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BIOSORPTION OF COPPER

BY ACTIVATED SLUDGE

by

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A thesis presented in partial fulfilment of the requirements for the degree of Master of Philosophy in Environmental Engineering at Massey University, Palmerston North, New Zealand

1994

ABSTRACT

Biosorption of copper by sludge from a lab-scale activated sludge was studied. S-typed isotherms were found in almost all cases. This revealed the importance of reversible sites on the cell surfaces. Hydroxyl groups on the neutral polymers of the cell surfaces were likely to be the biosorption sites.

The equilibrium time of biosorption could be divided into two phases. The fast initial phase was observed within thirty minutes. The second phase went to an equilibrium after six hours. The biphasic equilibrium time was explained by the adsorption on the cell surfaces and active uptake, respectively.

Freundlich isotherms were found to describe the biosorption fairly. From constants of Freundlich equation, it was found that unwashed sludge could biosorb about 16 mg copper per gram dry weight of sludge.

Washing of sludge by various concentrations of EDTA and 0.85% NaCl did not show any difference from unwashed sludge. Anyway the optimum washing time in this study was three hours. The specific biosorptions were decreased after the long period of washing. The high concentration of EDTA (1% EDTA) gave the lowest biosorption capacity.

Sludge characteristics play the most important role in copper biosorption. Type of organisms influenced the biosorption capacity. The population proportion was changed due to the operation conditions of the reactor and the biological interaction among species. Effects of hydraulic retention time (HRT) and solids retention time (SRT) were discussed. Although they could not control the biosorption directly, they influenced sludge characteristics and the performance of exocellular polymers.

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Behaviour of the lab-scale activated sludge was monitored during the operation period in order to compare the adsorption with the biological characteristics of sludge. At the high dilution rate (0.042 hr⁻¹) the solids in the reactor fluctuated and did not reach a steady state after a prolonged period of six months. In contrast, the solids concentration of 0.021 hr⁻¹ dilution rate went to a stable state after one month.

The interrelationship of three groups of organisms in the reactor was proposed in order to explain the transient behaviour of the system. The combination of dilution and predation separated the fast and slow growing bacteria resulting in the instability of the system.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Associate Professor S.M. Rao Bhamidimarri, my supervisor for his remarkable guidance and supervision.

I am also gratefully indebted to Professor R.L. Earle, Head of the Department of Process and Environmental Technology, Dr Graham Manderson, Associate Professor Robert Chong, Associate Professor Ian Maddox, Dr Noemi Gutierrez, Mrs Anne Leonard for their help and suggestions in this project. The assistance of Dr A.J. Mawson in attempts to analyze the system mathematically is also thankfully acknowledged.

I also thankfully acknowledge the New Zealand Ministry of Foreign Affairs and Trade for the financial support, the Royal Thai Government and Khon Kaen University for allowing me the time to pursue my academic advancement.

My sincere thanks to Mr John Alger, Mr Bruce Collins and Mr Don McLean for their excellent and willing assistance with all the related technical matters and laboratory equipment fabrication requirement that arose during the project.

The assistance in the laboratory work of Mr John Sykes, Mrs A.-M. Jackson, Mr Mike Steven, Mr Mike Sahayam and Mrs Judy Collins are greatly appreciated.

Thanks are extended to Mr Wayne Mallet for assistance in all the computer related works.

I extend my appreciation to office-mates, and postgraduate colleagues, Dr Pinthita Mungkarndee, Ms Sirichom Leangon, Ms Tania Ngapo, Mr Roger Ludbrook, Ms Bongkot Noppon, Mr Narongrit Wongsuwan, Mr Wirut Ampun, Mr Thongchai Soommart, Ms Pattanaree Phourangk, Ms Payothorn Keosuk, Mr Thanit Wongpiyasatit, Ms Jutha Debahasatin, and all my Thaifriends in New Zealand for their time and friendship.

The consistent supports and encouragements of my parents, brother, and sisters are deeply acknowledged.

Finally, my best friend, Ms Sorujsiri Amornpunt for her understanding, love and encouragement. Her moral support is deeply appreciated.

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CHAPTER 1

INTRODUCTION

Biosorption in this thesis means the uptake of a solute by biomass either living or dead cells. The two mechanisms of the biosorption are the physicochemical, and biological process. These mechanisms are often named passive and active immobilization, respectively. The important physico-chemical processes are adsorption, and ion exchange on cell surfaces. With living organisms, active transport also contributes to the process. Surprisingly microorganisms, bacteria, fungi and algae, can biosorb significant amounts of metals. Therefore, biosorption was proposed to decontaminate the industrial effluents containing metals (Tsezos, 1990).

Due to some specific properties of copper, such as the good thermal and electrical conductivity, softness and resistance to most environments, mankind knew how to utilize it for a very long time. It is also an essential element for living organisms. The important role of copper in the intracellular metabolism is another reason that its properties are studied widely. Copper in the earth's crust down to 45 kilometres is estimated to be 14 * 10²⁰ g (National Academy of Science, 1975). However, that amount is only 0.001 percent of the total mass in the lithosphere. Most of the copper sources are in the sedimentary rocks and ocean (Nriagu, 1979).

Moreover, copper is a trace element but it is an essential element in enzymetric processes in living organisms. On the other hand, it can inhibit or can even be lethal to cells at the excessive concentrations. Each organism has a limit of tolerance depending upon type and adaptability individually. Some animals are more sensitive to copper than others. For example, copper is highly toxic to fish and most aquatic organisms but it is less toxic to terrestrial species both plants and animals. If the amount of copper in the metabolic pathways is not appropriate, this might cause diseases. The main sources of high copper contaminated effluents are from mining processes and the effluent of industrial wastewater, particularly, electroplating and dyeing. After the wastewater treatment processes, high copper contaminated sludge cannot be applied to land because it maybe leached by biogeochemical processes resulting in the bioaccumulation in food chain. The contaminated sludge can cause problems to both plants and livestock. Furthermore, the leachate from contaminated sludges finds its way to ground water.

Brooks (1985) highlighted the incentives for the recovery and recycling of metals from waste effluents. They are; (a) minimizing the environmental impacts of metal waste on the receiving water because of their toxicity; (b) maximizing the conservation of raw materials; (c) minimizing the cost of disposal of the contaminated sludge.

Wastewater treatment plants dealing with metal contaminated effluents in future are likely to use the biosorption technology at some stage of the treatment process. Some inactivated bacterial products and some synthetic polymers have been patented and have been applied commercially by some factories (Tsezos, 1990). The treated biomass can be regenerated and possibly the biosorbed metals, particularly, precious metals for example gold, uranium etc., could be recovered by the bioleaching or chemical processes. Although the process is applied commercially, there is little understanding of the process.

This thesis can be separated into two main parts. A lab-scale model activated sludge system in order to maintain the consistent sludge was operated. Biosorption experiments of copper by sludge microorganisms were studied at different points during the activated sludge operation.

Interrelationships among organisms that directly affect the biosorption were studied. Filamentous bacteria, which are the main competitors in the system and cause bulking sludge, also were evaluated by an abundance score. The important changes in a simulated activated sludge system during the cultivation period will be discussed. The effects of sludge characteristics on biosorption will be discussed. Finally, the possibilities in bioleaching based on the biosorption data are proposed.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Since biological processes tend to separate soluble and particulate heavy metals, they are accumulated in the sludge. As copper is a widely used metal, it is normally found in sludges. The typical copper concentration in sewage sludge varies from 45 to 16,030 mg/l on dry weight basis (mean 1250, median 700 mg/l)(Jewell,1983).

The heavy metals contaminated sludge can cause environmental problems. Its leachate was concentrated in the ecological food chain. The limit on the amount of metals is considered based on the translocation of metals from sludge into various plants and animals. The influencing parameters of assimilation are cation exchange capacity (CEC) and specific adsorption of metal on sludge. However, the land application of sludge is still recommended only for the crops that are not directly related to human food chain.

The cost of the contaminated sludge disposal was estimated to be half of the wastewater treatment cost. Because the highly contaminated sludge is treated like a toxic waste. Land application is unlikely to match the high concentration sludge. The effective and economical alternatives of utilization and disposal of sludge are proposed to minimize wastewater treatment cost. Simultaneously, the applications must not deteriorate the environment. Beyond that the basic science of biosorption and leaching should be thoroughly studied. The more information available, the more precise decision about the treatment technology can be made.

2.2 Theory of adsorption

2.2.1 Definition

Adsorption is a surface phenomenon. As the result of surface forces between adsorbent and adsorbate, the molecules or atoms of adsorbate are bound to the surface of the adsorbing material. The terms "adsorption" and "absorption" are not clearly defined although the common concept is widely accepted. Adsorption is typically without a permanent chemical reaction at the interface while absorption refers to a permanent chemical reaction or changes of the phase.

The adsorbent is generally a porous solid but the external surface is only a small part of the total surface. Diffusion of adsorbate into the internal surface of adsorbent is sometimes confused with the absorption process. However, as long as the adsorbate does not react permanently with the adsorbent, it is considered to be adsorption.

2.2.2 Physical and chemical adsorption

The nature of surface forces are still incompletely understood. Generally, there are two main surface forces found in adsorption. A weak interaction between mass is van der Waals or physical adsorption. Another force is the electrostatic or coulombic force between the different charges of the dipole moment of the unbalanced molecule. The latter force causes chemisorption or activated adsorption.

Table 2.1 compares the difference of the physical adsorption and chemisorption. In chemisorption, the collision energy must be higher than the activation energy so that a chemical bond can be formed. The chemisorption sites are the various types of the functional groups on the surface of the adsorbent which participate in adsorption through electron-sharing reaction.

Molecules removed by physical adsorption are held to the adsorbent by weak van der Waals' forces. The attraction by π -bonding under certain condition is thought to be the physical force (Benefield *et al.*, 1982).

Most adsorption processes in wastewater treatment are neither absolutely physical nor chemical adsorption. Designs for adsorption are based on the experimental data of pilot plants.

Physical adsorption	Chemical adsorption
Heat of adsorption less than	Heat of adsorption greater than
about 40 kJ mol ⁻¹	about 80 Kj mol ⁻¹
Adsorption appreciable only at	Adsorption can occur at high
temperatures below the boiling	temperature
point of the adsorbate	
Incremental increase in amount	Incremental increase in amount
adsorbed increases with each	adsorbed decreases with each
incremental increase in pressure	incremental increase in pressure of
of adsorbate	adsorbate
Amount of adsorption on surface	Amount of adsorption
is a function more of adsorbate	characteristic of both adsorbate
than adsorbent	and adsorbent
No appreciable activation energy	Activation energy may be involved
involved in adsorption process	in adsorption process
Multilayer adsorption occurs	Adsorption leads to a monolayer,
	at most

Table 2.1 Comparison of physical adsorption and chemisorption.

Source : Barrow (1979)

2.2.3 Factors influencing adsorption

Molecules of solute are removed from the solution by the adsorbent during the adsorption process. Simultaneously, the adsorbed molecules are desorbed into the solution. The process will continue until the concentration of solute remaining in solution is in equilibrium with the concentration of the adsorbed molecules. As the reversible reaction, the adsorption still occur although it is in the equilibrium stage. The rate of adsorption is equal to the rate of desorption at that point.

Some factors influencing the adsorption are reviewed in this part. Several of the more important factors are discussed.

2.2.3.1 Characteristics of the adsorbent

The first consideration of adsorption is the characteristic of the adsorbent. Some properties of adsorbent are thoroughly studies before using in wastewater treatment processes. They are: a.) surface area; b.) particle size; c.) uniformity coefficient; d.) bulk density; e.) particle density f.) iodine number; and g.) molasses number.

Since the adsorption is a surface phenomenon, all parameters related to the surface area also influence the adsorbability. The larger surface area, the greater adsorption capacity.

Iodine number and molasses number are the standard tests for measuring the volume of the adsorbent pore size. Iodine number uses to determine the pore size from 10 to 28 Å in diameter whereas molasses number measures the pore size greater than 28 Å. These numbers show the adsorbability for low and high molecular weight molecules respectively.

2.2.3.2 Characteristics of the adsorbate

Although the adsorbability of wastewater on adsorbent is too complicated because of the complexity of the composition, the adsorption data of individual adsorbates is still useful. An understanding of the behaviour of pure compound on a particular adsorbent can assist to predict the adsorption of the mixture in wastewater.

