentration. It was further found that the metal biosorption capacity of aerobic granules increased with an increase of the relative metal concentration--- C_0/X_0 ratio.

This study showed that aerobic granules can remove cadmium and copper ions from aqueous solution rapidly and efficiently, and the used aerobic granules could be easily separated from the treated effluent because of their excellent settleability. It appears that aerobic granules could be used as an innovative and efficient biosorbent for the removal of soluble heavy metals from industrial wastewater.

Chapter 4

Development of a General Isotherm Model for Heavy Metal Biosorption by Aerobic Granules

4.1 Introduction

The Langmuir and Freundlich sorption isotherms are two of the most widely used models to describe adsorption. The Freundlich model was derived empirically, while the Langmuir adsorption isotherm was developed from rational considerations. To date, the Langmuir and Freundlich equations have been considered as two independent equilibrium models. There is evidence that not all biosorption data show the clear maximum of the Langmuir isotherm, while the Freundlich isotherm can accommodate adsorption data only over a range of adsorbate concentrations (Morel and Hering, 1993; Metcalf and Eddy, 2003). In addition, other adsorption isotherm models except for the Langmuir and Freundlich models, such as Sips, Tóth, Radke-Prausnitz and Fritz-Schluender models, have been used to fit experimental data (LeVan et al., 1997). These models are derivatives of either Langmuir or Freundlich, and they are empirical or semi-empirical. Therefore, this study attempted to derive a general biosorption isotherm model for metal biosorption by aerobic granules through a thermodynamic approach.

4.2 Materials and Methods

Aerobic granules used were taken from the same SBR as described in Chapter 3.

4.2.1 Preparation of Stock Metal Solution

In this study, individual cadmium and copper were used as the model heavy metals in biosorption tests. The preparation of Cd^{2+} and Cu^{2+} solution was specified in Chapter 3.

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4.2.2 Batch Biosorption Experiments

The Cd^{2+} and Cu^{2+} batch biosorption experiments were carried out as described in Chapter 3. In these experiments, temperature was kept at 25°C and initial pH was adjusted to 7.0 ± 0.1 by 0.1 M of HNO_3 or NaOH solution.

4.2.3 Analytical Methods

Soluble metal concentrations were analyzed by Inductively Coupled Plasma Emission Spectrometry (ICP) (Perkin-Elmer, Plasma 2000, Perkin-Elmer Corporation, Norwalk, USA).

4.3 Results

4.3.1 Model Development

It has been proposed that the overall biosorption reaction could be regarded as a simple change in the state of metal ion (Morel and Hering, 1993), i.e.,

where C and C_{ads} is respective metal concentration in bulk solution, and that adsorbed at time t , while $\Delta G^{0'}$ is change of the effective free energy of biosorption, which may vary with the biosorption reaction. If the metal concentration (C) in bulk solution increases, its adsorption is more favorable, i.e. $\Delta G^{0'}$ would decrease with the increase of the metal concentration (Metcalf and Eddy, 2003). According to Morel and Hering (1993), $\Delta G^{0'}$ can be expressed as

$$\Delta G^{0'} = \Delta G^{\circ} - nRT \ln C \quad (4-2)$$

where n is positive coefficient, R is the gas constant with a value of 8.314 J/mol K, T is the temperature (K) and ΔG° is the standard free energy change. Evidence shows that the real driving force of biosorption is the difference between the amount adsorbed by unit biosorbent (Q) at a given metal concentration and the

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theoretical amount that could be adsorbed by unit biosorbent at that concentration (Q_{th}), and this driving force is disappearing when the biosorption reaction gradually approaches its equilibrium state (Metcalf and Eddy, 2003). As biosorption proceeds, the driving force decreases and the adsorption resistance will increase. It is a reasonable consideration that the overall change of free energy of the biosorption process (ΔG) would increase with the increase of adsorption resistance, and decrease with the increase of the driving force of adsorption reaction. We assume that the overall change of free energy of the biosorption reaction should be formulated as the function of the driving force and resistance of adsorption such that

$$\Delta G = \Delta G^{o'} + RT \ln \frac{\text{Resistance}}{\text{Driving force}} \quad (4-3)$$

In a theoretical sense, Eq. 4-3 is indeed consistent with the expression for free energy change of an ideal gas and solution, while this equation is also similar to the Maxwell/Boltzmann distribution law (Morel and Hering, 1993). As pointed out earlier, the adsorption reaction becomes less favorable as the adsorption proceeds, i.e. ΔG must increase accordingly. These seem to imply that Q would reflect the magnitude of adsorption resistance. On the other hand, the difference between Q_{th} and Q represents the actual driving force of the biosorption process, a larger difference leads to a smaller value of ΔG . Therefore, Eq. 4-3 can be written as follows:

$$\Delta G = \Delta G^{o'} + RT \ln \frac{Q}{Q_{th} - Q} \quad (4-4)$$

Equation 4-4 shows that when $Q=0.5Q_{th}$, $\Delta G^{o'}$ is equal to ΔG . This in turn implies that $\Delta G^{o'}$ can be defined as the overall free energy change at $Q=0.5Q_{th}$, i.e. the driving force of biosorption is equal to the resistance force. As Q approaches Q_{th} , ΔG goes to infinity and further adsorption becomes energetically impossible, this is indeed in agreement with that stated by Morel and Hering (1993). Substitution of Eq. 4-2 into Eq. 4-4 yields

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$$\Delta G = \Delta G^\circ - nRT \ln C + RT \ln \frac{Q}{Q_{th} - Q} \quad (4-5)$$

When the adsorption reaches its equilibrium, ΔG is zero. Hence,

$$0 = \Delta G^\circ - nRT \ln C_e + RT \ln \frac{Q_e}{Q_{th}^e - Q_e} \quad (4-6)$$

in which C_e , Q_e and Q_{th}^e are the respective value of C , Q and Q_{th} at equilibrium. Solving Eq. 4-6 for Q_e gives

$$Q_e = Q_{th}^e \frac{C_e^n}{\exp\left(\frac{\Delta G^\circ}{RT}\right) + C_e^n} \quad (4-7)$$

Equation 4-7 can be rearranged as

$$Q_e = Q_{th}^e \frac{C_e^n}{K_{ads} + C_e^n} \quad (4-8)$$

in which

$$K_{ads} = \exp\left(\frac{\Delta G^\circ}{RT}\right) \quad (4-9)$$

Analogue to a chemical reaction, the thermodynamic equilibrium constant of adsorption reaction (K_{eq}) can be defined as

$$K_{eq} = \exp\left(-\frac{\Delta G^\circ}{RT}\right) \quad (4-10)$$

Comparison of Eq. 4-9 and Eq. 4-10 shows that

$$K_{ads} = \frac{1}{K_{eq}} \quad (4-11)$$

Equation 4-11 reveals the real physical meaning of K_{ads} . In this study, a simple least square-based computer program was developed to estimate the constants involved in Eq. 4-8.

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4.3.2 Model Verification

The biosorption isotherms of Cd^{2+} and Cu^{2+} at a constant granule concentration of 150 mg/l are shown in Figs. 4.1 and 4.2. It can be seen that the proposed model (Eq. 4-8) can provide a satisfactory description for the experimental data obtained from Cd^{2+} and Cu^{2+} biosorption, indicated by high value of correlation coefficient (R) which was 0.935 and 0.989 for Cd^{2+} and Cu^{2+} , respectively. For the Cd^{2+} biosorption on aerobic granules, the value of n estimated is 0.71, while 0.51 for Cu^{2+} biosorption. These n values seem to suggest that the Langmuir model could be suitable for the Cd^{2+} biosorption, but may not fit to the Cu^{2+} biosorption data. The respective K_{ads} value obtained is 3.47×10^{-3} for Cd^{2+} biosorption and 2.21×10^{-2} for Cu^{2+} biosorption by aerobic granules.

To further verify the proposed model, literature data were used. Figure 4.3 shows that Eq. 4-8 can satisfactorily predict the experimental data obtained in biosorption experiments with various metal species, and different kinds of biosorbents. The estimated constants are summarized in Table 4.1. In fact, many other literature data also support Eq. 4-8 even though they are not shown here (Ruiz-Manriquez et al., 1998; Zhou, 1999; Aksu, 2001; Wang et al., 2001).

Figure 4.1 Biosorption isotherm of Cd^{2+} by aerobic granules. Equation 4-8 prediction is shown by solid curve. $n=0.71$ and $K_{\text{ads}}=3.47 \times 10^{-3}$.

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Figure 4.2 Biosorption isotherm of Cu^{2+} by aerobic granules. Equation 4-8 prediction is shown by solid curve. $n=0.51$ and $K_{\text{ads}}=2.21 \times 10^{-3}$.

Table 4.1 Equilibrium and thermodynamic parameters estimated for literature data.

Metal	n	K_{ads}	Q_{th}^e (mg/g)	R	Reference
Ni	1.51	1.60×10^{-6}	31.88	0.975	Selatnia et al. (2004)
Pb	1.31	9.64×10^{-7}	136.45	0.997	Pagnanelli et al. (2003)
Cr	0.702	1.84×10^{-2}	180.55	0.996	Loukidou et al. (2004)
U	0.95	1.50×10^{-4}	296.47	0.994	Yang and Volesky (1999)

R=correlation coefficient

Figure 4.3 Biosorption isotherms for literature data. a: Ni^{2+} on *Sorepoomyees rimosus* biomass (Selatnia et al., 2004); b: Pb^{2+} on *Sphaero tilas natans* (Pagnanelli et al., 2003); c: Cr^{6+} on *Aeromonas caviae* (Loukidou et al., 2004); d: U on *Sargassum* biomass (Yang and Volesky, 1999).

Equation 4-8 prediction is shown by solid curve.