Solubility of the adsorbate is the main factor believed to influence the adsorption. The more soluble compound generally adsorbs less than the insoluble compound. Because the more soluble compound has stronger affinity between the solute and the solvent than the less soluble one. However, efforts to determine a quantitative relationship between the adsorbability and solubility have met with only limited success (Benefield *et al.*, 1982)

Molecular size of the adsorbate is another factor affecting the adsorbability. Since the adsorbate molecule must enter the micropore of the adsorbent, molecular size would be important for adsorption. Hassler (1974) reported that adsorption usually increases as the size of the molecule becomes greater in a homologous series of aliphatic acids, aldehydes, or alcohols. Benefiled *et al.* (1982) debated that if the molecule is too big to fit in the micropore, the adsorption will decrease because the bulky molecule will block the pore size.

2.2.3.3 Agitation

Benefield *et al.* (1982) reported that rate of adsorption depends on the amount of agitation in the system. If relatively little agitation occurs, the surface film of liquid around the particle will be thick and film diffusion will be the ratelimiting step. In contrast, if adequate mixing is provided, the rate of film diffusion will increase and the pore diffusion will be the rate-limiting step.

2.2.3.4 Physico-chemical parameters (pH and temperature)

The *p*H and temperature are the most important parameters influencing the adsorbability. The hydrogen ions are strongly adsorbed on the surface of adsorbents. In addition, *p*H influences the ionization of many compounds. According to Benefield *et al.* (1982) organic acids are more adsorbable at acidic condition, whereas the adsorption of organic bases prefers higher *p*H. However, the optimum *p*H for any adsorption must be determined by laboratory testing.

Since the adsorption is an exothermic reaction, the adsorption at lower temperature is better than at the higher ones. However, the rate of adsorption depends on the temperature. At higher temperature, the kinetic energy of molecules is high and the collision energy between adsorbate molecules and the adsorbent is high enough to cross the activation energy hill.

2.2.4 Adsorption isotherms

As the adsorption depends on temperature, most of the adsorption data are obtained at a constant temperature. The relationships of the solute distribution between the solid and liquid phases have been developed in order to interpret the adsorption data. They are referred to as "adsorption isotherms".

There are three common adsorption isotherms applied largely in wastewater treatments. They are: a.) Langmuir isotherm; b.) Freundlich isotherm; and c.) BET isotherm.

2.2.4.1 Langmuir isotherm

Langmuir's isotherm is based on the assumption that each site on the adsorbing material can adsorb one molecule of adsorbates. So, the adsorbed

layer is only one layer thick. Furthermore, each site has equal affinity. The presence of an adsorbed molecule at one site will not affect the adjacent molecule. The Langmuir equation is commonly written as follow (Langmuir, 1918):

$$\frac{x}{m} = \frac{abc}{1+ac}$$

or

$$\frac{1}{(x/m)} = \frac{1+ac}{abc}$$

or

$$\frac{1}{(x/m)} = \frac{1}{b} + \frac{1}{abc}$$

2.2.4.2 Freundlich isotherm

Freundlich's isotherm is based on the assumption that the adsorbent had a heterogeneous surface composed of different adsorption groups. Each group of adsorption sites is followed Langmuir equation. Freundlich theory still believes that the adsorption layer is one layer thick but the surface of the adsorbent was not energetically homogeneous. Therefore, the surface of the adsorbent consists of regions that adsorb with different strengths. The Freundlich's equation is written as follow (Freundlich, 1926):

$$\frac{x}{m} = KC^{1/n}$$

$$\log\left(\frac{x}{m}\right) = \log K + \frac{1}{n} \log C$$

where

 x = amount of solute adsorbed (mg or g)
m = weight of adsorbent (mg or g)
C = concentration of solute remaining in solution at equilibrium (mg/l)
n and K = constants

2.2.4.3 BET isotherm

Brunauer, Emmett, and Teller (1938) developed a mathematical model based on the assumption that molecules can adsorb more than one layer thick. Beside the multi-layer adsorption, the model still relies on the assumption of Langmuir model. The adsorbent surface is composed of uniform, localized sites, and adsorption at one site does not affect the adjacent sites. Their equation, known as "BET equation", is written as follow:

$$\frac{x}{m} = \frac{ACx_m}{(C_s - C) \left[1 + (A - 1)\frac{C}{C_s}\right]}$$

or

$$\frac{C}{(C_s - C) \frac{x}{m}} = \frac{1}{Ax_m} + \frac{A - 1}{Ax_m}$$

where

x = amount of solute adsorbed (mg or mole)
m = weight of adsorbent (mg or g)
x_m = amount of solute adsorbed in forming a

- complete monolayer (mg/g or mole/g)
- C_s = saturation concentration of solute (mg/l or mole/l)
- C = concentration of solute in solution at equilibrium (mg/l or mole/l)

2.3 Copper in the environment

Nriagu (1979) developed a global model of copper in presumed spheres or reservoirs. The copper burden in the lithosphere was estimated to be $14*10^{20}$ g. Most of the copper sources in this sphere are in sedimentary rocks $(0.75*10^{20} \text{ g})$ followed by copper in soil ($6.7*10^{15}$ g). Copper in the hydrosphere was estimated to be $5*10^{15}$ g. In addition, very small amount of copper compared to the others was found in the atmosphere ($2.6*10^9$ g.)

In New Zealand, copper level in soils developed from serpentine parent material ranges from 10 to 91 ppm. (mean 50 ppm) (Lyon *et al.*, 1970). Some organic and alluvial soils derived from hydrothermal pumice in the central of the North Island are enriched in copper, arsenic, and several other elements (Wells and Whitton, 1966).

Organic matter is the main factor of heavy metal adsorption by soil. Borah *et al.* (1992) found that after removal of organic matters, all the soil showed a decrease in the maximum adsorption and selectivity coefficient for trace elements. Compared to zinc and manganese, copper has a lower adsorption capacity with removal of organic matter.

The concentration of copper in the environment is quite high. National Water and Soil Conservation Authority of New Zealand (1986) reported that copper in rain water distributed spatially. It was 41, 5.4 and 0.06 mg/m³ in urban, rural and remote area respectively.

2.4 Sources of copper in wastewater

Since copper is a widely used material, there are many actual or potential sources of copper pollution. The main source of copper in wastewater is the effluent from industries, such as, dyeing process, copper piping, copper plating, wood preserving, inorganic pigment plants and landfill leachates. Among these sources, inorganic pigment plant has the highest concentration of copper in the effluent (Barrett *et al.*, 1974). Copper level in plating baths can be as high as 50,000 mg/l, while the concentration of the metal in rinse waters is approximately 0.02 to 1 percent of the process bath concentration (Golomb, 1972). Klein *et al.*(1974) studied metals in wastewater in the New York City and summarized the order: fur dressing and dyeing > ice cream > soft drink and flavouring > laundry.

In New Zealand, the concentration of copper in the wastewater effluent is different from town to town. Palmerston has a high copper concentration (1010 mg/m³ in raw sewage and 240 mg/m³ in treated sewage). Piggery waste is a very high copper source in New Zealand. Overcash *et al.*(1978) found that 14,000 mg/m³ of copper was detected in piggery effluent.

2.5 Copper related microorganisms

Bacteria are the most important group of microorganisms for biosorption and also for bioleaching. Kasen and Baecker (1989a) pointed out that some metal-tolerant bacteria in the activated sludge can remove metal from broths. Brierley (1990) reported that copper was biosorbed by various *Pseudomonas*, *Aeromonas* and *Zoogloea*. Some acidophilic thermophiles, e.g., *archaebacteria*, *sulfolobus*, can recover metals from ore through a bacterial leaching process.

The microbial products were found to be effective biosorbents. Capsules and extracellular polymers are frequently reported that can biosorb metals better than the whole cells. Pradhan and Levine (1992) summarized that the extracellular material from *Actinomycetes* possessed a greater capacity of binding metals than their cells.

Sulphate-reducing bacteria play an important role in biosorption and bioleaching processes. Apel *et al.*(1990) found that sulphate reducing bacteria,

were capable of using lactate as a carbon source and produced sulphide that reacted with soluble metals to form insoluble metal sulphide.

Thiobacilli are very tolerant to copper and can leach copper from low grade ore and sewage sludge. At least, *Thiobacillus* can survive in the solution that contain several grams of copper per litre. However, some heavy metals, e.g., silver and molybdenum are particularly toxic to iron-oxidizing *Thiobacilli* (Tuovinen, 1990).

Norris (1990) summarized the morphology of *Thiobacillus ferrooxidans*. This gram-negative and rod-shaped bacterium is usually 0.5 to 1.5 μ m long. They are found singly or in pairs. It is the dominant organism to oxidise mineral sulphides in most acidic environment at the temperature below 40 °C. It is a mesophilic bacterium. The optimum temperature for most strains is probably about 30 to 35 °C. The upper temperature limit for the growth of them was raised with decreasing acidity. If the acidity of the media was decreased (higher *p*H), the maximum tolerance temperature will be higher.

Similarly, *Thiobacillus thiooxidans* is an acidophilic and mesophilic bacterium. This rod-shaped, gram-negative bacterium oxidizes sulphur and uses carbon dioxide as a carbon source at the optimum temperature about 30 °C. It usually has a polar flagellum giving consistent motility.

Norris(1990) reported that the physiological diversities of all strains of sulphate-reducing bacteria have not been examined in taxonomy except the difference of temperature tolerance. The main different characteristic of *T. thiooxidans* and *T. ferrooxidans* is the lower GC-content in its DNA. *T. thiooxidans* has percentage GC content 52 percent whereas *T. ferrooxidans* has 58 percent GC content. In pure culture, *T. thiooxidans* is unable to effectively degrade pyrite and chalcopyrite concentrate but it can degrade zinc sulphide (Khalid and Ralph, 1977; Lizama and Suzuki, 1988). In mixed culture with iron-oxidizing bacteria, it can contribute to the dissolution of chalcopyrite

concentrate through the oxidation of sulphur. Sulphuric acid from the sulphur oxidation plays an important role in bioleaching. This process was also applied in mining commercially.

The growth rate of iron-oxidizing bacteria is not necessarily related to the rate of mineral oxidation. For example, *T. ferrooxidans* grows twice as fast as *L. ferrooxidans* on a ferrous ion medium but these bacteria degrade pyrite at the same rate.

Fungi also have effective heavy metal removal capability. *Gonoderma*, a wood rotting fungus and *Aspergillus niger* were reported to have effective copper and chromium removal ability. Rao *et al.*(1993) compared three waste biomass on the biosorption capacity and reported that *G lucidum* is more suitable to remove copper from industrial effluent than *A. niger* and activated sludge. Yeast also can adsorb copper from wastewater. Ehrlich and Fox (1967) found that *Rhodotorula* and *Trichospon* were capable of precipitating copper due to hydrogen sulphide production. In addition, Huang *et al.* (1990) reported that *Saccharomyces cerevisiae* was capable of copper biosorption.

Wong and So (1993) selected an assorted strain of *Pseudomonas putida* from an activated sludge that treats electroplating effluent. This strain could biosorb copper as high as 6.5 percent of its biomass. They also reported that bacterial cells grown in the sulphate-limiting medium had the highest copper removal capacity.

2.6 Inhibition of copper in activated sludge

Netzer and Beszedits (1979) reported that copper in the sewage effluent is in the range of 0.4 to 25 mg/l. These amounts of copper in the treatment plant reduced the efficiency of the BOD removal by up to 7 percent, even after the plants were acclimatized to copper. Dilek and Yetis (1992) evaluated the toxic effects of various heavy metals $(Cu^{2+}, Ni^{2+} \text{ and } Cr^{2+})$ on activated sludge process. They pointed out that the treatment efficiency was not adversely affected up to 10 mg/l of copper or nickel and up to 50 mg/l of chromium. However, the maximum specific growth rate and half substrate coefficients were changed.

Among heavy metals, copper is less toxic than many others. Cimino and Caristi (1990) studied the toxicity of heavy metals on activated sludge and suggested the decreasing order of toxicity. The sequence is $Hg^+ > Cd^{2+} > CrO_4^{2+} > CrO_4^{2+} > Cr^{3+} > Cu^{2+}$. However, the strong synergistic effect of copper and zinc was discovered. Therefore, it is suggested the water quality standard should not be set based on a single element.