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4.4 Discussion

Equation 4-11 shows that the value of $1/K_{ads}$ is related to the magnitude of the thermodynamic equilibrium constant, K_{eq} . When the value of K_{ads} is small, i.e. the thermodynamic equilibrium constant, K_{eq} is large. In this case, the biosorption reaction would proceed far towards completion, and the position of equilibrium lies far toward the biosorption of metal. On the other hand, when the value of K_{ads} is very large, i.e. K_{eq} is very small, thus the position of equilibrium lies far towards the soluble metal. It is most likely that the value of K_{ads} is determined by the natures of both biosorbent and the physicochemical characteristics of heavy metal. Consequently, the magnitude of K_{ads} value may represent the equilibrium position of a biosorption process.

The Langmuir and Freundlich sorption isotherms have been commonly used to describe the equilibrium behavior of biosorbents. When n equals 1, Eq. 4-8 is reduced to the following form, which is the same as the well known Langmuir adsorption isotherm:

$$Q_e = Q_{th}^e \frac{C_e}{K_{ads} + C_e} \quad (4-12)$$

Equation 4-12 shows that the Langmuir adsorption isotherm would be a particular case of Eq. 4-8 when n has a value of 1. When the value of C_e^n is much less than the value of K_{ads} , Eq. 4-8 is simplified to the Freundlich adsorption isotherm:

$$Q_e = \frac{Q_{th}^e}{K_{ads}} C_e^n \quad (4-13)$$

Equation 4-13 reveals that the Freundlich constant indeed equals Q_{th}^e/K_{ads} . It appears from Eq. 4-12 and 4-13 that Eq. 4-8 can be regarded as a generalized form of the Langmuir and Freundlich models. Meanwhile, Eq. 4-8 can also be arranged to Eq. 4-14 which is similar to the Sips or so-called Hill model (Roels, 1983; LeVan et al., 1998):

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$$Q_e = Q_{th}^e \frac{C_e^n}{\left(\frac{1}{k_{eq}}\right)^n + C_e^n} \tag{4-14}$$

in which k_{eq} is intrinsic equilibrium constant. As Roels (1983) noted, the Hill model is a purely empirical equation. Equation 4-14 seems to provide a theoretical interpretation for the empirical Hill model.

The Gibbs free energy change (ΔG^0) indicates the degree of spontaneity of the biosorption process and the higher negative value reflects a more energetically favourable biosorption. In the sense of reaction thermodynamics, change in free energy (ΔG^0) of biosorption can be calculated in a way such that

$$\Delta G^0 = RT \ln K_{ads} \tag{4-15}$$

in which T is temperature in Kelvin and R is the gas constant with a value of 8.314 J/mol K. Table 4.2 summarized the estimated constants involved in Eq. 4-8 and free energy change (ΔG^0) calculated from Eq. 4-15 for the Cd^{2+} and Cu^{2+} biosorption by aerobic granules. It was found that the ΔG^0 of Cd^{2+} and Cu^{2+} biosorption was -13.93 and -9.49 kJ/mol, respectively. The negative values of ΔG^0 show that the Cd^{2+} and Cu^{2+} biosorption processes would be spontaneous. The larger values of ΔG^0 for Cd^{2+} may imply that the degree of spontaneity of Cd^{2+} biosorption is higher than that of the Cu^{2+} biosorption. This is in agreement with the observation that the maximum biosorption capacity (Q_{th}^e) of Cd^{2+} by aerobic granules is higher than that of Cu^{2+} .

Table 4.2 Equilibrium and thermodynamic parameters estimated for Cd^{2+} and Cu^{2+} biosorption by aerobic granules.

Metal	n	K_{ads}	Q_{th}^e (mg/g)	ΔG^0 (kJ/mol)	R
Cd	0.705	3.47×10^{-3}	234.6	-13.93	0.935
Cu	0.508	2.21×10^{-2}	115.8	-9.49	0.989

R=correlation coefficient.

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The biosorption capacity of aerobic granules at equilibrium was 234.6 mg/g for Cd^{2+} and 115.8 mg/g for Cu^{2+} . Aksu (2001) reported that the biosorption capacity of Cd^{2+} on *C. vulgaris* was 85.3 mg/g, while the biosorption capacity of Cu^{2+} by *Thiobacillus ferrooxidans* was found to be 40 mg/g (Ruiz-Manriquez et al., 1998). These seem to indicate that the biosorption capacities of cadmium and copper by aerobic granules are comparable with those conventional suspended biosorbents in the forms of microbial flocs or dispersed bacterial species.

4.5 Conclusion

A general model (Eq. 4-8) for the biosorption of heavy metals was derived, and was verified by the experimental data obtained from the biosorption tests of Cd^{2+} and Cu^{2+} by aerobic granules. The proposed model can reduce to the well-known Langmuir, Freundlich and Hill models in some special cases. The ΔG^0 values of Cd^{2+} and Cu^{2+} biosorption by aerobic granules were found to be negative, i.e. these processes would be spontaneous.

Chapter 5

pH- and Temperature-Effect on Ni²⁺ Biosorption by Aerobic Granules

5.1 Introduction

As shown in Chapters 3 and 4, aerobic granules would be an effective biosorbent for the removal of heavy metal ions from aqueous solution. However, the effects of various key operating parameters on the metal biosorption by aerobic granules have not yet touched in previous chapters. Nickel is usually present in the wastewater discharged from mine drainage, table ware plating, metal finishing and forging industries. Moreover, nickel and its related compounds are carcinogenic and may constitute danger to human being and other lives (Patterson, 1985; Volesky, 1990). Thus, in this chapter nickel was used as a model heavy metal and key factors affecting the nickel biosorption by aerobic granules were investigated.

5.2 Materials and Methods

5.2.1 Batch Biosorption Experiments

The cultivation of aerobic granules was as described in Chapter 3. The stock Ni²⁺ solution (1g/l) was obtained by dissolving 2.208 g of nickel chloride (NiCl₂) in one liter of de-ionized water. The Ni²⁺ biosorption experiments at different initial pH values and temperatures are described below.

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Phase 1: This phase investigated the effect of initial pH ranging from 2 to 7 on the Ni^{2+} biosorption isotherm by aerobic granules at a temperature of 25°C, while the initial granules concentration was kept at 1 g/l.

Phase 2: In the 2nd phase, the effect of temperature (25, 35, 45 and 55°C) on the Ni^{2+} biosorption isotherm was studied when the initial granules concentration was fixed at 1.5 g/l. Initial pH was adjusted to 6.0 ± 0.1 by 0.1 M of HNO_3 or NaOH solution.

In the above two phases of study, the initial of Ni^{2+} concentration was controlled in the range of 2 to 150 mg/l.

5.2.2 Analytical Methods

Two kinds of aerobic granules were used in EDX analysis, i.e. fresh aerobic granule without Ni^{2+} biosorption and the Ni^{2+} -contaminated aerobic granule that were taken from the biosorption experiment conducted at an initial Ni^{2+} concentration of 150 mg/l, initial biomass concentration of 1 g/l and initial pH 6.0. The intact aerobic granule and its microscopic slices for SEM analysis were prepared according to the procedure used by Jiang et al. (2004). Aerobic granule slides with depth of 50 μm were cut by cryo-microtome (CM3050S; Leica). The prepared samples were freeze dried overnight at -20°C before SEM analyses. A scanning electron microscope (SEM) model JSM-6360 (JEOL, Japan) equipped with Energy Dispersive X-ray (EDX) elemental composition analyzer (model JED-2300) was used to analyze both the microscopic structure and composites of intact aerobic granule and aerobic granule slides. The upper limit of the detected elements depended on the maximum acceleration voltage (30 kV) for the SEM model JSM-6360. The accelerating voltage used during the investigation in this study was 15 kV while the spot size used was 41. Total scanning time during elemental map generation was

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10 min. The composites were sputter coated with aurum to prevent charging when analyzed by the electron beam. The SEM/EDX system was controlled by JED Series Analysis Program. The X-ray peaks generated during the scanning were used to record the elements in the composites, and then elemental maps were generated.

Soluble Ni^{2+} , K^+ , Ca^{2+} and Mg^{2+} concentrations in solution before and after the biosorption experiments were determined by Inductively Coupled Plasma Emission Spectrometry (ICP) (Perkin-Elmer, Plasma 2000, Perkin-Elmer Corporation, Norwalk, USA). Zeta potential of aerobic granules was measured with Zetasizer (Malvern Model 3000).

5.3 Results

5.3.1 Effect of Initial pH on Ni^{2+} Biosorption

So far, little information is available for the pH-effect on the Ni^{2+} biosorption by aerobic granules. In this study, a series of batch biosorption experiments were carried out at different initial pH values of 2 to 7. Equation 4-8 developed in Chapter 4 was used to describe the Ni^{2+} biosorption isotherms at different initial pH values and the results were shown in Fig. 5.1. It can be seen that Eq. 4-8 can satisfactorily fit the Ni^{2+} biosorption data in the pH range studied. Obviously, the Ni^{2+} biosorption was pH-dependent. The equilibrium constants at different initial pH values were calculated by Eq. 4-8.

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Figure 5.1 The Ni^{2+} biosorption isotherms by aerobic granules at various initial pH values. Equation 4-8 prediction is shown by solid curve.

5.3.1.1 Effect of Initial pH on Zeta Potential of Aerobic Granules

Figure 5.2 shows the zeta potentials of aerobic granules determined in the pH range of 2 to 7. At pH 2, the aerobic granules had a positive zeta potential of 11.5 mV, while the zeta potential decreased steeply in the pH range of 2 to 5 and they carried a negative zeta potential of 31.50 mV at pH 5. Beyond pH value of 5, the negative increase of zeta potential became slowly. It can be seen that at a pH value above 3, aerobic granules would be negatively charged.

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Figure 5.2 Zeta potential of aerobic granules at various initial pH.