Anaerobic digestion is much more seriously affected from high copper contamination than aerobic processes. Lin (1992) found that Cd and Cu were the most toxic substances on volatile fatty acid degradation in anaerobic digestion. Moreover, some combinations of synergistic effect were detected. The similar result was discovered in decreasing order of Ni > Cu > Cd > Cr > Pb. The result showed that 1,000 g/m³ of copper caused irreversible inhibition of acid production.

The biosorption is recommended to solve the problem in activated sludge. Lawrence and McCarty (1975) suggested that the toxicity of heavy metal on anaerobic digestion can be decreased by adding soluble sulphide. In addition, the increasing concentration of suspended solid was frequently applied to reduce toxicity of heavy metals in activated sludge process.

Another point of interest is the problem of the reaction of heavy metal with the intermediates from the process. For example, Ammonia from the nitrification of aerobic activity reacts with metals to form complexes that will increase toxicity to the process. The complexes of copper-amine were discovered during the process. Then the efficiency of the treatment system was reduced. (Sato *et al.*, 1988)

2.7 Immobilization of heavy metals by microorganisms

Biosorption of metals by microorganisms has two main processes. They are active and passive immobilization of metal. Active biosorption is the living microorganism activity. On the other hand, the passive immobilization is the non-living microorganism adsorption.

2.7.1 Active immobilization

Brierley (1990) reviewed literature and concluded that there are four mechanisms classified to be an active biosorption. They are precipitation, intracellular uptake and complexation, oxidation and reduction, and methylation and demethylation. These mechanisms are explained below.

First, precipitation is the process in which metal ions were precipitated by the reaction with the extracellular products of microorganisms. Several heavy metals that are toxic to microorganisms are detoxified by this process. Prime reactions of this process are sulphide and hydrogen phosphate precipitation. Insoluble salts are formed by precipitation between metals and microbial products, mainly, sulphide and phosphate (Aiking *et al.*, 1984). Apel *et al.*(1990) reported that *Consortium* utilized lactate as a carbon source and produced significant quantity of sulphide that reacted with soluble metal to form insoluble metal sulphide. However, removal efficiencies varied from metal to metal.

Secondly, Intracellular accumulation is an absorption process. Some metals, particularly, the essential elements are carried into cells by active transport. Most of the elements take part in intracellular metabolism.

Metal-complexes are formed during the biosorption. The stability of the complexes related directly to the biosorption efficiency. Lake *et al.* (1989) reported that the stability of complexes formed in each metal on each sludge type followed the general order: copper > lead > cadmium. In addition, copper formed more stable complexes in the treated sludge than in the raw sludge.

The third method, oxidation and reduction of metals by microorganisms, was not much studied. Lithotrophic autotroph can oxidize some metals such as, Fe, Fe²⁺, Mn²⁺, and U⁴⁺. Alternatively, metals or metal ions can act as electron acceptors during anaerobic bacterial growth (McLean and Beveridge, 1990).

The last method, methylation and demethylation are the ways by which microorganisms detoxified the toxicity of mercury and tin. This method is a very important pathway of bio-magnification of mercury through food chains. However, the fate of them does not correspond to the biosorption of copper.

2.7.2 Passive immobilization

Passive metal immobilization is due to the dead organisms. Metals are bound by excreted products or cell surfaces by physico-chemical forces. The process is largely adsorption and can be defined adequately by Langmuir or Freundlich adsorption isotherm model. This phenomenon is explained by the interaction of cationic metal and anionic cell surfaces of microorganisms. Therefore, it is no doubt that gram positive bacteria that have more anionic charge from the peptidoglycan layer will be useful as adsorbents.

MacNicol and Beckett (1989) studied the distribution of heavy metals in four components in activated sludge. They concluded that 85-95 percent of copper accumulated in biofloc (bacterial detritus), 5-15 percent accumulated in mineral grains and organic fragments and not more than 3 percent adsorbed by soluble and colloidal organic matters.

Ion Exchange is another important mechanism of biosorption. Metals can act as electron acceptors and exchange with protons from some macro-molecules on cell surfaces. Fletcher and Beckett (1987b) reported that copper can replace more protons from the soluble organic matter than the other ions (Ca, Mg, Co, Ni, Zn, Mn, Pb, Fe). Ion exchange with protons is a basic principle in binding of metals and organic matter and they predicted that over 99 percent of the presenting copper will be bound to the soluble organic matter at pH 7.

Goldstone *et al.* (1990) pointed out that metals were primarily associated with suspended solids. However, solubilization of copper occurs during activated sludge treatment due to the adding of anaerobic solids from the wasted activated sludge.

The biosorption equilibrium consists of two phases. The first phase is the rapid direct surface adsorption and follows by the second much slower step is an intracellular uptake.

Desorption of metals from biomass was not extensively studied. This depended on the eluent and the elution condition. An important elution parameter is the ratio of metals charged biomass to the volume of an eluent (solid to liquid, S/L). For the recovery of metals, this number has to be the highest. However, the concentration of the solvent molecule in the desorption solution must be high enough to recovery metals from bound cells.

2.8 Biosorption of copper by activated sludge

Removal of heavy metals from wastewater by biosorption was studied extensively. Lowe and Gaudy (1989) pointed out that the heterogeneous microbial population developed in the modified activated sludge process could biosorb at least as good as those culture processes. Moreover, the metal-sorbing biomass can be reused by the desorption regenerating processes. Stephenson and Lester (1984) reported that the major removal mechanism for metals including copper was the entrapment of precipitated metals by settleable biological solids. Goldstone *et al.* (1990) pointed out that metals in wastewater were primarily associated with suspended solids. The removal of metals increased with decreased sludge solid concentration and pH (Tyagi *et al.*, 1988).

Efficiency of metal biosorption by activated sludge varies at different treatment sites. Oliver and Carey (1976) studied the heavy metal removal efficiency at Southern Ontario and pointed out that 72 percent of heavy metals were removed by activated sludge system. Netzer and Beszedits (1979) found that the activated sludge system was 50-79 percent efficiency in removal of copper and the undetected concentration on the treatment efficiency was 1 mg/l. The result was comparable to Barth *et al.* (1963) who suggested that the total concentration of four metals (Cu, Cr, Zn and Ni) equal to 8.9 mg/l did not affect the efficiency of the activated sludge treatment system. No synergistic effect among metals was detected. Furthermore, approximately 54 percent of copper was removed by the process. However, the nitrification of the system was totally inhibited, so, the final effluent contained excess ammonia.

Norberg and Rydin (1984) reported that the bacterium Zoogloea ramigera plays an important role in sludge flocculation because of its exopolysaccharides. It can remove about 3 mM copper per gram dry weight when determined at less than 1 g dry weight per litre. Other extracellular producing organisms also showed high biosorption possibilities, for example, *Krebsiella aerogen*, *Arthrobacter viscosus* and *Pseudomonas sp.* (Fujii *et al.*, 1990; Scott and Palmer, 1990).

The stability of the metal-exopolymer complexes was studied by some investigators recently (Cheng *et al.*, 1975; Rudd *et al.*, 1984; Sterritt and Lester, 1985; Karapanagiotis *et al.*, 1990). These experiments based on the reversible

reaction that the biosorbed metals not only adsorb but also desorb from the cell surfaces or any organic substances, such as exopolymers or humic substances, simultaneously. The stability constants will be calculated from the equilibrium constants of the formation. These constants are useful for the valid comparisons of affinities of different exopolymers for metal ions. However, these constants vary with environmental conditions such as pH, ionic strength, polymer concentration, and temperature.

To date, only a few stability constants have been determined for bacterial exopolymer-metal complexes (Geesey and Jang, 1990). Jang *et al.* (1989) proposed the intrinsic stability constants that are independent of reaction conditions. Actually, the determination of these constants requires an accurate estimation of the activities of the interaction at the biosorption sites. Such information is difficult to obtain in case of the anionic polysaccharides.

2.9 Sludge characteristics

The biosorption of copper by activated sludge depends on both sludge characteristics and the properties of copper itself. Although the properties of copper in aqueous solution are quite well understood, the characteristics of sludge are highly variable. In this section, the physical, chemical and biological characteristics of sludge are reviewed. Interrelationships of organisms in activated sludge are an important factor to change the community structure. All characteristics will relate to the biosorption of copper.

2.9.1 Physical characteristics

The primary sludge from the primary sedimentation tank is a dense, and granular structure, while the secondary sludge containing mostly bacterial cells that are viscous and difficult to dewater. Healthy activated sludge has a brownish and flocculant appearance. The sludge resulting from wastewater treatment typically contains 0.25 to 12 percent solid by weight depended on the operations and processes (Tchobanoglous and Burton, 1991) The distribution of water type in sludge was estimated by Bjorkman (1969). 70 percent is the water between cells, 22 percent is the adhesion and capillary water and 8 percent is the absorption and intracellular fluids.

Size of secondary sludge was estimated by Patterson *et al.* (1973). They predicted that 99 percent of sludge have the size less than 9 mm. McCalla *et al.* (1977) estimated the density of the sewage sludge. They reported that the density of heat-dried sludge was 0.58 g/cm^3 , 1.01 g/cm^3 for liquid digested sludge, 1.08 g/cm^3 for lagoon digested sludge, and 1.2 g/cm^3 for aged Imhoff sludge.

2.9.2 Chemical characteristics

The chemical properties of sludge are widely studied because the chemicals in sludge are directly affected its suitability for land application and other beneficial use. It depends on the chemical composition of the influent. Generally, the sludge from industrial wastewater treatment plant accumulates more heavy metals than municipal treatment plant. Therefore the typical metal content in activated sludge varies widely.

Another interesting point is the plant nutritional elements. The average nitrogen and phosphorus in United State are 5.27 percent and 0.5 percent, respectively, on a dry weight basis (Gschwind and Pietz, 1992). The typical plant nutrients in activated sludge were reported by Tchobanoglous and Burton (1991). The typical nitrogen, phosphorus and potassium range are 2.4-5.0, 2.8-11.0 and 0.5-0.7 percent, respectively. Thus, it can be said that sludge is an excellent source of nitrogen and phosphorus.

2.9.3 Biological characteristics

Mostly the biological characteristic of sludge relies on the type of organisms and number of organisms in the sludge. Due to the type and also the number of each species change all the time, the sludge characteristic also changes even in a steady state. In this section, the biological characteristics of sludge will be reviewed in terms of community structure of organisms and the interrelationships of the organisms in sludge.

2.9.3.1 Types of organism in sludge

Pipes (1966) classified the organisms in activated sludge into four major classes. They are: a) floc-forming organisms, b) saprophytes, c) predators, and d) nuisance organisms. A particular organism may be classified into more than one group at a time depended upon its role. The roles of these organisms in activated sludge will be illustrated below.

a). Floc-forming organisms

Floc-forming organisms play an important role in the sedimentation. Without them the sludge cannot separate properly from the treated water. A variety of bacteria are capable of flocculation including *Pseudomonas, Achromobacter, Flavobacterium, Alcaligenes, Arthrobacter, Citromonas,* and *Zoogloea* (Dias and Bhat, 1964). Beside microorganisms, activated sludge flocs contain organic and inorganic particles from the influent and the extracellular polymers from bacteria. The extracellular polymers typically are consisted mostly carbohydrates and contribute 15 percent to 20 percent by weight of MLSS (Jenkin *et al.,* 1993). They play a role in the bioflocculation of the activated sludge. However, the mechanism of bioflocculation in activated sludge is complex and the influencing factors are poorly understood.

b.) Saprophytes

The saprophytes play a role in biodegradation of organic matter in activated sludge. They are a variety of bacteria including the floc-formers. The primary saprophytes are responsible for the degradation of original substrates. In addition, the secondary saprophytes feed on the metabolic products of the primary ones. Most literature reported that the eubacteria are gram-negatives bacilli belong to the genera *Pseudomonas*, *Flavobacterium*, and *Archromobacter* (Allen, 1940; Leslie *et al.*, 1980). Recent study reported in contrast that grampositive bacteria predominated in the most activated sludge (Bux and Kasan, 1994). Rod-shape bacteria are more frequently found than cocci (McKinney and Weichlein, 1953; Dias and Bhatt, 1964; Bux and Kasan, 1994).

c.) Predators

The main predators in activated sludge are protozoa that feed on bacteria. Pike and Curds (1971) reported that about 230 species have been found and they may constitute as much as 5 percent of the biological solids. The most important role of protozoa in activated sludge is their removal of nonflocculated bacteria by their feeding activities. This yields a better clarified effluent (Curds *et al.*, 1968; Curds and Fey, 1969).

d.) Nuisance organisms

All organisms that interfere with the proper operation of the activated sludge are classified to this group. High proportion of filamentous bacteria and fungi cause major problems in plant operations. The presence of excess filamentous bacteria reduces the specific gravity of biofloc and it is very difficult to separate by gravity settling. Mycelium of fungi also has a filamentous structure and behaves like filamentous bacteria in the treatment system.
Jenkin *et al.* (1993) summarized the factors that influence the growth of filamentous bacteria in activated sludge. They can be classified into general factors and specific factors. The general factors are including high SRT or low F/M, and presence of an unaerated zone in the aeration basin. The specific factors are: low DO concentration, deficiency of nitrogen and phosphorus, low *p*H, high sulphide, nature of substrate, seeding from tank surfaces, and surface trapping of foam and foam recycle.

2.9.3.2 Interrelationships of organisms in sludge

Microorganisms always interact not only at a physico-chemical level but also with the other cells in surroundings. Although the classification and terminology of bacteria interrelationships sometimes cause confusion, they can be generally classified into seven groups (Gooday, 1988). They are: neutralism, competition, amensalism, predation, parasitism, commensalism, and mutualism. Only competition, mutualism, and predation will be clearly illustrated here because they are more important in the mixed population of activated sludge.

a.) Competition

The competition is an interaction among the organisms that needs the same substrates or the same condition for survive. In the general ecological rule, there are no two species of organisms share exactly the same ecological niche. Only the one, which is the fittest to the niche, survives and others disappear. This also happens in activated sludge. Only the highest specific growth rate organism is a dominant species in the system.

Competition occurs when two or more individuals, either the same or different species need the common positive factors to survive. The terms of intraspecific and interspecific are the prefixes of competition for the same and different species, respectively. In fact, intraspecific competition is more serious, because the same species needs the same growth factors.

In biological treatment system, two or more species also compete for a limited supply of nutrient in a closed system. According to Meer and Tempest (1968) the faster growing bacteria will become a higher proportion in the aeration basin. After a sufficiently long period, the slower growing population under a certain dilution rate will be washed out from the system. Only the faster growing population left. Normally, the primary saprophytes in activated sludge have the highest competition for the same materials.

A competition can cause a natural selection. The higher specific growth rate organisms have higher chance to utilize a greater proportion of limited nutrients. This causes the domination of some species while the lower specific growth rate organisms disappear. The natural system also relies on this basis. When the environment change or in the other words, the ecological niche changes, then the new species, which wins the competition, becomes predominant the system.

b.) Mutualism

Mutualism in activated sludge is a complex phenomenon. Most bacteria need the growth factors from other organisms. Only 8 percent of the isolated bacteria from the activated sludge can grow without the additional growth factors (Pike and Curds, 1971). This indicated that the mutualism plays an important role in activated sludge systems. Metabolites from one species are perhaps growth factors for another species.

c.) Predation

Ciliates are the most important group of predators in activated sludge. The role of removing dispersed bacteria from the effluent assists the clarity of the

effluent. A large number of bacteria are mainly reduced by this group of organisms. A good operation has to maintain a balance between preys and predators in the system. Too many predators can cause the inefficiency of the treatment process because of the reduction of solids in the system. In contrast, too few ciliates result in too high solid concentration in the effluent.

2.9.4 Effect of sludge characteristics on biosorption

Types of organisms in the activated sludge fluctuate because of the interrelationships of the population in the system. Since the sludge characteristics vary depending on the types of organisms, biosorption of sludge depends on the type of dominant organisms. For example, the specific surface area of bacteria is much higher than protozoa or nematodes.

2.10 Effect of operating parameters on biosorption

In wastewater treatment, the operating parameters have been shown to affect the removal of metals from the wastewater. These factors can either stimulate or inhibit the function of the system. The removal of metal ions from the waste treatment plant is directly related to the suspended solids. Therefore, all operating parameters that influence the amount of suspended solids will also affect the metal removal efficiency.

2.10.1 Solids retention time (SRT)

Tsezos (1990) reported that age of microorganisms may affect the metal uptake capacity. This might be the result of excreted products and the structure of the cell wall and cell surface. Daigger and Grady (1977) pointed out that as SRT (solids retention time) influences the production of volatile suspended solids, increasing SRT causes increasing metal uptake in activated sludge. This was supported by Patterson (1978) who developed a mathematical model that relates metal uptake by activated sludge to MLSS

(mixed liquor suspended solids), soluble TOC (total organic carbon), and metal stability constants. The metal stability constants depend directly on the SRT results in a higher metal uptake at a higher SRT. Steritt and Lester (1981) also reported that increasing the SRT increased metal uptake by activated sludge.

Some investigators debated the effect of SRT on metal biosorption. Elenbogen *et al.* (1987) studied two pilot plants of three and ten-day SRT. They reported that there was no statistical difference in metal uptake between them. Melcer *et al.* (1992) also reported that SRT did not influence metal removal efficiency over the tested range. A reason may be the nitrification at the high SRT (Nelson *et al.*, 1981). The complexes of metal and nitrogen compounds ligands can change the metal removal efficiency.

The biosorption in pure culture also supported this hypothesis. Cotoras *et al.* (1992) studied biosorption of seven strains of *Bacillus* and *Micrococcus* and reported that culture age of cells had little or no effect on their sorption capacity.

2.10.2 Hydraulic retention time (HRT)

Hydraulic retention time of the activated sludge system was also reported to be another factor influencing the biosorption. Gordon *et al.* (1988) showed that the quantity and composition of slime polysaccharide by *P. atlantica* varied with dilution rates in the chemostat culture. At low dilution rates (0.005 hr⁻¹) the uronic content is lower than at high dilution rates (0.01-0.04 hr⁻¹). This implied that the properties of metal-binding sites depended on the surrounding environment.

2.10.3 Solids removal

Brown *et al.* (1973) pointed out that metal removal is related exponentially to the suspended solids removal. Cheng *et al.* (1975) reported that metal uptake increases with increased mixed liquor volatile solids.

2.11 Effects of physico-chemical parameters on biosorption

Biosorption efficiency depends not only on the substrates and microorganisms themselves but also the physico-chemical conditions.

2.11.1 Acidity and Basicity of the solution

Acidity and basicity of the solution play an important role in biosorption. Competition of protons at the low *p*H level is the main reason of decreasing of biosorption. Normally, binding of metal ions on microorganism surfaces will not be detected below a threshold *p*H (McLean and Beveridge, 1990). The exact value of this threshold *p*H varies with the affinity of the particular metal to the surface of microbes (Jean and Bancroft, 1986). However, the mechanism of the process was still not quite understood.

Aksu *et al.* (1991) studied the biosorption of Cu(II), Ni(II) and Cr(IV) ions to activated sludge bacteria. An equation of sorption process of copper(II) as a function of pH, temperature and metal concentration is established in activated sludge system. This model revealed that Cu(II) at a concentration of 150-200 mg/L gave the highest initial adsorption rate. Recently, they studied the effect of some physico-chemical parameters on the biosorption and found that the optimum initial pH for the biosorption of copper (II) by *Chlorella vulgaris* and *Zoogloea ramigera* is between 4.0 and 4.5 (Aksu *et al.*, 1992).

Adams (1985) found that the uptake of copper from solution by uncontaminated sewage sludge increased with increasing pH between four and seven, with added metal concentration up to a likely saturation value. Unfortunately, the adsorption data did not fit closely to Langmuir or Freundlich adsorption isotherms. Furthermore, Huang *et al.* (1990) determined the biosorption of Cu(II) by *Saccharomyces cerevisiae* and found that the adsorption was strongly affected by *p*H. In addition, Frimmel and Geywitz (1987) showed that Pb, Cu, Zn and Cd would co-precipitate with ferric hydroxide at *p*H 7.5.

2.11.2 Inactivation Techniques

Techniques of microorganism inactivation affected the efficiency of metal biosorption. Among them, heat is the best technique to inactivate the microorganism biomass (Somers, 1963; Horikoshi *et al.*, 1979, 1981; Nakajima *et al.*, 1981; Tsezos *et al.*, 1988). Cheng *et al.* (1975) compared the efficiency of metal uptake by viable and treated sludge and reported that the uptake efficiency of activated sludge was higher than the inactivated sludge. The biosorption reaction of inactivated sludge is time independent. This led to the conclusion that only a single step was found for inactive sludge.

Indeed, several studies have shown that dead microorganisms will often bind greater quantity than do the living cells in the same condition (Somers, 1963; Horikoshi *et al.*, 1979, 1981; Nakajima *et al.*, 1979, 1981; Sakaguchi *et al.*, 1979; Kurek *et al.*, 1982; Dispirito *et al.*, 1983). McLean and Beveridge (1990) concluded that the number of metal binding sites increased after the autolysis of cells.

2.11.3 Composition of the culture media

Composition of culture media can change the biosorption efficiency. Tsezos *et al.* (1988) studied the uranium biosorption capacity of *Penicillium spp.* on

various carbon source media and found that the metal binding properties of microbial biomass were affected by medium composition. However, Tsezos and Keller (1983) found that the radium binding capacity of activated sludge from wastewater treatment plant in different cities remains quite similar.

Initial metal concentration affects the biosorption rate. The initial biosorption rate increased by increasing metal ion concentration. The highest initial copper (II) concentration for *C. vulgaris* was 150-200 mg/l while for *Z. ramigera* was 100-125 mg/l (Aksu *et al.*, 1992)

2.11.4 Concentration of other metal ions

The presence of alkali or alkali earth metal ions in the solution reduces the efficiency of the biosorption process. Due to a passive biosorption is the electrostatic reaction of the different charges by the dipole moment of the unbalance bonding. Therefore, heavy metal and other metals compete to react with the anionic charge on the same cell surface. This causes the reduction of the metal removal efficiency (Brierley, 1990).

2.11.5 Chelating agents

Chelating agents such as CN⁻, EDTA, NTA or NH4⁺ affect the biosorption activity. Alibhai *et al.* (1985) concluded that chelating agents can both influence and inhibit the biosorption. Four possible effects of EDTA on cell surfaces are described below; (a) extract the present metals; (b) react with the extracellular polymer; (c) change the nature of the binding sites; and (d) inactivate cells. EDTA was found to extract some anionic ligands such as phosphoryl, sulhydryl, or hydroxyl groups from the cell surface. Therefore, the biosorption by the passive process is reduced at a high concentration of EDTA (Nelson *et al.*, 1981). Most often the metal solution must be pretreated by strong oxidization to destroy the chelating agents before the biosorption. However, the removal of copper from the EDTA complex was relatively insignificant (Cheng, et al., 1975).

2.11.6 Temperature

Temperature has the least effect compared to any other factors. Tsezos (1990) reported that temperature does not affect the binding of copper by microorganisms. However, Kasan and Baecker (1989b) pointed out that biosorption by treated sludge of copper(II), nickel(II), chromium(III) and iron(II) was increased independently of temperature when applied as single solution. Basically, the biosorption capacity is slightly increased in higher temperature.

2.12 Biosorption sites

Binding of copper on the surface of microorganisms was explained by the structure of the cell surface. Principally, the anionic character of the bacterial surfaces acts like a sponge that it can soak up metal ions (McLean and Beveridge, 1990). There are two main active functional groups on cell wall or exopolymer. The carboxyl groups are available to react with cationic metal ions. The electronegative charge from lone-pair electrons of oxygen in hydroxyl groups is another active site for metal binding. For the high producing exopolymer sludge, the latter active site is likely more effective than carboxyl groups.

The mechanism of biosorption is much more complicated than normal binding with electrostatic force. Current knowledge and information are limited, although researchers believe that bacterial exopolymer controls the metal biosorption efficiency. This mechanism is described below.

Gram positive bacteria have a thick layer of peptidoglycan on the cell wall. This structure will have at least one carboxyl group on the terminal of amino acid of peptide chain. Every carboxyl group gives a negative charge along the repeating units of the chain. Phosphate groups are also the other chance to give anionic characters on the chain. These positive charges will react with the negative charges of the cationic metals by the chemisorption process.