5.3.1.2 Effect of Initial pH on Ni^{2+} Maximum Biosorption Capacity

Figure 5.3 shows the maximum biosorption capacities (Q_{th}^e) of Ni^{2+} by aerobic granules at different initial pH values. Q_{th}^e was found to be pH-dependent, i.e. the amount of nickel adsorbed by granules tends to increase with the increase of pH until a plateau was reached at a pH value above 6. These seem to indicate that the optimum initial pH value for the Ni^{2+} biosorption by aerobic granules would be around 6. As shown in Appendix, no Ni precipitation would not form in the pH range of 2 to 6.

It is well known that both the cell surface binding sites and the availability of metal ions in solution are pH-related. At low pH, the cell surface binding sites should be protonized, thereby making them unavailable for the other cations. However, with

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an increase in pH, there is an increase in ligands with negative charges which in turn would result in increased binding of cations as shown in Fig. 5.3.

Figure 5.3 Q_{th}^e of Ni^{2+} at various initial pH values.

5.3.1.3 Relationship between Isotherm Constants and Initial pH

Figure 5.4 shows the dependence of K_{ads} and n on the initial pH, i.e. both K_{ads} and n are positively related to the initial solution pH. The values of K_{ads} and n at different pH fell into a range of 0.87×10^{-3} to 2.8×10^{-3} and 0.72 to 0.92, respectively.

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Figure 5.4 K_{ads} and n at various initial pH values

5.3.1.4 Release of Light Metals at Various Initial pH

It appears from Fig. 5.5 that some light metals, such as K^+ , Mg^{2+} and Ca^{2+} , were released from the aerobic granules during the Ni^{2+} biosorption experiments. The respective amount of K^+ and Mg^{2+} ions released tended to increase with the increase of pH from 4 to 6. However, the Ca^{2+} released from aerobic granules was the least at pH of 5. The respective content of K^+ , Ca^{2+} and Mg^{2+} in granules was also determined by ICP before and after the biosorption experiments, and the mass balance showed 99.8% of recovery for Ca^{2+} , 110% for Mg^{2+} and 98.4% for K^+ . Figure 5.5 clearly showed that the amount of light metal ions released from aerobic granules was in the order of $\text{Ca}^{2+} > \text{Mg}^{2+} > \text{K}^+$ at a given initial pH, e.g. at pH 6, the percentage of Ca^{2+} , Mg^{2+} and K^+ released over the amount of the adsorbed Ni^{2+} was 48.4%, 8.3% and 6.2%, respectively.

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Figure 5.5 Effect of pH on release of Ca^{2+} , Mg^{2+} and K^{+} from aerobic granules during the Ni^{2+} biosorption experiment at an initial Ni^{2+} concentration of 50 mg/l.

5.3.2 Temperature-effect on Ni^{2+} Biosorption

Figure 5.6 presented the isotherm profiles of the Ni^{2+} biosorption by aerobic granules at various temperatures. It appears that a high temperature would favour Ni^{2+} biosorption by the aerobic granules. It was also found that the Ni^{2+} biosorption could be predicted by Eq. 4-8 very well at various temperatures in the range of 25 to 55 °C.

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Figure 5.6 Ni^{2+} biosorption isotherms by aerobic granules at various temperatures.
Equation 4-8 prediction is shown by solid curve.

5.3.2.1 Dependence of Ni^{2+} Maximum Biosorption Capacity on Temperature

According to Fig. 5.6, the maximum biosorption capacity (Q_{th}^e), K_{ads} and n were calculated using Eq. 4-8. Figure 5.7 shows that Q_{th}^e was increased with the increase of temperature, i.e. the Ni^{2+} biosorption by aerobic granules would be an endothermic process. The temperature-effect might be due to the fact that an increase in active sites would occur due to bond rupture at high temperatures (Aksu, 2002). This is supported by the increased n value at high temperatures (Fig. 5.8).

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Figure 5.7 Q_{th}^e of Ni^{2+} at various temperatures.

5.3.2.2 Relationship between Temperature and Isotherm Constants

The respective relationship between the isotherm constants (K_{ads} , n) and temperature was shown in Fig. 5.8. It was found that K_{ads} was inversely related to the temperature, while a positive effect of temperature on n was observed. When the temperature was increased from 25 to 55 °C, n increased from 0.65 to 0.92, which mean that more binding sites would be available for the Ni^{2+} biosorption at high temperature.

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Figure 5.8 K_{ads} and n at various temperatures.

5.3.3 Distribution of the Adsorbed Ni^{2+} in Aerobic Granule

The fresh (before biosorption) and Ni^{2+} -contaminated (after biosorption) aerobic granules were analyzed by SEM coupled with EDX, and results were shown in Figs. 5.9 and 5.10. It can be seen that C, O and Ca would be three major elements detected in the fresh as well as in the Ni^{2+} -contaminated aerobic granules. It is logical that no Ni signal was detected in the EDX spectrum of the fresh aerobic granule (Fig. 5.9), i.e. there was no Ni present on the fresh aerobic granule or the amount of Ni was not detectable. After the Ni^{2+} biosorption, Ni signal clearly appeared in Fig. 5.10. This indicates a certain amount of Ni was biosorbed on aerobic granules during the biosorption experiment.

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Figure 5.9 SEM image and EDX spectrum of fresh aerobic granule.

Figure 5.10 SEM image and EDX spectrum of Ni-contaminated aerobic granule.

In order to look into the distribution of the adsorbed Ni^{2+} in aerobic granule, the Ni^{2+} -contaminated granule was sectioned from the surface to the centre of granule by a thickness of 50 μm, and the sectioned granule samples were further analyzed by EDX. In this study, depth-0 μm (at surface), depth-150, depth-300 and depth-450 μm from surface of granule, namely No.1 to No. 4, were defined to represent four slices of one aerobic granule at depth of 0, 150, 300 and 450 μm

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from the surface, respectively. The mean size of aerobic granules used was about 1mm.

Figures 5.11 to 5.14 showed the SEM image, EDX spectra and elemental mapping of the granule slices No. 1 to 4, respectively. It can be seen that an EDX characteristic peak corresponding to nickel was detected in all four samples, i.e. Ni^{2+} could penetrate into aerobic granule through the channel or pore of aerobic granules and biosorbed on interior sites. In comparison with the images of depth-0, depth-150 and depth-300, the Ni element mappings indicated that biosorbed Ni^{2+} almost uniformly distributed over these three slices. For slice of depth-450, the Ni^{2+} distribution was similar to what were observed in other three slices except that more Ni ions were concentrated on a small part where the density of Ni pixel was much higher than that on the other parts of the slice. The above observations seem to show a nearly uniform distribution of Ni^{2+} across aerobic granule.

The atomic mass percentages of C, O, Ca and Ni obtained from the EDX analysis were summarized in Table 5.1. The data indicated that the biosorbed Ni percentage fell into the range of 3 to 3.75 percent, i.e. 30 to 37.5 mg Ni was biosorbed by per gram aerobic granules. This finding is in accordance with the maximum biosorption capacity for Ni experimentally determined (32.35 mg Ni/g dried granules) at initial pH of 6. It was also observed that there was no significant difference between the Ni percentages in the four granule slices, i.e. the biosorbed Ni^{2+} ions would be uniformly distributed along the radii of aerobic granules.

Figure 5.11 SEM image, EDX spectrum and element mapping of depth-0 of aerobic granule.

Figure 5.12 SEM image, EDX spectrum and element mapping of depth-150 of aerobic granule.

Figure 5.13 SEM image, EDX spectrum and element mapping of depth-300 of aerobic granule.

Figure 5.14 SEM image, EDX spectrum and element mapping of depth-450 of aerobic granule.

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Table 5.1 Semi-quantitative elemental analysis of different cross sections for aerobic granule.

Element	Atomic Mass (%)			
	depth-0	depth-150	depth-300	depth-450
C	43.27	45.06	46.78	37.45
O	43.35	45.09	39.36	45.11
Ca	9.76	6.11	10.86	14.05
Ni	3.61	3.75	3.00	3.34

5.4 Discussion

Zeta potential is one of the most useful parameters to characterize the surface charge of biomaterials. Figure 5.2 shows an inverse relationship of the zeta potential of aerobic granule to the solution pH. In fact, previous research also reported that surface charges of activated sludge and biofilms were pH-dependant (Schiewer and Volesky, 1995; Nuhoglu and Oguz, 2003).

The results of Ni²⁺ biosorption indicated that aerobic granules lose biosorption capacity at initial pH value of 2, which might be related to their positive surface charge. At low pH, the cell surface binding sites should be protonized, thereby making them unavailable for the other cations (Zhou, 1999; Nuhoglu and Oguz, 2003; Tang et al. 2003; Selatnia et al., 2004). It can be seen in Fig. 5.3 that pH has a significant effect on the Ni²⁺ biosorption by aerobic granules, i.e. a increasing pH seems to favour the Ni²⁺ biosorption. As the pH increases, more function groups on the cell surface would be exposed, and result in an increase of negative charges with subsequent attraction of metallic ions with positive charge (Fig. 5.2). Therefore, at

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pH 3 to 5, the amount of Ni^{2+} removed by aerobic granules increased sharply due to the steep decrease of the zeta potential of aerobic granules (Fig. 5.2). It should be pointed out that when the zeta potential attained a plateau at pH 6 and 7, there was no significant change in the Ni^{2+} biosorption capacity of aerobic granules (Fig. 5.3). The relationship between zeta potential of aerobic granules and Ni^{2+} biosorption capacity granules seems to indicate that electrostatic attraction might be involved in the mechanism of Ni^{2+} biosorption by aerobic granules. It has been widely reported that metal ion sorption by biomaterial is pH-dependent, e.g. cadmium uptake capacity by *D. potatorum* was low at the pH value less than 2, and a sharp increase of biosorption capacity was occurred from pH 2 to 4, while there was no significant difference on the biosorption capacity at pH above 4 (Matheickal et al., 1999).

Figure 5.5 showed that 2.41 meq of Ca^{2+} and 0.68 meq of Mg^{2+} were released into solution from aerobic granules when 3.51 meq of Ni^{2+} was adsorbed on aerobic granules. It may imply that ion exchange would be one of the possible mechanisms responsible for the Ni^{2+} biosorption by aerobic granules. In fact, rich Ca^{2+} and Mg^{2+} contents in aerobic granules had been reported (Qin et al., 2004a). In their study of biosorption by brown marine macroalgae, Raize et al. (2004) also reported that main nickel binding mechanism is probably ion exchange because its binding process caused a significant decrease in calcium and magnesium concentrations. This and previous research confirms that ion exchange would be a main mechanism involved in the Ni^{2+} biosorption by biomaterials.

As discussed in Chapter 4, the value of $1/K_{\text{ads}}$ is related to the magnitude of the thermodynamic equilibrium constant, K_{eq} . When the value of K_{ads} is small, i.e. the thermodynamic equilibrium constant, K_{eq} is large. In this case, the biosorption process would proceed far towards completion, and the position of equilibrium lies far toward the biosorption of metal. As shown in Fig. 5.8, K_{ads} tended to decrease

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with the increase of temperature. The smaller K_{ads} at higher temperature implies that the Ni^{2+} biosorption by aerobic granules would be more favourable at high temperature (Figs. 5.7 and 5.8). In fact, Dilet et al. (2002) also found that the Ni^{2+} adosorption by *Polyporous versicolor* was favoured by increased in the temperature in the range of 20-35 °C.

As discussed in Chapter 4, free energy change (ΔG^0) could be calculated from the isotherm constant (K_{ads}) using Eq. 4-15. The estimated ΔG^0 at various initial pH values were listed in Table 5.2 for the Ni^{2+} biosorption by aerobic granules at 25 °C. It can be seen that the estimated ΔG^0 values were negative at all studied initial pH values and the lowest ΔG^0 value of -14.5 kJ/mol was observed at the initial pH value of 3.0. Moreover, ΔG^0 was negatively related to the initial pH value. It in turn indicates that the degree of spontaneity of the Ni^{2+} biosorption would increase at increasing initial pH value.

The ΔG^0 values of the Ni^{2+} biosorption by aerobic granules at different temperatures were computed as well at the initial pH 6.0 (Table 5.3). It was found that the ΔG^0 values of the Ni^{2+} biosorption process at all temperatures studied were negative and increased with the increase in temperature. It indicates that the Ni^{2+} biosorption process would be spontaneous in the nature and the spontaneity would be increased with temperature.

Table 5.2 ΔG^0 of the Ni^{2+} biosorption at different initial pH values.

Initial pH value	ΔG^0 (kJ/mol)
3	-14.5
4	-15.0
5	-15.3
6	-17.1
7	-17.5

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Table 5.3 ΔG^0 of the Ni²⁺ biosorption at different temperatures.

Temperature (°C)	ΔG^0 (kJ/mol)
25	-11.6
35	-16.2
45	-17.2
55	-19.7

In the sense of process thermodynamics, the relationship of ΔG^0 to enthalpy change (ΔH^0) and entropy change (ΔS^0) can be expressed as follows:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \tag{5-1}$$

Substituting Eq. 4-15 into Eq. 5-1 gives

$$\ln(K_{\text{ads}}) = \frac{\Delta H^0}{RT} - \frac{\Delta S^0}{R} \tag{5-2}$$

The plot of $\ln(K_{\text{ads}})$ against $1/T$ will theoretically yield a straight line that allows to calculate ΔH^0 and ΔS^0 from respective slope and interception (Fig. 5.15).

Chapter 5 pH- and Temperature-Effect on Ni^{2+} Biosorption By Aerobic Granules Figure 5.15 $\ln(K_{\text{ads}})$ versus $1/T$.

The ΔH^0 estimated from Fig. 5.15 was 63.8 kJ mol^{-1} , and 0.26 kJ mol^{-1} for ΔS^0 . It had been reported that ΔH^0 of adsorption of Direct Red 84 by chitin was about 55.5 kJ/mol (McKay et al., 1982). Basically, the heat evolved during the physical adsorption is of the same order of magnitude as the heat of condensation, i.e., 2.1 to 20.9 kJ/mol (Sag and Kutsal, 2000), while the heats of chemisorption generally fall into a range of 80 to 200 kJ/mol (Hayward and Trapnell, 1964; Smith 1981). Therefore, it seems that the Ni^{2+} biosorption by aerobic granules would be attributed to a physico-chemical adsorption process rather than a pure physical or chemical adsorption process. The positive value of ΔH^0 indicates that the Ni^{2+} biosorption would be an endothermic process, which in turn provides a plausible explanation for the temperature-enhanced Ni^{2+} biosorption as shown in Fig. 5.6. The low value of ΔS^0 may imply that no remarkable change in entropy occurred during the Ni^{2+} biosorption by aerobic granules. In fact, the positive value of ΔS^0 reflects the increased randomness at the solid–solution interface during biosorption.

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The intercorrelations of Q_{th}^e , K_{ads} , n , pH and temperature were analyzed, and the degree of association between each pair of variables was evaluated by the respective correlation coefficient. Table 5.4 listed the correlation coefficients between constants calculated from Eq. 4-8 and initial pH values. The correlation coefficients between constants calculated from Eq. 4-8 and temperatures were presented in Table 5.5. The correlation coefficients between Q_{th}^e -pH, K_{ads} -pH and n -pH were 0.974, 0.998 and 0.973, respectively. While the correlation coefficients between Q_{th}^e -T, K_{ads} -T and n -T were 0.988 -0.839 and 0.948, respectively. It implies that Q_{th}^e , K_{ads} and n would be all closely related to initial pH value and temperature.

Table 5.4 Correlation coefficients between the estimated constants from Eq. 4-8 and pH values.

	pH	n	K_{ads}	Q_{th}^e (mg/g)
pH	1			
n	0.998	1		
K_{ads}	0.973	0.967	1	
Q_{th}^e (mg/g)	0.974	0.977	0.938	1

Table 5.5 Correlation coefficients between the estimated constants from Eq. 4-8 and temperatures.

	T (°C)	n	K_{ads}	Q_{th}^e (mg/g)
T (°C)	1			
n	0.948	1.		
K_{ads}	-0.839	-0.940	1	
Q_{th}^e (mg/g)	0.988	0.960	-0.822	1

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Aerobic granules would have porous structure with water channels (Tay et al, 2002e). The porous structure would favour the diffusion of Ni^{2+} ions into aerobic granule. It has been believed that the metal adsorption would be described as a three-step process, i.e. (i) mass transfer of metal ions from the bulk solution to the particle surface; (ii) intraparticle diffusion; and (iii) adsorption at an interior site. In this study, the presence of Ni^{2+} at interior of aerobic granules indicated that Ni^{2+} could diffuse through whole granule (Figs. 12 to 14). It was further demonstrated that the binding sites of Ni^{2+} were available both outside and inside of aerobic granules and their distribution did not change with depth of aerobic granules (Figs. 11 to 14). More recently, Van Hellebushch et al. (2004) reported that cobalt ion was able to penetrate into the core part of anaerobic granule, and a homogeneous distribution of adsorbed cobalt was observed as well.

5.5 Conclusion

The Ni^{2+} biosorption capacity of aerobic granules tended to increase with an increase in initial Ni^{2+} concentration. The Ni^{2+} biosorption by aerobic granules was pH- and temperature-dependent. The optimal pH for the Ni^{2+} biosorption by aerobic granules was found to be around 6.0 and the highest Ni^{2+} biosorption capacity was obtained at 55°C. The pH and temperature also affected the isotherm equilibrium constants. The estimated n increased with temperature in the range of 25 to 55°C and pH 3-6. However, K_{ads} increased with the increase of initial pH value and decreased with the increase of temperature. It was shown that the electrostatic attraction mechanism could provide a good explanation for the relationship between the zeta potential of aerobic granules and the Ni^{2+} biosorption, while the large quantity of K^+ , Mg^{2+} and Ca^{2+} released during the Ni^{2+} biosorption indicated that an ion-exchange mechanism would be involved in the Ni^{2+} biosorption by aerobic granules. The EDX analysis further revealed that Ni^{2+} ions could penetrate into and

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adsorbed at the interior of aerobic granule, and the adsorbed Ni^{2+} was almost uniformly distributed in the aerobic granule.

Chapter 6

Mechanisms of Cd^{2+} , Cu^{2+} and Ni^{2+} Biosorption by Aerobic Granules

6.1 Introduction

The kinetics and thermodynamics of heavy metal biosorption by aerobic granules were studied in Chapters 3 to 5, while the mechanisms of heavy metal adsorption by aerobic granules still remain unclear. Therefore, this chapter looked into how the model heavy metal ions including cadmium, copper and nickel would be adsorbed by aerobic granules.

6.2 Material and Methods

6.2.1 Preparation of Aerobic Granules

Cultivation of aerobic granules was presented in Chapter 3.

6.2.2 Extraction of Extracellular Polymers

In this study, two kinds of aerobic granules were used as biosorbents, i.e. fresh aerobic granules and extracted aerobic granules. Aerobic granules taken from the parent SBR were gently washed three times using de-ionized water, and were called as fresh aerobic granules. Part of fresh aerobic granules further underwent extraction of extracellular polymers (ECP), mainly consisting of extracellular polysaccharides (PS) and proteins (PN), and this part of granules was referred to as the extracted aerobic granules.

The ECP of aerobic granules were extracted by the cold aqueous extraction method (Zhang et al., 1998a). In this method, a 20-ml biomass sample was centrifuged at

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12,000 rpm for 10 minutes. The harvested biomass was then re-suspended in a 8.5% NaCl solution containing 0.22% formaldehyde. The mixture was chilled in ice and was homogenized for 3 minutes. After then, the supernatant was recovered by high speed centrifugation at 12,000 rpm for 30 minutes for determination of ECP. Polysaccharides (PS) and proteins were analyzed by the method of Dubois et al. (1956) and the method developed by Lowry et al. (1951), respectively. Glucose and bovine serum albumin were used as polysaccharide and protein standards, respectively.