Although, the thick layer of peptidoglycan is not found in gram negative bacteria, the region between outer membrane and plasma membrane contains a thin layer of peptidoglycan. In contrast the structure of this thin layer peptidoglycan is different from the thick one. Therefore, gram-negative bacteria have more limited metal-binding capacity (McLean and Beveridge, 1990).

Extracellular polymers (slime and capsule) are the important active sites for metal binding. Anionic groups of exopolymer are different from the cell wall. Composition of capsules is different among bacteria. Most of the capsules consist of linear polymers of polysaccharide or amino acid repeating units (Sutherland, 1977). They may sometimes contain other anionic groups, such as carboxyl group and occasionally, phosphate and sulphate groups (Smiley and Wilkinson, 1983). Carboxyl groups in capsules can react with metal ions like in the cell wall.

Only difference between cell wall and capsule is the rigid structure of the cell wall. Cross-linked bonding between adjacent molecules fixes the structure of the wall, while the structure of capsules is loose. The amount of bound metal by bacterial extracellular polymers is quite high. McLean and Beveridge (1988) reported that copper binding by *Bacillus licheniformis* capsules and *Klebsiella pneumoniae* K-20 capsules are 0.89 and 0.003 micro mole bound copper/ mg dry weight of capsule respectively.

Geesey and Jang (1990) reported that exopolymer is the most important biosorption site. The subunit composition and structure of extracellular polymers determine their physical properties. Most microbial exopolysaccharides are extremely hygroscopic. Greater than 99 percent of the volume of a hydrated polymer is contributed by water.

The capsules of most bacteria contain uronic acids from 5 to 25 percent (Sutherland, 1980). Certain species produces ketal-linked pyruvate or sialic acid capsules (Boyle and Reade, 1983; Dewitt and Rowe, 1961). These subunits of capsules show an anionic character because of their carboxylic groups. The cationic metal ions can react with bacterial capsules at the partially oxidized carboxylic acids.

Furthermore, polysaccharides also contain an abundance of hydroxyl groups in their sugar rings. The electronegative oxygen atom of the hydroxyl groups is likely to participate in metal ions. This hydroxyl site was shown to be the most important biosorption site by Brown and Lester (1979)

The ionic radii of metal ions can affect the binding affinity. The affinity of metals decreases with increasing ionic radii of metals for neutral polysaccharide. In contrast, ionic polysaccharides prefer to bind with large ionic radii (McLean and Beveridge, 1990).

2.13 Biosorption isotherms

Two main groups of biosorption isotherms are the S-type and L-type. The Ltype shows that the adsorption sites on the adsorbent are occupied by the solute molecules permanently. Therefore, the other solutes have less chance beyond the initial stage. On the other hand, the S-type isotherm behaves differently. The more solute adsorbs on the solid surfaces, the more additional adsorption happens. This implies that the S-type isotherm is assisted by the previously bound molecules.

Giles *et al.* (1960) suggested that S-type adsorption will occur under three conditions. They are; (a) the solute molecule should be mono-functional

group and adsorbed as a single unit rather than a micelle; (b) the interaction forces should be moderate and result in vertical packing; c) there should be strong competition for adsorption sites either from the solvent molecules or other solutes.

Dugan (1975) has reported that the metal adsorption sites for the L-isotherm are carboxyl groups. This biosorption is practically irreversible at normal pH values. The adsorption of the S-type isotherm was proposed to be the hydroxyl groups on the hexose or pentose ring on bacterial capsules. The metal ions are attached by weak electrostatic forces of the lone pair electron zone. As the hydroxyl groups would almost be hydrated, metal adsorption would result in a decrease in the bound water content of the exopolymer (Forster, 1976). This leads us to believe that the S-type isotherm of polysaccharide to metal binding is the more important mechanism than the L-type isotherm (Brown and Lester, 1979).

Langmuir or Freundlich adsorption isotherm model described adequately the adsorption equilibrium of microorganisms from a single solution when all other solution parameters, such as, *p*H and temperature remain constant. However, the equilibrium is influenced significantly by other ions, for example, radium adsorbed by activated sludge was strongly influenced by calcium ion (Tsezos *et al.*, 1986). This effect is also call "co-ionic effect".

Langmuir's model is more frequently used for explaining the metal biosorption than Freundlich's. Cotoras *et al.* (1992) reported that Langmuir isotherm is suitable to determine the maximum sorption capacity , and the affinity of heavy metals biosorption by seven strains of *Bacillus* and *Micrococcus*.

2.14 Summary

Copper is a widely used metal. Some industrial wastewater and agricultural effluents contain very high copper concentration. After the wastewater treatment process, copper mostly accumulates on the sludge. The contamination of sludge, not only limits its beneficial use but also increases the cost of disposal.

Activated sludge is a very efficient heavy metal removal treatment. It tolerates metals at relatively high concentrations and also reduces the metals to an acceptable level to meet the standard.

Microorganisms are capable of immobilization of copper by both active and passive routes. Passive biosorption has many more advantages than the active one. Some passive biosorbents are patented and are supplied commercially but the mechanisms are not well understood.

Copper biosorption sites are both cell walls and exopolymers. The carboxyl groups on the peptidoglycan are the biosorption sites on cell walls. The hydroxyl groups of polysaccharide on the exopolymers are the other sites. Many workers suggested that exopolymer sites are more important than sites on bacterial cell walls.

Both Langmuir and Freundlich isotherms are known to describe the adsorption equilibrium, although, Langmuir's model is more frequently used. The S-type isotherm of polysaccharide to metal binding is the main mechanism. The bound hydrated water on the hydroxyl groups will be decreased by the new bond of copper and hydroxyl group, thereby assisting additional binding.

The biosorption of copper by activated sludge depends on the sludge characteristics. The problem is the characteristics of sludge rely on the types of organisms that are dynamic. In addition, the operations, the composition of influent and also the environmental factors also vary from site to site. Therefore, it is difficult to specify the exact behaviour of sludge at the different stages or different sites.

Lately, some investigators explored the concept of intrinsic stability constant of metal and sludge exopolymer complexes. Unfortunately, it requires an accurate measurement of activities of the interaction sites and therefore, its application to describe biosorption is limited.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Activated sludge system

A model activated sludge system (Figure 3.1, 3.2 and 3.3) consisted of a well mixed 14-litre Pyrex glass vessel (New Brunswick Scientific Co., New Jersey, USA) with *p*H and temperature control. The working volume of the reactor was kept constant at 10 litres. The *p*H of the reactor was controlled to 6.5-7.5 by a *p*H controller (Type 5997-20, Chemtrix, Inc., Oregon, USA). 1.0 M Sodium hydroxide solution was used to increase *p*H whenever required. Air flowrate of 1 VVM was maintained. Agitation speed was constant at 100 rpm. The temperature was controlled at $25\pm1^{\circ}$ C.

Seed of the activated sludge microorganisms was originally taken from the Palmerston North Wastewater Treatment Plant. The wastewater treated at the plant is mainly domestic waste. The seed sludge was then acclimatised in an incubator shaker (Lab-line, Illinois, USA) at 25°C 250 rpm. The spent medium was decanted and then 400 ml of fresh synthetic media were added daily. After one week of incubation, the seed was transferred to the reactor. The influent was fed to the reactor at a rate of 5 1/d until the reactor was filled up to ten litres. Hydraulic retention time (HRT) used in the experiment was one or two days. These correspond to dilution rate of 0.042 and 0.021 hr⁻¹ respectively.



Figure 3.1 Photograph of the lab-scale activated sludge system



Figure 3.2 Photograph of the clarification and recycle system in the activated sludge



Figure 3.3 Schematic diagram of the activated sludge

Modified synthetic wastewater based on Dilek *et al.* (1991) was used throughout this study. The composition of the medium is shown in Table 3.1. Feed medium was pumped continuously by a Masterflex peristaltic pump (Cole Palmer Instrument Co., Illinois, USA) into the reactor.

Figure 3.2 shows the clarification and returned sludge system. The mixed liquor from the reactor was pumped simultaneously at a calculated rate into a 1.5 l circular clarifier (a). At the bottom, 1 rpm scrapper was used for collecting the sludge from the centre of the clarifier and then pumped back to the recycled sludge tank (b) The sludge was recycled at a rate of 1.5 l/d throughout the experiment. The excess sludge was collected in the waste sludge tank (c) for the copper biosorption studied. The overflow from the clarifier was collected in the effluent tank (d) for averaging the effluent COD concentration.

3.1.2 Chemicals

All chemicals used for media preparation and all analytical work were of analytical reagent grades. The chemicals, which were used in the experiments and sources, are listed below. Deionized water was used for reagent preparation and the chemical blank throughout the experiments.

Ajax Chemicals (Sydney, Australia)

Copper sulphate

Component	Concentration (mg/l)
Proteose-Peptone	1221.7
NaCl	407.4
Na ₂ SO ₄	44.6
K ₂ HPO ₄	44.6
MgCl ₂ . 6 H ₂ O	3.7
FeCl ₂ . 2 H ₂ O	3.7
CaCl ₂ . 2 H ₂ O	3.7
MnSO ₄	0.057
Na ₂ MoO ₄	0.039
ZnSO ₄	0.046
CoCl ₂	0.041
CuSO ₄	0.076

Table 3.1 Composition of the synthetic wastewater

Source: adapted from Dilek et al. (1991)

BDH Chemicals Ltd (Poole, England)

Calcium chloride; Cobalt(II) chloride; Copper nitrate; di-Potassium hydrogen phosphate; EDTA (ethylene diamine tetraacetic acid disodium salt); Ferrous chloride; Magnesium chloride; Manganese sulphate; Potassium dichromate; Sodium hydrogen phthalate; Sodium hydroxide; Sodium molybdate; Sodium sulphate; Sulphuric acid; Zinc sulphate

Difco Proteose peptone

Scientific Supplied Ltd.

Sodium chloride

3.2 Analytical methods

3.2.1 Methods for activated sludge process study

Parameters, soluble COD, solids (MLSS and MLVSS), and sludge volume index (SVI), were determined every two days during the first nine months. When the system reached a steady state, they were measured once a week. The composition of community in the reactor was surveyed once every four days.

3.2.1.1 Filamentous Bacteria

Air dried smears of one drop (0.05 ml) mixed liquor from the reactor were prepared on microscopic slides. They were studied under a light microscope (Olympus, Model CH, Japan). The filamentous bacteria abundance score based on Jenkin *et al.* (1993) was used for determining the number of these bacteria in the mixed liquor. The less abundance is zero while the highest is six. The abundance scores used are shown in Table 3.2.

Appearance	Abundance	Abundance score
Filaments are not observed	none	0
Filaments present, but only observed in an occasional floc	few	1
Filaments commonly observed, but not present in all flocs	some	2
Filaments observed in all flocs, but at low density (1-5 filaments per floc)	common	3
Filaments observed in all flocs at medium density (6-20 filaments per floc)	very common	4
Filaments observed in all flocs at high density (>20 filaments per floc)	abundant	5
Filaments observed in all flocs- more filaments than flocs	excessive	6

Table 3.2 Abundance score of filamentous bacteria in biofloc

Source : Jenkin et al. (1993)

3.2.1.2 Solids concentration

The solids concentrations were measured by the method described in the Standard Methods (APHA, 1992). Both mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were determined. Solids in the reactor were taken directly from the reactor while the recycled solids and effluent solids were withdrawn from the recycled sludge tank and the effluent tank, respectively. At very high solid concentrations, the mixed liquor was diluted.

3.2.1.3 Chemical Oxygen Demand (COD)

Chemical oxygen demand was measured following the Standard Methods (APHA, 1992). The mixed liquor from the reactor and the return sludge was withdrawn freshly from the vessel and the return sludge tank, respectively. The effluent COD was taken from the effluent tank after the clarifier. The soluble COD was measured by filtering the liquor through either GF-paper or 0.45 µm membrane (Millipore, type HA). The filtrates were digested by a closed reflux method in a COD reactor (HACH company, USA), and then the colour was measured by the colorimetric method.

3.2.1.4 Sludge Volume Index (SVI)

Volume of the settleable sludge was evaluated in an Imholf cone. One litre of the mixed liquor from the reactor was placed in an Imholf cone. The sludge volume was read at 30 min. Then the SVI was calculated by the equation below.