6.2.3 Batch Biosorption Experiments

In this study, Cd^{2+} , Cu^{2+} and Ni^{2+} were used as model metal ions. Stock solutions of cadmium, copper and nickel with a concentration of 1.0 g/l were prepared as described in Chapters 3 and 5. Biosorption experiments were carried out in 300 ml Erlenmeyer bottles, which were agitated on a shaker at 150 rpm for 5 hours. The initial aerobic granules concentration was kept at 1.0 g/l and the initial metal ion concentration was fixed at 100 mg/l. The initial pH was kept at 6.0 ± 0.1 , and all the experiments were conducted at 25°C. The samples were taken during the batch experiments and were then centrifuged at 3200 rpm for 15 minutes. Subsequently, the supernatant was used to determine the concentrations of soluble Cd^{2+} , Cu^{2+} , Ni^{2+} as well as released light metals, such as like Ca^{2+} , Mg^{2+} and K^{+} in solution.

6.2.4 Analytical Methods

Analysis of granule elemental composition

In order to determine the elemental compositions of fresh and heavy metal contaminated aerobic granules, granule samples were dried to a constant weight at 103°C, and were then pulverized according to standard method (APHA, 1998). 1.0 mg of the prepared sample was used to determine the cell C, H, N, S and O contents by CHNS/O analyzer (CHNS/O 2400 II, Perkin-Elmer). Meanwhile, 0.1 g of the prepared sample was digested by nitric acid (APHA, 1998) and multi-elemental

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analysis was performed by an inductively coupled plasma emission spectrometer (Perkin-Elmer, Plasma 2000).

X-ray diffraction analysis (XRD)

Aerobic granules before and after the metal biosorption tests were freeze-dried for overnight, and were subsequently examined by X-ray powder diffraction (Bruker XRD D8 Advance) coupled with a copper X-ray tube. The scans were collected in a range of 2θ from 5 to 90° at a rate of $0.02^\circ/\text{min}$. The original diffractograms were processed for background subtraction as well as for peak identification using the software EVA.

Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectroscopy (Perkin-Elmer) was employed to detect vibration frequency changes in the aerobic granule before and after heavy metal biosorption. The freeze dried granule sample was mixed with KBr in the ratio of 1:100 and compacted to pellet form under high pressure. The IR spectra were collected by a Perkin-Elmer spectrometer within the range $400\text{--}4000\text{ cm}^{-1}$. The background obtained from the scan of pure KBr was automatically subtracted from the sample spectra. All spectra were plotted using the same scale on the absorbance axis.

X-ray photoelectron spectroscopy (XPS)

The XPS measurement for freeze-dried aerobic granules samples before and after metal biosorption tests were performed in the ultrahigh vacuum chamber of a Kratos Ultra XPS system (Kratos Analytical, Surface Analysis Product Group, Manchester, England). The XPS spectra were recorded by using the energy source of monochromatic Al K_α radiation (1486.71 eV) operated at 15 kV and 10 mA . The survey scans were conducted from 0 to 1200 eV with a pass energy of 160 eV . The elements to be analyzed were finally scanned over different energy with the pass

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energy of 40 eV. For calibration, the binding energy of the spectra was standardized with the C 1s peak at 284.2 eV. The XPS spectra peaks were decomposed into subcomponents by fixing the 0% Lorentzian–Gaussian curve-fitting program with a linear background to the spectra through an XPSpeak 4.1 software package.

6.3 Results and Discussion

6.3.1 Elemental Composition of Fresh Aerobic Granules

Table 6.1 summarizes elemental composition of fresh aerobic granules. Results indicated that fresh aerobic granules mainly comprised seven major elements, i.e. C, H, O, N, S, P and Ca. As for heavy metals, element Cd was not detected in the fresh aerobic granules. However, very small amounts of Cu, Ni and Zn at the respective levels of 0.25, 0.027 and 0.239 mg/g were found in the fresh aerobic granules. These are simply due to the fact that Cu, Ni and Zn were added to synthetic wastewater as the trace elements required for microbial growth. The Ca content in aerobic granule was as high as 150.36 mg/g, while 1.37 and 3.47 mg/g for Mg and K respectively. It seems that aerobic granules would contain a significant amount of light metal ions. In fact, Qin et al. (2004b) also reported a high Ca content of 180 mg/g in aerobic granules. XRD was used to analyze the compounds phase presented in the fresh aerobic granules. The main crystal compounds detected in the fresh aerobic granules were ragonite (CaCO_3) and magnesium calcite synthesis ($(\text{Mg}_{0.03}\text{Ca}_{0.97})(\text{CO}_3)$) (Fig. 6.1). Both would contribute to the ash content of the fresh aerobic granules as reported in Table 3.1 as CaCO_3 and $(\text{Mg}_{0.03}\text{Ca}_{0.97})(\text{CO}_3)$ would not be decomposed at 550 °C.

It appears from Table 6.1 that the amount of Ca^{2+} in fresh aerobic granules was 150.36 mg/g. If all the Ca^{2+} was in the form of CaCO_3 and $(\text{Mg}_{0.03}\text{Ca}_{0.97})(\text{CO}_3)$, the amount of CaCO_3 and $(\text{Mg}_{0.03}\text{Ca}_{0.97})(\text{CO}_3)$ could be around 375.6 mg/g, i.e. the calculated amount of CaCO_3 and $(\text{Mg}_{0.03}\text{Ca}_{0.97})(\text{CO}_3)$ would be much larger than the ash content in fresh aerobic granules (Table 6.1). Obviously, such estimate is not reasonable because CaCO_3 and $(\text{Mg}_{0.03}\text{Ca}_{0.97})(\text{CO}_3)$ may only represent part of

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the total granule ash. This in turn implies that Ca^{2+} could also bind to negatively charged functional groups present on bacterial surfaces and extracellular polymeric substances, and act as a bridge to interconnect these components (Jiang et al., 2003). Davis et al. (2003) reported that untreated biomass generally contained the light metal ions, such as K^+ , Na^+ , Ca^{2+} and Mg^{2+} , which were originally bound to the acid functional group of biomass.

Table 6.1 Elemental compositions of different kinds of aerobic granules (mg/g dry weight of granules).

Element	Types of AG			
	Fresh AG	Cd-AG	Cu-AG	Ni-AG
C	315.830	314.730	298.330	320.200
H	44.470	43.100	40.030	44.300
N	49.930	57.100	53.200	56.900
S	4.770	4.370	4.930	4.730
O	421.390	358.740	419.060	404.100
P	4.344	3.920	3.790	4.100
Cd	0.000	84.520	0.000	0.000
Cu	0.250	0.229	44.140	0.263
Ni	0.027	0.005	0.005	21.720
Zn	0.239	0.140	0.162	0.217
Ca	150.360	129.860	134.380	140.280
Mg	1.370	0.780	0.312	0.804
K	3.470	1.365	0.390	1.131
others	3.550	1.141	1.270	1.255

AG = Aerobic Granules

Fre Granules

Figure 6.1 XRD analysis of fresh aerobic granules (before heavy metal biosorption).

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6.3.2 Elemental Composition of Aerobic Granules after Heavy Metal Biosorption

The XPS survey scanning spectra of aerobic granules before and after Cd^{2+} , Cu^{2+} and Ni^{2+} biosorption were showed in Figs. 6.2 to 6.5, respectively. The main elemental peaks detected in the spectrum of fresh aerobic granules are O 1s, Ca 2s, Ca 2p, N 1s and C 1s (Fig. 6.2), while the peaks of Cd, Cu and Ni were not presented in the XPS survey scanning spectrum of fresh aerobic granules. However, the peaks of Cd 3p and Cd $3d_{3/2}$ and Cd $3d_{5/2}$ appeared in the wide scanning spectrum of aerobic granules after Cd^{2+} biosorption, which demonstrated that Cd was really biosorbed on the aerobic granular sludge (Fig. 6.3). It is also clear that Cu and Ni peaks were detected in the XPS survey scanning spectra of aerobic granules after Cu and Ni biosorption, respectively (Figs. 6.4 and 6.5). The presence of these peaks provided the evidence that Cd, Cu and Ni were adsorbed on the aerobic granules.

Comparison of the XPS survey scanning spectra of aerobic granules before and after heavy metal biosorption confirmed the presence of Cd, Cu and Ni on the Cd^{2+} , Cu^{2+} and Ni^{2+} -contaminated aerobic granules, respectively. To determine the amount of biosorbed heavy metals in the metal contaminated aerobic granules, the granule samples were further digested for the multi-elemental analysis, and results were presented in Table 6.1. On the other hand, the elemental mass balance between those interested elements presented in solution and aerobic granules was conducted (Tables 6.2 to 6.4). For example, the Cd^{2+} removed by aerobic granules was 1.607 meq/g, while 1.504 meq/g Cd^{2+} was recovered from the Cd^{2+} -contaminated aerobic granules after the biosorption experiment, i.e. a Cd^{2+} recovery efficiency of 93.57% was achieved. Tables 6.2 to 6.4 indeed provide evidence that ion exchange would be one of the mechanisms for metal biosorption by aerobic granules.

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Table 6.2 Metal mass balance in the Cd^{2+} -contaminated aerobic granules.

	Cd (meq/g)	Ca (meq/g)	Mg (meq/g)	K (meq/g)
Fresh AG (Dig.)	0.000	7.518	0.114	0.089
Cd-AG (Dig.)	1.504	6.493	0.065	0.035
Difference	1.504	1.025	0.049	0.054
Removal from solution	1.607	-	-	-
Released into solution	-	1.116	0.045	0.051
Percentage (%)	93.57	91.84	108.89	105.88

Dig. = Digestion

AG = Aerobic Granules

Table 6.3 Metal mass balance in the Cu^{2+} -contaminated aerobic granules.

	Cu (meq/g)	Ca (meq/g)	Mg (meq/g)	K (meq/g)
Fresh AG (Dig.)	0.008	7.518	0.114	0.089
Cu-AG (Dig.)	1.389	6.719	0.026	0.010
Difference	1.381	0.799	0.088	0.079
Removal from solution	1.403	-	-	-
Released into solution	-	0.835	0.090	0.076
Percentage (%)	98.46	95.74	97.78	103.95

Dig. = Digestion

AG = Aerobic Granules

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Table 6.4 Metal mass balance in the Ni^{2+} -contaminated aerobic granules.

	Ni (meq/g)	Ca (meq/g)	Mg (meq/g)	K (meq/g)
Fresh AG (Dig.)	0.009	7.518	0.114	0.089
Ni-AG (Dig.)	0.740	7.014	0.067	0.029
Difference	0.731	0.504	0.047	0.060
Removal from solution	0.764	-	-	-
Released into solution	-	0.524	0.044	0.061
Percentage (%)	95.73	96.77	106.82	98.37

Dig. = Digestion

AG = Aerobic Granules

Figure 6.2 XPS survey scanning spectrum of fresh aerobic granules (before heavy metal biosorption).

Figure 6.3 XPS survey scanning spectrum of Cd^{2+} -contaminated aerobic granules (after Cd^{2+} biosorption).

Figure 6.4 XPS survey scanning spectrum of Cu^{2+} -contaminated aerobic granules (after Cu^{2+} biosorption).

Figure 6.5 XPS survey scanning spectrum of Ni^{2+} -contaminated aerobic granules (after Ni^{2+} biosorption).

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6.3.3 Chemical Precipitation-Associated Biosorption

The crystal phases of aerobic granules before and after the Cd^{2+} , Cu^{2+} and Ni^{2+} biosorption were determined by X-ray powder diffraction (Fig. 6.1 and Figs. 6.6 to 6.9). It can be seen that the sample crystallinity was available as the clear peaks appeared in the patterns of all samples. As shown in Fig. 6.8, the peaks position and ratio of aerobic granules after the Ni^{2+} biosorption were the same as those of fresh aerobic granules before metal contamination. This in turn implies that there was no new crystal precipitated in the Ni^{2+} -contaminated aerobic granules or the amount of new crystal precipitation generated could be negligible. For the Cd^{2+} and Cu^{2+} -contaminated aerobic granules, their peaks positions and ratios are different with those of fresh aerobic granules. The appearances of some new peaks imply that there were different crystals precipitated in the Cd^{2+} and Cu^{2+} -contaminated aerobic granules and more complicated mechanisms would be involved in the Cd^{2+} and Cu^{2+} biosorption.

The further XRD phase matches reveal that otavite (CdCO_3), aragonite (CaCO_3) and magnesium calcite synthesis ($(\text{Mg}_{0.03}\text{Ca}_{0.97}) (\text{CO}_3)$) could be the main compounds in the Cd^{2+} contaminated aerobic granules (Fig. 6.7). The appearance of otavite (CdCO_3) seems to indicate that precipitation would contribute to the removal of Cd^{2+} by aerobic granules.

From the XRD analysis of the Cu^{2+} -contaminated aerobic granules (Fig. 6.8), it was found that aragonite (CaCO_3) could not be detected in the other samples. However, magnesium calcite synthesis ($(\text{Mg}_{0.03}\text{Ca}_{0.97}) (\text{CO}_3)$) can be detected in the Cu^{2+} -contaminated aerobic granules. The new compound formed in the Cu^{2+} -contaminated aerobic granules was found to be copper metallic synthesis clinoatacamite ($\text{Cu}_2(\text{OH})_3\text{Cl}$), i.e. the removal of Cu^{2+} by aerobic granules could be partly attributed to the microprecipitation of copper in the form of $\text{Cu}_2(\text{OH})_3\text{Cl}$. For Ni^{2+} contaminated aerobic granules, no new crystal compounds were detected indicated that chemical precipitation did not occur during the Ni^{2+} biosorption or its quantity was lower than

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the detection limit (Fig. 6.9). As shown in Figs. 6.7 and 6.8, the intensity of precipitations otavite CdCO_3 and clinoatacamite ($\text{Cu}_2(\text{OH})_3\text{Cl}$) was very low (the intensity major reflection line less than 300 counts). Scheckel (et al., 2005) reported that chloropyromorphite standard was diluted to 10 mg/g soil, the intensity of XRD major reflection line identifying chloropyromorphite was around 700 counts. Since intensity is proportional to concentration in the mixture (Ouhadi and Yong, 2004), the amount of otavite CdCO_3 and clinoatacamite ($\text{Cu}_2(\text{OH})_3\text{Cl}$) in the Cd^{2+} and Cu^{2+} contaminated aerobic granules would be very small, i.e. the contribution of precipitation to Cd^{2+} and Cu^{2+} removal by aerobic granules would be minor.

As shown in Fig. 6.7, otavite (CdCO_3) was found in the Cd^{2+} -contaminated aerobic granules, i.e. Cd^{2+} existed in the form of CdCO_3 precipitate. After the Cu^{2+} biosorption, clinoatacamite ($\text{Cu}_2(\text{OH})_3\text{Cl}$) was precipitated out on the Cu^{2+} -contaminated aerobic granules, which also contributed to the Cu^{2+} removal by aerobic granules. The presence of clinoatacamite ($\text{Cu}_2(\text{OH})_3\text{Cl}$) could satisfactorily explain the phenomena that the color of aerobic granules was changed from yellow brown (before) to green (after). In fact, previous studies also reported that the mechanisms involved in heavy metals removal by biomaterial might also include chemical precipitation and sorption onto minerals such as calcium carbonate (Garcia-Sanchez and Alvarea-Ayuso, 2002; Prieto et al. 2003; Hellebusch et al., 2004).

Figure 6.6 X-Ray Diffraction patterns of aerobic granules before and after Cd^{2+} , Cu^{2+} and Ni^{2+} biosorption. From the top to bottom are: Cd^{2+} -contaminated aerobic granules, Cu^{2+} -contaminated aerobic granules, fresh aerobic granules.

Cd_granules

Figure 6.7 XRD analysis of the Cd^{2+} -contaminated aerobic granules.

Cu_granules

Figure 6.8 XRD analysis of the Cu²⁺-contaminated aerobic granules.

Ni_Granules

Figure 6.9 XRD analysis of the Ni^{2+} -contaminated aerobic granules

6.3.4 ECP-Associated Biosorption

Aerobic granules before and after the ECP extraction were used as biosorbents for the removal of Cd^{2+} , Cu^{2+} and Ni^{2+} from aqueous solution. Figure 6.10 shows that the specific Cd^{2+} , Cu^{2+} and Ni^{2+} biosorption capacities of fresh aerobic granules (before ECP extraction) were 1.607, 1.403 and 0.764 meq metal/g dried aerobic granules, respectively, while after the ECP extraction, the corresponding specific biosorption capacities for Cd^{2+} , Cu^{2+} and Ni^{2+} were found to decrease to 1.296, 1.176 and 0.656 meq/g. These may imply that the granule ECP also involved in the Cd^{2+} , Cu^{2+} and Ni^{2+} biosorption by aerobic granules (Table 6.5).

Figure 6.10 The ECP contributions to the metals biosorption by aerobic granules.

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6.3.5 Ion Exchange-Associated Biosorption

In order to investigate the mechanisms of heavy metal biosorption by aerobic granules, concentration analyses of light metal ions in the solution before and after the biosorption experiments were conducted. Figure 6.11 shows the amounts (meq/g) of biosorbed heavy metals on aerobic granules as well as light metal ions (Ca^{2+} , Mg^{2+} and K^{+}) released into the solution during the biosorption experiment. It can be seen from Figure 6.11 that light metal ions Ca^{2+} , Mg^{2+} and K^{+} released into aqueous solution accompanied with the uptake of heavy metal ions. As shown in Fig. 6.11, the amount of released Ca^{2+} is far larger than the other two light metal ions Mg^{2+} and K^{+} . Accompanied with 1.607, 1.403 and 0.764 meq/g of Cd^{2+} , Cu^{2+} and Ni^{2+} uptake on the aerobic granules, the respective released Ca^{2+} amounts are 1.117, 0.835 and 0.524 meq/g. It seems that a larger heavy metal uptake would associate with a larger Ca^{2+} release. Table 6.5 further shows the ratio of released individual and total light metal to biosorbed heavy metal in terms of meq/meq or percentage. It can be seen that the ratio of total released Ca^{2+} , Mg^{2+} and K^{+} to the biosorbed heavy metal was 75.51, 71.31 and 82.43% for Cd^{2+} , Cu^{2+} and Ni^{2+} , respectively. The simultaneous release of light metals with the uptake of heavy metals by aerobic granules may indicate that an ion exchange mechanism would be involved, but the observed non-stoichiometric exchange of ion also shows that the ion exchange mechanism was not the sole mechanism involved in the Cd^{2+} , Cu^{2+} and Ni^{2+} biosorption by aerobic granules.

Figure 6.11 shows that release of light metals, such as Ca^{2+} , Mg^{2+} and K^{+} from aerobic granules during the biosorption of Cd^{2+} , Cu^{2+} and Ni^{2+} by aerobic granules, and Table 6.5 further indicates that 71.31 to 82.43% of heavy metal uptake would be associated with the release of light metals. It seems that ion exchange could be one of the main mechanisms involved in metal removal by aerobic granules. In a study of metal biosorption by anaerobic granules, Hawari and Mulligan (2006a) reported that 77%, 82% and 50% of adsorbed copper, cadmium and lead would be attributed to an ion exchange mechanism. Furthermore, Schnierder et al. (2001) demonstrated that the

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surface group responsible for metal exchange was primarily the carboxylate group, while Tsezos and Volesky (1981) reported the bivalent metal ions exchange with counter ions of polysaccharides.