SVI= settled sludge volume (30min) (ml/l)×1000 (mg/g) Suspended solids (mg/l)

3.2.2 Methods for copper biosorption

3.2.2.1 Sludge collection

Sludge was collected freshly from the wasted sludge tank. Then it was centrifuged at 5,000 rpm for 10 min at 20-25°C by refrigerated centrifuge (Du Pont Company, Sorvall Products, Delaware, USA). The clear solution on top of the centrifuge tubes was decanted. Where required, the same amount of washing solution was replaced and then recentrifuged at the same rpm. The water content in sludge was measured by the gravimetric method.

Copper concentrations of 5, 10, 20 and 50 ppm in 0.85 percent NaCl were studied with duplicates of 0.5 percent and 1.0 percent wet weight of activated sludge. The experiment was carried out in the incubator shaker at 250 rpm and 25°C.

At 0.25, 0.5, 2, 4, 6, 8, 10, and 24 hr samples were withdrawn and then filtered through 0.45 µm membrane filter paper (Whatman, type HA, 47 mm diameter). The remaining copper concentration in the filtrate was determined immediately by flame atomic absorption spectrophotometer (GBC atomic absorption, Australia). Graphs between remaining copper concentration versus time (Figure 3.4 and Figure 3.5) were plotted. An appropriate equilibrium time was selected.

3.2.2.3 Washing reagent and washing time

Activated sludge was washed by washing reagent before measuring the biosorption. EDTA (ethylene diamine tetraacetic acid disodium salt) at different concentration used to chelate the bound metal ions from the cell surface. Normal saline (0.85 percent NaCl) was used instead of water to prevent osmotic effect.

Concentrations of EDTA at 0.15, 0.25, 0.75, and 1.00 percent in normal saline were. Deionized water (Millipore Co., Massachusetts, USA) was used as a control. 0.85 percent NaCl solution was also used for washing the sludge. A fixed 1 g wet weight of washed sludge in every flask was used as adsorbent. Then, all flasks were shaken in an incubator shaker at 25°C, 250 rpm. After the equilibrium time (10 hr), the mixed liquor was taken out, filtered and determined the copper concentration as the same method as described in 3.2.2.2



Figure 3.4 Equilibrium time for 0.5% sludge at various initial copper concentrations



Figure 3.5 Equilibrium time for 1.0% sludge at various initial copper concentrations

3.2.2.4 Biosorption Isotherms

Various sludge concentrations of 0.5, 1.0, 1.5, 2.0 and 2.5 g wet weight were transferred into 250 ml flasks. 100 ml of 50 ppm copper solution in saline solution were added. Each flask was well shaken and covered with cotton wool before put into the incubator shaker.

After 10 hr of shaking, the mixed liquor was withdrawn, then filtered through 0.45 µm membrane. The copper concentration was measured by flame atomic absorption spectrophotometer as described in 3.2.2.2

3.3 Data analysis

Simple linear regression and asymmetric sigmoid equation of the biosorption isotherm were calculated by a computer programme (Fig. P. version 6.0c, Fig P. software Corporation, USA). The coefficients of the biosorption isotherm were estimated.

CHAPTER 4

CHARACTERISTICS OF THE MODEL ACTIVATED SLUDGE

The performance of the activated sludge depends on the operating conditions, particularly, dilution rate, and solids retention time. At the low dilution rate, the activated sludge reaches a steady without much oscillation. On the contrary, the MLVSS and the substrate concentration fluctuate at high dilution rates for prolonged periods of operations.

In this chapter the performance of the model activated sludge will be discussed with the operating conditions and the biological structure of the organisms in the reactor. Predation is likely to be the most important factor in the operation of the system, however, competition and mutualism will also be discussed.

The transient pattern will be discussed based on the specific growth rate and the interrelationships among the species. In this case, three groups of organisms are assumed. Fast-growing bacteria, slow-growing bacteria, and predators are expected in the aeration tank.

4.1 Biological characteristics of sludge

Types of organisms in the reactor were observed regularly. The community structure changed depending on the types of organisms. Floc forming, filamentous bacteria, ciliates, and others were the main groups of organisms. The ecological succession of each group was detected. Some common organisms found in the activated sludge are shown in Figure 4.1.



Figure 4.1 Photographs of some common organisms in the reactor (a) and (b) cocci and rod shape bacteria. (c) and (d) filamentous bacteria (e) and (f) protozoa cysts (g) ciliates (h) stalked ciliates (i) rotifers (j) nematodes

The succession of the population in the experiment is similar to the simulated model of Curds (1973). He showed a theoretical succession of dominating population in an activated sludge unit. The sludge bacteria increased in an oscillating cycle. Another interesting feature in his model was the morphology of organisms influenced the persistence. Attached ciliates could survive, while free-swimming and crawling ciliates were washed out from the system.

4.2 Interrelationships in the model activated sludge

The population in activated sludge is the combination of different interactions among mixed populations. Most workers only reported the interactions between pure cultures. In fact, the performance of an activated sludge system is a combination of all interactions. However, interrelationships of each pair of organisms will be discussed below.

4.2.1 Competition

In many instances successions of species in a mixed population system occur as the chemical environment changes with time. Fast-growing organisms, which are able to grow faster than others, predominate the system. The selection happens by the maximum specific growth rate of the species involved (Meers, 1974; Bailey, 1986). Therefore, although a mixed population was used in this study, only some species predominate the culture in the aeration tank.

In laboratory-scale activated sludge, the competition for survival is very high because the positive and negative growth factors are stable all the time. Therefore, only one species or a group of species that have the affinity for the substrate and conditions will survive while others will disappear. That is quite different from a real situation. The larger number of substrate in the raw wastewater causes more diversity of the organisms due to less competition for the same substrate.

Filamentous bacteria are the most important competitors. They have more beneficial characters than biofloc. But, the filamentous bacteria only bloom in some conditions such as low Do. This causes the bulking of sludge due to the low density. The relationship of F/M and SVI in Figure 4.2 reveals a reverse relationship of SVI on F/M. When the system was in starvation period, the filaments outcompete the floc-forming bacteria resulting in poor flocculation of sludge.



Figure 4.2 Relationship between SVI and F/M in the reactor at HRT= 1.0 d

Although most of the physico-chemical parameters are controlled, there are some factors that influence the growth of filamentous bacteria in this experiment. Food to microorganisms ratio (F/M) is the most important factor that cannot be effectively controlled. This parameter depends on the biomass in the reactor. F/M went down, while the SRT was high. The filaments survive better in this condition. Due to their morphology, they can survive at lower levels of oxygen and nutrients. After a short period of operation at high SRT, the sludge bulking occurred resulting in high SVI.

Not only the problem of the different species but also the mutation of the same species is considered. Because the same substrate is used for a long period, the organisms can mutate to best utilise the substrate. In this case, the mutants may be considered as new species. If the new one has a much higher specific growth rate, then the old species will be washed out.

4.2.2 Predation

The oscillating cycles of populations in Figure 4.3 and 4.4 reveal the predation phenomena. At the low bacteria concentration, the high density of ciliates population were detected. Although the filamentous bacteria also changed simultaneously, the concentration of MLVSS gradually increased. This revealed that the competitors are unable to take over the system.

The specific preference of protozoan cause the reduction of the preferred bacteria while the population of the non-preferred organisms was still high. The normal feeding mechanisms of ciliate skim all materials and filter them. Only the limited size is filtered. The oversize or too small bacteria or detritus are not consumed. Some bacteria may have other mechanisms to avoid the predation by protozoa. Curds and Vandyke (1966) reported that the bacteria pigments of *Chromobacterium violacium* are toxic and may be rejected by bacterivores although the avoidance mechanism was unknown.

The predation in the system changes not only the number of population but also the structure of organisms. In many cases the number of bacteria and predators are dependent on each other. In the mixed population, if one species was removed then other species may take over. The new species may not act in the same way as the former. Species that depends on the primary



Figure 4.3 Average MLSS in the activated sludge at HRT= 1.0 d



Figure 4.4 Average MLVSS in the activated sludge at HRT= 1.0 d

species in this case will disappear. However, the predation has never removed all their preys, if the preys have a high specific growth rate than other, they will become predominant again.

4.3 Effect of operating conditions on the activated sludge process

4.3.1 Hydraulic retention time (HRT)

The experimental hydraulic retention time was set very high. The HRTs were limited at one and two days or the dilution rates were 0.042 and 0.021 hr⁻¹, respectively. These numbers were very high compared to the normal activated sludge conditions. Typical activated sludge systems were implemented based on the design at 0.1 to 0.4 d HRT.

In this experiment, the soluble COD of the influent has to take into account. The initial COD was $1300 \pm 50 \text{ mg/l}$ while the COD of typical activated sludge that treated domestic waste are approximately 450 mg/l (200-700 mg/l) (Bailey, 1986). Therefore, every day the organic loading is approximately 1300 and 650 mg COD/l for the one-day and two-day HRT respectively. The typical activated sludge receives a load at the similar range. The loading range is 1100-4500 mg COD/l/d. This means that the soluble CODs in the experiment were within the normal activated sludge range.

As the hydraulic retention time influences the biological sludge characteristics, the biosorption of copper by sludge also varies based on HRT. The bacteria that their specific growth rates are lower than the dilution rate of the system are washed out. In the experiment, the proportion of the type of organisms maybe varied based on the HRTs.

In addition, bacteria in activated sludge cannot grow without the mutualism with other cells (Pike and Curds, 1971). Therefore, the lower diversity at the

high dilution rate causes the fluctuation of the cell mass in the system when they were attacked by the predators.

In conclusion, both slow and fast growing bacteria can grow at the low dilution rate while only the fast growers left at the high dilution rate. Since the number of bacteria species or the variety of the bacteria at the high dilution rate was less than the diversity in the lower dilution rate. Moreover, the diversity is the index of the resistance to change and the stability of the system. Therefore, the MLVSS performance of one-day HRT fluctuated more than two-day HRT.

4.3.2 Solids retention time (SRT)

Solids retention time of the two-day HRT was quite stable, while the one-day HRT fluctuated markedly (Figure 4.5 and 4.6). The main problems are the changing of MLVSS in the recycle sludge and the different growth rate of bacteria in the reactor at the high dilution rate.

4.3.3 Sludge volume index (SVI)

Filamentous bacteria were the most important group of the competitor in the reactor. They bloom under certain conditions. This causes the bulking of sludge due to their low density. The relationship of F/M and SVI in Figure 4.2 revealed the reverse relationship of SVI on F/M. The substrate loading of the system was fixed but the concentration of bacteria increased, therefore, the F/M went down. The filaments survive better under F/M conditions because they have more specific surface area. They outcompete the floc-formation bacteria, resulting in the poor flocculation of sludge and consequent settling problems.



Figure 4.5 Average SVI and SRT in the reactor at HRT= 2.0 d



Figure 4.6 Average SVI and SRT in the reactor at HRT= 1.0 d

Average soluble COD concentration in the reactor, effluent and returned sludge are quite similar (Figure 4.7 and 4.8). During the high growth rate, the high substrate utilization rate was also detected. There is an exception during the bulking of sludge. The COD removal efficiency also reduced.

4.3.5 Temperature

Although the temperature in the growth vessel was controlled at 25 °C, all the peripherals were not maintained at this value. The volume of the clarifier plus the returned sludge tank was two litres. The mean HRT for the system outside the reactor was 4.8 hour throughout the experiment. The temperature in those parts varied between 10 °C to 30 °C in winter and summer, respectively. As organisms rely on the seasonal change, besides the specific growth rate, the selection of the organisms perhaps was affected by the temperature outside the aeration tank as well. Some organisms, which were more affected by the temperature change than others, could eventually disappear.



Figure 4.7 Average soluble COD in the activated sludge at HRT= 2.0 d



Figure 4.8 Average soluble COD in the activated sludge at HRT= 1.0 d

4.4 Transient behaviour in the activated sludge

The biological solids are quite constant at 0.021 hr⁻¹ dilution rate (Figure 4.9 and 4.10). Surprisingly, the growth pattern of the 0.042 hr⁻¹ dilution rate is not stable after a prolonged period (six months) of operations (Figure 4.3 and 4.4). Every time the MLVSS in the reactor was reduced to a minimum level then the growth increased significantly (Figure 4.4). Therefore, concentrations of cells in the system increased.