Figure 6.11 Release of the light metals during the biosorption experiments.

Table 6.5 Contributions of different mechanisms to metal biosorption by AG.

Metal Type	Ion-Exchange				Other (%)
	Ca (%)	Mg (%)	K (%)	All (%)	
Cd	69.50	2.83	3.18	75.51	24.49
Cu	59.50	6.40	5.41	71.31	28.69
Ni	68.60	5.85	7.98	82.43	17.57

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6.3.6 Contribution of Granule Functional Groups to Metal Biosorption

6.3.6.1 Evidence from FTIR Analysis

The FTIR spectra of aerobic granules before and after the Cd^{2+} , Cu^{2+} and Ni^{2+} biosorption are shown in Figs. 6.12 to 6.14. These spectra were obtained from scanning in the range of $400\text{--}4000\text{ cm}^{-1}$. Table 6.6 lists the main functional groups corresponding to the peaks observed on IR spectra of fresh aerobic granules. The band at $3200\text{--}3400\text{ cm}^{-1}$ represented O-H stretching of polymeric compounds in fresh aerobic granule, and the band at 2928 cm^{-1} would be due to an asymmetric vibration of CH_2 , while the band at 2852 cm^{-1} resulted from a symmetric vibration of CH_2 . The bands at 1725 and 1261 cm^{-1} could be attributed to the stretching vibration and deformation vibration of C=O of carboxylic acids, respectively. A 1648 cm^{-1} band was the result of the stretching vibration of C=O and C-N (amide I) peptidic bond of protein, while a 1520 cm^{-1} band would show stretching vibration of C-N and deformation vibration of N-H (amide II) peptidic bond of protein. Bands in the range of $1130\text{--}1160$ and at 1056 cm^{-1} could be attributed to stretching of C-O-C and OH of polysaccharides, respectively. Some bands observed in the “fingerprint” zone could be attributed to the phosphate or sulphur groups. In fact, it appears from Figs. 6.12 to 6.14 that different functional groups would be responsible for the biosorption of Cd^{2+} , Cu^{2+} and Ni^{2+} . Compared to the spectra of fresh and Cd^{2+} -contaminated aerobic granules (Fig. 6.12), the broad stretching absorption band at 3407 cm^{-1} shifted to 3414 cm^{-1} after the Cd^{2+} biosorption. The band intensity at 1725 cm^{-1} clearly decreased after the Cd^{2+} biosorption, i.e. there would be an interaction of Cd^{2+} with carboxylate groups (Fig. 6.12). The shoulder band at 1520 cm^{-1} became sharper in the spectra of the Cd^{2+} -contaminated aerobic granule, and the band at 1384 cm^{-1} only appeared in the spectra of Cd^{2+} -contaminated aerobic granules and could be assigned to bending vibration of $-\text{CH}_3$ (Fig. 6.12). Low intensities of the bands in the range of $1200\text{--}1320\text{ cm}^{-1}$ in the spectra of fresh aerobic granules became smoother and a band at 1245 cm^{-1} appeared in the spectra of Cd^{2+} -contaminated aerobic granules (Fig. 6.12). The enhancement of the band intensities at 1520 and 1245 cm^{-1}

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would result from the complexation of Cd^{2+} with the functional groups from protein (Fig. 6.12). Comparison of the IR spectra between fresh and Cu^{2+} -contaminated aerobic granules is shown in Fig. 6.13. After the Cu^{2+} biosorption, the peaks on the spectra of Cu^{2+} -contaminated aerobic granules show a series of changes as compared to those of fresh aerobic granules. The broad and strong bands ranging from 3200 to 3600 cm^{-1} split into two sharper bands at 3446 and 3335 cm^{-1} , which could be assigned to NH stretching of amine and OH stretching of polymeric compounds. The band at 1082 cm^{-1} could be assigned to C-O from polysaccharide, while the band at 1787 cm^{-1} disappeared (Fig. 6.13). On the other hand, the band at 1488 cm^{-1} would be attributed to the C-H bending shifted to 1468 cm^{-1} (Fig. 6.13). The new bands at 1535 and 1240 cm^{-1} could be assigned to -NH and C=O, respectively (Fig. 6.13). Fig. 6.14 further shows the IR spectra of fresh and Ni^{2+} -contaminated aerobic granules. The bands ranging from 3200 to 3600 cm^{-1} were shifted by 4 cm^{-1} . The peaks at 1725 cm^{-1} turned to a shoulder after the Ni^{2+} biosorption, and this may indicate a possible Ni^{2+} interaction with C=O (Fig. 6.14). A band new band at 1385 cm^{-1} appeared after the Ni^{2+} biosorption, which could be attributed to the bending mode of C-O-H that would occur in an alcoholic group or a protonated alcoholic group or a protonated ether group (Chen et al., 2002).

The respective intensity of band at 1725 and 982 cm^{-1} was found to decrease after the Cd^{2+} and Ni^{2+} adsorption (Figs. 6.12 and 6.14), which indicated that C=O stretching band and O-H out-of-plane band of the carboxyl group would be attributed to the biosorption of the Cd^{2+} and Ni^{2+} ions. Lin et al. (2005) also reported similar phenomenon in the Ag^+ biosorption by *Lactobacillus* sp. strain A09, and they thought that both the carboxylate anion and the hydroxyl group from the peptidoglycan layer of the cell wall would play the key roles in binding of Ag^+ to the biomass. In fact, the decrease of intensity of these bands may represent a typical complexation of the carboxylate anion functional group by coordination with metal cation (Lin et al., 2005). The shift of absorbance peak of -OH after Cd^{2+} , Cu^{2+} and Ni^{2+} biosorption provided the evidence that alcoholic groups would be one of the biosorption sites for

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removing these three metal ions. In addition, Zhou et al. (2005) also found that the wave number of hydroxyl group shifted from 3415 cm^{-1} before lead biosorption to 3427 cm^{-1} after lead sorption. For the Cu^{2+} biosorption by aerobic granules, the main changes of spectra are different with Cd^{2+} and Ni^{2+} . In the protein zone, the band at 1787 cm^{-1} vanished, thus this band was assigned to the $\nu_{\text{C=O}}$ of the carboxylic acid anhydride formed by dehydration of both carboxyl groups (Lin et al., 2005). A clear shift of $\nu_{\text{O-H}} + \nu_{\text{C-O}}$ from 1082 to 1056 cm^{-1} was found after Cu^{2+} biosorption by aerobic granules (Fig. 6.13) due to the interaction of Cu^{2+} with alcoholic group. The new band at 1385 cm^{-1} appeared after Cd^{2+} , Cu^{2+} and Ni^{2+} biosorption by aerobic granules (Figs 6.12 to 14). According to Chen et al. (2002), this would indicate the interaction between the metals and the bending mode of C-O-H from alcoholic groups.

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Table 6.6 Main functional groups on fresh aerobic granule detected by the FTIR.

Wave Number	Vibration type	Functional type
3200-3600	Overlapping of stretching vibration of OH and NH	OH into polymeric compounds and amine
2928	Asymmetric stretching vibration of CH_2	
2850	Symmetric stretching vibration of CH_2	
1725	Stretching vibration of $\text{C}=\text{O}$	Carboxylic acids
1648	Stretching vibration of $\text{C}=\text{O}$ and $\text{C}-\text{N}$ (amide I)	Protein (peptidic bond)
1520	Stretching vibration of $\text{C}-\text{N}$ and deformation vibration of $\text{N}-\text{H}$ (amide II)	Protein (peptidic bond)
1261	Deformation vibration of $\text{C}=\text{O}$	Carboxylic acids
1130-1160	Stretching vibration $\text{C}-\text{O}-\text{C}$	polysaccharides
1082	Bending vibration of $\text{C}-\text{O}$	polysaccharides
1056	Stretching vibration of OH	polysaccharides
<1000	“Fingerprint” zone	Phosphate or sulphur functional groups

Figure 6.12 FTIR spectra of aerobic granules before and after Cd^{2+} biosorption. Top: Fresh aerobic granules; Bottom: Cd^{2+} - contaminated aerobic granules.

Figure 6.13 FTIR spectra of aerobic granules before and after Cu^{2+} biosorption. Top: Fresh aerobic granules; Bottom: Cu^{2+} -contaminated aerobic granules.

Figure 6.14 FTIR spectra of aerobic granules before and after Ni^{2+} biosorption. Top: Fresh aerobic granules; Bottom: Ni^{2+} -contaminated aerobic granules.

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6.3.6.2 Evidence from XPS Analysis

The X-ray photoelectron spectroscopy was used to attain the elemental information of aerobic granules as well as to illustrate the interaction between the organic functional groups in aerobic granules and the metals adsorbed. The functional groups were characterized by the binding energy of C 1s, while the metal ions adsorbed on these functional groups were also analyzed. The changes of binding energy (BE) of the coordination carbon atom (C 1s) in aerobic granules before and after the metal biosorption are shown in Figs. 6.15 a-d, and their area ratios were summarized in Table 6.7. The C 1s spectra of these samples comprise four peaks with corresponding BE of 285.60, 287.07, 288.82 and 290.23 eV that were identified via the deconvolution.