The MLSS and MLVSS pattern in the reactor depended on the dilution rate. At the low dilution rate, the pattern closely followed the theoretical pattern. After a period, the biomass and substrate concentrations were constant (Figure 4.9 and 4.10). In contrast the patterns of MLSS and MLVSS at the 0.042 hr⁻¹ dilution rate were different. The concentrations of MLSS and MLVSS were oscillatory (Figure 4.3 and 4.4). This behaviour is likely to be the result of unstable growth and predation cycle.



Figure 4.9 Average MLSS in the activated sludge at HRT= 2.0 d



Figure 4.10 Average MLVSS in the activated sludge at HRT= 2.0 d

It is assumed that three groups of organisms are present in the reactors at a dilution rate near the steady state, with no preferential predation (see Figure 4.11). Let H be the removal level of the summation between the washed-out by a fixed dilution rate and the predation by bacterivores.

At a limited substrate concentration (S_1) , both species A and species B will disappear after a sufficiently long time. Since the population of A and B are less than H. On the other hand if the growth rate is less than the combination of the dilution rate and the consumption rate, species A and B will be washed out and/or eaten by the predators. In this case, all organisms will disappear after a period.

At the second substrate concentration (S₂), where μ_{B2} is less than H but μ_{A2} is greater than H, therefore at anytime species B will be removed from the system while species A will remain. This selection will remove the slow-growing bacteria from the system while the fast-growing bacteria are still there.


Figure 4.11 Hypothetical curves for two microbial species (A,B) and a predator (H) in a continuous culture. When $\mu_A > \mu_B$

In the second case, if only the fast-growing bacteria remain in the reactor, the substrate will be utilized more efficiently and the concentration of MLSS will be higher than normal. Before the slow-growers disappear, the biomass depends on the proportion of both species. This was confirmed from the results which showed that the MLVSS increased and the soluble COD decreased continuously until the maximum growth rate of the population or the carrying capacity of the system was reached.

Although the dilution level is fixed, the predation level relies on the number of preys. Therefore, the H level will oscillate at the opposite end of the growth cycle of bacteria. That is the normal one predator-one prey interrelationship. When the concentration of predators is maximum, the numbers of bacteria are minimum. On the other hand, when the numbers of predators go down then the numbers of preys will increase. In this case, the level H will be either higher and lower than both slowgrowers and fast-growers depending on the numbers of bacteria. If the predators do not prefer types of bacteria, the higher numbers of fast-growers will be ingested more than the slow-growing bacteria. When the level H is maximum, the both species A and B are at their lowest concentration either by wash out or through consumption. Compared with the conventional theory, the removal level is constant. If the level is higher than the slowgrower, they will be washed out until they disappear. This perhaps is the reason that why the slow-growing bacteria still survive at the high dilution rate in a mixed population. The total removal of the slow-growers is possible when the removal level is much higher than the specific growth rate of the slow-grower.

The difference of the pattern at low and high dilution rates also can be explained by this hypothesis. Although the specific growth rate of such pure culture is constant, the density of bacteria at the high dilution rate is higher than the lower dilution rate. According to Figure 4.11, the level of H will be low during the growth of bacteria. The small fluctuation of bacteria number in the reactor at the low dilution rate causes also the small fluctuation of the cell mass. Therefore, the growth pattern was more oscillatory at high dilution rate than the low dilution rate.

However, the dilution rate also affects the concentration of predators. If the specific growth rates of the predators are lower than the dilution rate, they will be also washed-out.

4.5 Genetic change and adaptation in the transient growth

Other explanation is a genetic change and an adaptation of the organisms. Some investigators tried to explain this phenomenon in pure culture by the enzyme synthesis (Kjeldgaard *et al.*, 1958; Kjeldgaard, 1961; Barford *et al.*, 1982). They showed some evidence that the rate of RNA synthesis was increased. Such processes of DNA synthesis, protein synthesis and nuclear and cell division implied the control of cell mass. This explains the increasing of biological solids in their reactors. The processes of transient growth in this case also take a long time to stabilise (up to 50 to 100 generations).

CHAPTER 5

COPPER BIOSORPTION BY SLUDGE

Biosorption in this thesis means the uptake of a solute by biomass either living or dead cells. There are two mechanisms of the biosorption reported. They are the physico-chemical, and the biological process. These mechanisms are often named passive and active immobilization, respectively. The important mechanisms of physico-chemical processes are adsorption, and ion exchange on cell surfaces. With living organisms, active transport also contributes to the process.

Surprisingly microorganisms, i.e. bacteria, fungi and algae, can biosorb significant amounts of metals. Therefore, biosorption was proposed to decontaminate the industrial effluent containing metals (Tsezos, 1990). To do so, the basic studies of biosorption mechanisms must be investigated before it can be applied to wastewater treatment.

In this chapter the copper biosorption by sludge based on the biosorption isotherms will be discussed. Various factors involved in the biosorption, such as sludge characteristics, washing of sludge, and biosorption capability, are discussed. The biosorption sites on the sludge surfaces are also studied.

5.1 Equilibrium time

The time required for equilibrium of copper biosorption by activated sludge is shown in the Figure 3.4 and 3.5. Copper was biosorbed by activated sludge rapidly in the initial stage. Within thirty minutes, half of the total biosorbed amount was bound by sludge and followed by a much slower step, which reaches the equilibrium after six hours. The biosorption process can be divided into two phases. The first step was observed within thirty minutes. This step was believed to be because of the adsorption on the cell surfaces themselves. This was followed by a very much slower step implying that the adsorption and desorption were taking place simultaneously. This second stage was the uptake by living cells. A similar bi-phasic adsorption was also reported by Cheng *et al.* (1975). Their first phase was three to ten minutes and the equilibrium was reached after three hours. The first-phase time in a pilot scale of mercury, cadmium and zinc was three hours. The equilibrium time was achieved after about two weeks (Neufeld and Hermann, 1975).

In addition, the every two-hour data showed the remaining concentrations seem to be an oscillating cycle. However, the difference between the maximum and minimum was less than 10 percent of the initial solution. This result revealed that the stability of the complexes between the sludge surfaces and copper ions was loose. This will be explained by the extracellular polymer binding hypothesis later.

5.2 Biosorption isotherms

S-typed biosorption isotherm was found to describe the biosorption as shown in Figure 5.1 and 5.2. These isotherms mean that the bound complexes help the adsorption of other ions, which exist as free ions, to attach to the cell surfaces. This can be explained by the bond energy theory. As the bonding is the exothermic reaction, the energy will be utilized to break down other bonds, either bonds between surface and water, or bonds of the hydrated cationic molecules. Geesey and Jang (1990) also pointed out that the binding affinity is related more to the entropy change than to the bond strength. Binding will occur as long as there is a greater release of water.

The biosorption isotherm from the two-day HRT was a different case. The graph between the specific biosorption and the equilibrium concentration was

a straight line in contrast to the sigmoid from the others. Perhaps, the concentration of exopolymer under different operation conditions may be a factor. The exopolysaccharide plays an important role in reversible biosorption. If the system did not have much exopolymers then, the biosorption will rely on the irreversible carboxylic sites of cell surfaces.

Some researches showed that cells can produce lower extracellular polymers at the lower HRT than at the higher HRT (Gordon *et al.*, 1988). Tait *et al.* (1986) also reported that exopolysaccharide synthesis by *X. campestris* decreased as the growth rate decreased. The composition and proportion of extracellular polymers under different operating conditions were also different. Exopolymers isolated at high dilution rates had higher acetyl content but higher pyruvate content than at the low dilution rates (Tait *et al.*, 1986). The isotherms of biosorption may therefore be affected by these operating conditions.



Figure 5.1 Biosorption isotherms of copper by unwashed sludge at 25 ° C at low MLVSS





Both Langmuir's and Freundlich's model were fitted (Figure A.1-A.8). The Freundlich's isotherm was found to describe the biosorption of copper better. Isotherm constants of both Langmuir's and Freundlich's models are calculated from the average of all data presented. The biosorption coefficients of copper are shown in Table 5.1 below. The K value of the Freundlich can relatively show the biosorption capacity of the sludge under different conditions. Constant 1/n normally indicates the energy or the intensity of the biosorption reaction. The result showed that the 0.85% NaCl washed sludge biosorbed copper to a higher degree than others.

Some workers also reported the biosorption by the exopolymers of some bacteria (Sutherland, 1977, 1980; McLean and Beveridge, 1990). Thus, the mass of MLVSS itself may be inadequate to explain the biosorption phenomena. The surface area, the content of exopolymers, and the affinity of each polymer must be taken into account. The specific adsorption (the ratio of weight of adsorbed metals to the weight of adsorbents) may not be applied in this case. The intrinsic stability coefficients are proposed by some researchers (Geesey and Jang, 1990).

Flowrate	Washing Solution	Langmuir Coefficient		Freundlich Coefficient	
(l/d)		a	b	n	K
5	0.85% NaCl	0.073	0.016	2.480	2.802
	Unwashed	0.003	-0.010	1.071	0.632
10	0.85% NaCl	0.087	0.015	3.442	4.026
	0.15% EDTA	0.012	-0.007	1.468	0.907
	0.50% EDTA	-0.001	-0.389	0.961	0.276

Table 5.1 Biosorption coefficients of copper by sludge

5.3 Biosorption capability

The capability of biosorption by activated sludge was surprisingly high. The ratio of biosorbed copper to the biomass (X/M) in Table 5.2 revealed a high removal efficiency of copper by sludge. Every gram dry weight of unwashed sludge adsorbed approximately 16 mg of copper. Although other washed sludge biosorbed copper less than unwashed sludge, the average biosorption range is still high (between 11.5-12.84 mg Cu / g dry weight). In case of typical activated sludge system application, the normal mixed liquor concentration range in the activated sludge is 2-5 g/l. Therefore, the sludge can adequately remove the usual copper concentration in wastewater to below the usual discharge standards.

	Specific biosorption (mg Cu / g dry sludge)					
	unwashed	0.85% NaCl	0.15% EDTA	0.50% EDTA		
0.5% sludge	22.58	16.98	17.28	19.15		
1.0% sludge	17.19	11.88	11.61	13.91		
1.5% sludge	14.63	10.62	11.17	10.82		
2.0% sludge	13.20	10.65	9.85	10.40		
2.5% sludge	13.06	10.29	9.40	9.82		
3.0% sludge	12.76	8.80	-	-		
average	15.57	11.54	11.86	12.82		

Table 5.2 Specific biosorption of copper by unwashed and washed sludge at 25 ℃

Application of biosorption for wastewater treatment depends on three more factors. They are: a) competition with other metal species; b) toxicity of metal; and c) sludge characteristics. Several other metals can compete with copper ions for binding sites. Some of the heavy metals, especially in industrial effluents are highly toxic to biological systems beyond certain concentrations. The latest is the limitation of biosorbed copper to the cell mass. The change of type of organisms will directly affect the biosorption capability.

The X/M at high sludge concentration was lower than at the lower sludge concentration at the same initial copper concentration (Table 5.2). This is perhaps the result of exposure effect. Since sludge is likely to flocculate at high concentrations resulting in concentration gradients

Metal ions in the wastewater play an important role in bioflocculation. The usual solids have the same charge on the cell surfaces. So, the sludge in the mixed liquor unlikely aggregates. Metal ions will interact with the anionic species on the cell surfaces or other exopolymers. The neutral or different charge solids will therefore help flocculate to form bioflocs by physico-chemical forces. This is another reason that the high sludge concentration was likely to form biofloc. Once the free cell is trapped into the floc, the available biosorption sites will be reduced.

As excessive copper is toxic to sludge, most cells will be killed by the metal toxicity. The consequent autolysis of the cells increases the biosorption sites. The higher biosorption by dead cells also reported by other investigators (Somers, 1963; Horikoshi *et al.*, 1979, 1981; Nakajima *et al.*, 1979, 1981; Sakaguchi *et al.*, 1979; Kurek *et al.*, 1982; Dispirito *et al.*, 1983).

The rapid adsorption by the extracellular polymers was maybe an important mechanism of self-protection. Therefore, either low sludge concentration or bacteria which produce less extracellular products will have less protection than the others.

In the case of 5 ppm initial concentration remaining liquid-phase concentration at the equilibrium was lower than normal. The difference from the normal is possibly because of the uptake of copper by active transport into cells because copper is an essential element. Rudd *et al.* (1983) pointed out that capsular polymers can accumulate six to ten folds compared to the uptake by the cells.