According to the guidelines for XPS analysis, these peaks could be assigned to ether, alcoholic, carboxylate and carbonate groups (Biniak et al., 1997; Chen et al., 2002; Sheng et al., 2004; Chada et al., 2005). The area distributions given in Table 6.7 further reveal that ether and alcoholic would be the dominant carbon forms in aerobic granules. The ether carbon ratio was found to decrease after the metal biosorption (Table 6.7), which indicates that ether-metal species might be formed in aerobic granules. Consequently, the area ratios of alcoholic and carboxylate group carbon were also changed. The alcoholic group area ratio increased more after the Cd²⁺ biosorption. On the other hand, the carboxylate group area ratio increased after the Cu²⁺ biosorption. The carbonate carbon BE peak was not detected after the Cu²⁺ biosorption and there was no significant difference between the area ratios before and after the Cd²⁺ biosorption. These seem to indicate that the interaction and the coordination affinity between the functional groups and metals would be more complicated than expected. High-resolution of O 1s and N 1s spectra show no significant shifts before and after the Cd²⁺, Cu²⁺ and Ni²⁺ biosorption (data not shown).

Figures 6.16 a-c further showed the XPS spectra of elements Cd 3d, Cu 2p and Ni 2p for aerobic granules after Cd²⁺, Cu²⁺ and Ni²⁺ biosorption. As far as the adsorption of

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Cd^{2+} on the aerobic granules, the peak of Cd 3d_{5/2} at BE of 406.18 eV could be assigned to $(-\text{COO})_2\text{Cd}$ sorption species. Similar spectra of cadmium were observed in the biosorption of cadmium by marine algal biomass *Padina* and *Sargassum* (Sheng et al., 2004). As to the adsorption of Cd^{2+} by aerobic granules, the observed peak of Cd 3d_{5/2} at BE of 406.18 eV could be assigned to $(-\text{COO})_2\text{Cd}$ species. Similar spectra of cadmium were also reported in the biosorption of cadmium by marine algal biomass *Padina* and *Sargassum* (Sheng et al., 2004). Two sets of Cu core-level XPS spectra (Cu 2p_{3/2} and Cu 2p_{5/2}) were found on the Cu^{2+} -contaminated aerobic granules. The peak of Cu 2p_{3/2} could be differentiated into three subpeaks at BE of 933.3, 935.6 and 943.40 eV, while the peak of Cu 2p_{5/2} could be composed of three subpeaks at BE of 952.90, 955.27 and 962.97 eV. The component at BE binding energy of 943.40 and 962.97 eV would be the cupric complexation with functional group on aerobic granules, and the signals at BE of 933.3 and 955.27 eV might be due to the cuprous complexation with functional groups on aerobic granules. These observations are consistent with the alginate-bound Cu^{2+} peak characterized at BE of 932.8 eV (Chen et al., 2002), cupric xanthate at BE of 944 and 963 eV, cuprous xanthate at BE of 934 and 952.90 eV (Chang et al., 2002). The other two peaks at 935.6 and 952.90 eV could be attributed to the Cu^{2+} and Cu^+ ions with lower electron density in its valence shell, respectively. XPS spectra Ni 2p_{3/2} comprised two deconvoluted peaks at 856.75 and 862.38 eV, while Ni 2p_{1/2} comprised two deconvoluted peaks at 874.27 and 880.32 eV.

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Table 6.7 Summary of peaks number and area ratios of C 1s spectra for aerobic granules before and after heavy metal biosorption.

Peak (eV)	Peak area ratio (%)			
	Fresh AG	Cd^{2+} -AG	Cu^{2+} -AG	Ni^{2+} -AG
285.60	43.97	41.1	40.47	42.13
287.07	36.01	38.63	35.86	35.84
288.82	15.72	15.83	23.67	17.93
290.23	4.3	4.44	ND	4.09

AG=Aerobic Granules

ND=Not Detected

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Figure 6.15 XPS spectra (C 1s) of four kinds of aerobic granules. (a) Fresh; (b) Cd^{2+} -contaminated; (c) Cu^{2+} -contaminated; (d) Ni^{2+} -contaminated granules.

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Figure 6.16 XPS spectra of three kinds of aerobic granules. (a) Cd^{2+} -contaminated; (b) Cu^{2+} -contaminated; (c) Ni^{2+} -contaminated granules.

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6.4 Conclusion

The metal affinity with aerobic granules was found to be in the order of $\text{Cd}^{2+} > \text{Cu}^{2+} > \text{Ni}^{2+}$. The equivalent ratio of the released light metals, such as Ca^{2+} , Mg^{2+} and K^{+} , to the adsorbed metals was in the range of 0.71 to 0.82, which indicated an ion exchange mechanism involved in the removal of Cd^{2+} , Cu^{2+} and Ni^{2+} by aerobic granules. Meanwhile, the results showed that ECP would play a role in the uptake of the studied heavy metal ions by aerobic granules, while chemical precipitation would be another possible mechanism responsible for Cu^{2+} and Cd^{2+} biosorption by aerobic granules because of the formation of CdCO_3 and $\text{Cu}_2(\text{OH})_3\text{Cl}$ precipitates detected in the Cu^{2+} and Cd^{2+} contaminated granules. The analyses by FTIR and XPS demonstrated that chemical functional groups such as alcoholic, carboxylate and ether would be the main binding sites for the biosorption of the studied heavy metals by aerobic granules.

Chapter 7

Conclusions and Recommendations

7.1 Conclusions

In this study, cadmium, copper and nickel were used as model metals to investigate the kinetics, equilibrium, and mechanisms of metal biosorption by aerobic granules. The results of this investigation showed that aerobic granules could be an effective biosorbent for removing soluble heavy metals from aqueous solution. The following conclusions can be drawn from this study.

- The kinetic model developed for heavy metals biosorption by aerobic granules can provide a satisfactory prediction for the Cd^{2+} and Cu^{2+} biosorption data obtained under different experimental conditions. The respective Cd^{2+} and Cu^{2+} uptake capacities of the aerobic granules were in the range of 40.7-573.3 mg Cd^{2+} /g dried granules and 12.4-69.3 Cu^{2+} /g granules. Both the biosorption capacity and overall biosorption rate constant at equilibrium for the Cd^{2+} and Cu^{2+} biosorption by aerobic granules were related to initial metal and granule concentrations.
- A general isotherm model (Eq. 4-8) for the biosorption of heavy metals was derived, and was verified by the experimental data obtained in the biosorption tests of individual Cd^{2+} and Cu^{2+} . It was demonstrated that the proposed model was reduced to different well-known adsorption isotherm equations, such as the Langmuir, Freundlich and Hill models in some special cases. It is expected that one may gain good insights from Eq. 4-8 into the biosorption behaviors of heavy metals by aerobic granules.

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- The Ni^{2+} biosorption by aerobic granules and the zeta potential of aerobic granules were found to be pH-dependent. The Ni^{2+} biosorption capacity increased with the increase of pH in the range of 2 to 6, and gradually attained a maximum value at pH 6. The model (Eq. 4-8) proposed previously could predict the nickel biosorption equilibrium at a wide range of initial pH values very well. The maximum biosorption capacity, equilibrium constants K_{ads} and n were found to be closely related to the initial solution pH. It was revealed that electrostatic attraction mechanism could provide an good explanation for the relationship between the zeta potential of aerobic granules and the Ni^{2+} biosorption, while the large quantity of K^+ , Mg^{2+} and Ca^{2+} released during the Ni^{2+} biosorption indicated that the ion-exchange mechanism would be involved in the Ni^{2+} biosorption by aerobic granules.
- The general isotherm equation (Eq. 4-8) derived can satisfactorily describe the Ni^{2+} biosorption by aerobic granules at various temperatures. The thermodynamic analysis showed that the Ni^{2+} biosorption by aerobic granules was an endothermic process, which in turn explained the temperature-dependent feature of the Ni^{2+} biosorption. In the sense of process equilibrium and thermodynamics, this study provided a sound understanding of the biosorption of soluble heavy metals by aerobic granules. The EDX analysis further showed that Ni^{2+} ions could penetrate into and were adsorbed at interior of aerobic granules. The adsorbed Ni^{2+} was almost uniformly distributed in the aerobic granule.
- The results showed that the mechanisms of Cd^{2+} , Cu^{2+} and Ni^{2+} biosorption by aerobic granules were very complicated and more than one mechanism would be likely involved. The equivalent ratio of all released light metals

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such as Ca^{2+} , Mg^{2+} and K^{+} to the adsorbed heavy metals was in the range of 0.71 to 0.82, which implied that ion exchange was the main mechanism involved in the removal of Cd^{2+} , Cu^{2+} and Ni^{2+} by aerobic granules. It was also found that ECP would also contribute to the uptake of these three metals. The precipitation of CdCO_3 and $\text{Cu}_2(\text{OH})_3\text{Cl}$ would make minor contribution to the removal of Cd^{2+} and Cu^{2+} , while chemical precipitation would not be involved in the Ni^{2+} biosorption by aerobic granules. The analyses by FTIR and XPS demonstrated that chemical functional groups, such as alcoholic, carboxylate and ether, would be the main binding sites for the biosorption of the studied heavy metals by aerobic granules. The metal affinity to aerobic granules was in the order of $\text{Cd}^{2+} > \text{Cu}^{2+} > \text{Ni}^{2+}$.

The excellent settling ability of aerobic granules can ensure a rapid separation of biosolids from the treated effluent, which in turn can lead to a simple process design. This study probably for the first time shows that aerobic granules could be used as an effective biosorbent for efficient heavy metal removal from industrial wastewater.

7.2 Recommendations

This study provided the fundamental and in-depth insights into the biosorption of individual heavy metal by aerobic granules. Further study would be needed to investigate the influence of pH and temperature on Cu and Cd biosorption by aerobic granules, heavy metals removal in the multi-metals system and so on. Such information would be essential for developing a granules-based biosorber for the removal of soluble heavy metals from industrial wastewater. It is also suggested to look into reuse and regeneration of the spent aerobic granulation in future study.

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Appendix

Figure A Cd species distribution in the solution as a function of pH.
(Srivastava et al. 2005)

Figure B Cu species distribution in the solution as a function of pH.
(Srivastava et al. 2005)

Figure C Soluble Ni concentrations in the water as a function of pH. (Dyer et al. 1998)