5.4 Biosorption sites

The most important types of biosorption sites are the carboxyl site of the acidic polymers, and the hydroxyl site of the neutral polymers. The S-type isotherms in this experiments revealed that the later type was more important

in case of the sludge biosorption. This was supported by Brown and Lester (1979) who gave evidence that complexes between metal ions and the hydroxyl groups of capsules were the most important mechanism of metal removal in activated sludge. The mechanisms of binding are discussed below.

Both sites are potentially biosorbed copper ions. Carboxyl group (-COO⁻) has a free negative charge of an electron pair to give to a copper ion. The ionic bond in this case is a strong electrostatic force. So, it is an irreversible bond. On the contrary, hydroxyl group (-OH) has a neutral charge. As oxygen has a very high electronegativity compared to hydrogen, the electron cloud orbit close to the oxygen atom. Then the oxygen atom has a weak negative charge that cannot donate to other but only share with other atom by a weak coordinate covalent bond.

Since the isotherms are S-type, the free ions have more chances to bind with sludge than the others. The hydroxyl sites were suitable to explain this phenomenon than carboxyl sites. Since the carboxyl site is the irreversible bond. Therefore, the more binding, the less chance for other copper ions to bind.

Metal ions in activated sludge were bound by weak co-ordinate covalent bonds on the lone paired electron zone of the hydroxyl oxygen atoms. These bonds are the reversible bonds that contrast with the irreversible bonds on the carboxyl sites.

Therefore, some investigators tried to explain the reversible biosorption by the stability of the metal-organic ligand complexes (Cheng *et al.*, 1975; Rudd *et al.*, 1984; Sterritt and Lester, 1985; Karapanagiotis *et al.*, 1990).

5.5 Effects of washing on biosorption

The results of specific biosorption by EDTA washed sludge in Table 5.2 did not show any significant different from the unwashed sludge. That means the EDTA solution cannot remove the already bound copper from the biosorption sites. According to Alibhai *et al.* (1985) who summarized possible effects of EDTA on sludge surfaces. They are; (a) extract the present metals; (b) react with the extracellular polymer; (c) change the nature of the binding sites; and (d) inactivate cells. EDTA was found to extract some anionic ligands such as phosphoryl, sulhydryl, or hydroxyl groups from the cell surface. Therefore, the results might be a combination from those total effects or individually.

Figure 5.3 shows the optimum three hours of washing time at every EDTA concentration. At a given EDTA concentration, the possible process is proposed here. The initial step is the extraction of copper from the cell surface by EDTA. Next, the excess EDTA interacts with the free cell surfaces or extracellular polymers. Finally, the combined EDTA either deactivate cells or extract other biosorption sites.

Therefore, at the same concentration of the washing solution, the adsorption increased during the first three hours and then declined after that. It is likely that the removal of other ions from the cell surfaces is completed during the first three hours. The very long period (10 hr) of washing time revealed the lower biosorption yield. The reasons for this are unclear.

Comparison of results of the different EDTA washing solution concentration (Figure 5.4 and 5.5) shows an inverse relationship between concentrations of EDTA and the specific biosorption. 1.00% EDTA washed sludge give the lowest specific biosorption while the lower concentrations resulted in higher adsorption.

The specific biosorption of water washed and sodium chloride washed sludge were above all EDTA washed sludges. Normal saline (0.85% NaCl) seemed to be the best washing solution for sludge compared to any EDTA concentrations studied. The X/M of saline washed sludge is higher than others at every washing time. Deionized-water washed sludge also showed a very good copper adsorption. These mean sodium chloride and deionized water can remove some already attached ions from the cell surfaces.





The presence of alkali or alkali-earth metal ions in the solution normally reduces the efficiency of the biosorption process. Due to passive biosorption is the electrostatic force of the different charges. Therefore, heavy metal and other metals compete to react with the anionic charge on the cell surface. This causes the reduction of the metal removal efficiency (Brierley, 1990). In contrast, sodium ions in this case did not inhibit copper biosorption. Another point is copper has much higher biosorption affinity to sludge than sodium. So, all bound sodium ions will be replaced by copper ions at the equilibrium via the ion exchange process.

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Figure 5.4 Biosorption isotherms of copper by 0.15% EDTA washed sludge at 25 $^{\circ}\mathrm{C}$



Figure 5.5 Biosorption isotherms of copper by 0.50% EDTA washed sludge at 25 $^{\circ}\mathrm{C}$

5.6 Effect of sludge characteristics

Copper biosorption was studied at high and low sludge concentrations during the transient operation of the reactor. The results showed that the biosorption coefficients at each examining point were different (Figure 5.6 and 5.7). These reflected that the biosorption capacity depended on the type of organisms in the sludge rather than the biomass only.

Population dynamics was another factor that influenced the biosorption. There was some evidence to show that exopolymers producing bacteria will biosorb metal higher than those which do not produce exopolymers (Friedman and Dugan, 1968). Although the sludge in these experiments came from the same synthesis source, the population in the reactor was changing. At the low mixed liquor suspended solids (MLSS), the proportion of bacteria to other population, protozoa, rotifer, and sometimes nematodes, was significantly different from that at high MLSS. Therefore, the data were unlikely reproducible.

The gram-negative bacilli were believed to be the predominant species in activated sludge systems for a long time (Allen, 1940; Leslie *et al.*, 1980). Recent study by Bux and Kasan (1994) reported in contrast that ten activated sludge plants in their studies were dominated by gram-positive bacilli. As peptidoglycan is the cell surface composition, the anionic charges on the cell surfaces will react directly with the copper ions. Gram-positive bacteria have a thick peptidoglycan layer, so they may biosorb higher amounts of copper than the gram-negative species.



Figure 5.6 Biosorption isotherms of copper by 0.85% NaCl washed sludge at 25 °C at low MLVSS



Figure 5.7 Biosorption isotherms of copper by 0.85% NaCl washed sludge at 25 °C at high MLVSS

5.7 Effects of operating conditions

5.7.1 Hydraulic retention time (HRT)

The hydraulic retention time (HRT) is a likely factor in determining the removal efficiency. The X/M ranges (0.25-0.96 mg Cu/g dry weight sludge) at two-day HRT were significantly lower than the X/M at one-day HRT (8.80-25.5 mg Cu/g dry weight sludge). This phenomenon was supported by Gordon *et al.* (1988). They Showed that the quantity and composition of slime polysaccharides in the chemostat depended on the dilution rates. At the low dilution rate (0.005 hr⁻¹) the uronic content was lower than at high dilution rates (0.01-0.04 hr⁻¹).

Tait *et al.* (1986) also reported that exopolysaccharide synthesis by *X. campestris* decreased as the growth rate decreased. The composition and proportion of extracellular polymers under different operating conditions were also different. As the slime polysaccharides play the most important role in metal biosorptions, changing of the HRT will influence the metal biosorption.

5.7.2 Solids retention time (SRT)

Solids retention time (SRT) is one of the most debated parameters in related to biosorption. Many workers reported that the biosorption capability by sludge depended on the SRT (Daigger and Grady, 1977; Patterson 1978; Sterritt and Lester 1981). On the contrary, some investigators showed that SRT did not influence the removal efficiency (Elenbogen *et al.*, 1987; Melcer *et al.*, 1992; Nelson *et al.*, 1981). In this experiment, the SRT seemed to affect the biosorption capacity. At the average three-day SRT, the K of Freundlich coefficient is 4.026 while at the average 2.4 day SRT, the K coefficient is 2.802 reflecting higher capacity at higher SRT values.

CHAPTER 6

FINAL DISCUSSION AND CONCLUSIONS

Since copper is used widely, the wastewater of many industries contains high concentration of copper. It reduces the biological efficiency of the waste treatment system. Although some technologies are available to remove metals nowadays, they are still expensive or difficult to operate. Therefore, biosorption is a possibility to accumulate copper from wastewater and then the biosorbed sludge is treated to recover copper.

S-typed isotherms were found in almost all cases. These exhibited the important of reversible biosorption sites. The reaction between ions and the extracellular polymers was taken into account. The loose co-ordinate covalent bonds of oxygen atom and copper positive charge supposedly explained the phenomenon.

The reversible biosorption sites implied that the biosorbed copper can be easily removed. One of the possibilities is bioleaching by copper-tolerant bacteria. *Thiobacillus* are the most well-known genera and applied commercially in copper mining. Thus, it is feasible to recover copper from the contaminated sludge. Not only there is the benefit from recycling the metal but also the treated sludge can also be applied to land without deteriorating the environment.

The average slopes of the biosorption data were used for Langmuir and Freundlich constants calculation. Results show that Freundlich isotherms describe the copper biosorption better. The highest biosorption capacity from the Freundlich isotherms was from 0.85% NaCl washed sludge. In contrast, 0.50% EDTA washed sludge gave the least capacity. Unwashed sludge could biosorb copper approximately at 16 mg Cu per gram dry weight of sludge.

EDTA washed sludge surprisingly gave lower capacity than the unwashed sludge. However, the biosorption capability of copper by sludge increased proportionally with the sludge surface. The deviation pattern of very low and high concentration copper was detected. At the high concentration, the copper possibly deactivated cells. The active uptake of copper happened at the low copper concentration possibly for the intracellular metabolism.

As the Freundlich isotherm is based on the assumption that the adsorbent had a heterogeneous surface composed of different adsorption groups. Perhaps not only the carboxyl and hydroxyl sites but also other anionic species such as phosphoryl, sulhydryl etc., are also the biosorption sites.

The experiment showed that biosorption relied on the biological sludge characteristics. Type of organisms fluctuated during treatment state of the reactor. In this case, predation was an important interrelationship in the process. The oscillating cycle of the biomass for prolonged periods reveals the effect of consumption by the predators. The selection of the slow-growers was also found in the activated sludge. The proportion between the slow-growers and fast-growers changed according to the increase in biomass during the cultivation.

Bulking sludge also detected during the high SRT period. This reveals the main influencing factor for the growth of filamentous bacteria in this experiment is the food to microorganisms ratio.

The optimum time for sludge washing was three hours according to the specific biosorption. This leads us to believe that the EDTA can first remove the already bound ions from the active sites. After that it can replace or damage those sites results in the reduction of specific biosorption. In addition, the long washing time can also deactivate or damage the cells.

Sodium chloride and deionized washed sludge seem to give the highest adsorption. Therefore, some ions will be removed or replaced by sodium ions or protons from water molecules. The washing results also revealed that sodium ions have less binding affinity than copper ions.

The hydraulic retention time (HRT) of the activated sludge system influenced the copper biosorption. The specific biosorption of two-day HRT was lower than the one-day HRT. This was perhaps the effect of HRT on the production of extracellular polymers, which are directly related to the biosorption capacity of biomass.

In addition, SRT also influenced the biosorption capacity. The older cells can biosorb copper better than the younger ones. This maybe the effect of cell structure itself or cell products.

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APPENDICES
100 #7.931 x 1/(X/M) (mg/mg) 80 60 (a.) 40 0.0200 0.0225 0.0250 0.0275 0.0300 0.0325 0.0350 1/C [l/mg] 4ª - 8.771) 2316.231 x - 7.4861 80 70 1/(X/M) (mg/mg) 60 50 (b.) 40 30 0.025 0.030 0.020 0.035 0.040 1/C (I/mg)



Figure A.1 Langmuir biosorption isotherms of copper by unwashedsludge at 25 °Ca.) low MLSSb.) high MLSS



Figure A.2 Freundlich biosorption isotherms of copper by unwashed sludge at 25 ℃ a.) low MLSS b.) high MLSS



Figure A.3 Langmuir biosorption isotherms of copper by 0.15% EDTAwashed sludge at 25 °Ca.) low MLSSb.) high MLSS



Figure A.4 Freundlich biosorption isotherms of copper by 0.15% EDTAwashed sludge at 25 °Ca.) low MLSSb.) high MLSS



Figure A.5 Langmuir biosorption isotherms of copper by 0.50% EDTA washed sludge at 25 $^{\circ}\mathrm{C}$



Figure A.6 Freundlich biosorption isotherm of copper by 0.50% EDTA washed sludge at 25 $^{\circ}\mathrm{C}$



Figure A.7 Langmuir biosorption isotherms of copper by 0.85% NaClwashed sludge at 25 °Ca.) low MLSSb.) high MLSS



Figure A.8 Freundlich biosorption isotherms of copper by 0.85% NaClwashed sludge at 25 °Ca.) low MLSSb.) high MLSS