KINETIC STUDY OF BIOSORPTION AND BIOREDUCTION OF HEXAVALENT CHROMIUM BY UNMODIFIED SAWDUST

NG ZHUNG GIA

UNIVERSITI SAINS MALAYSIA

2013

KINETIC STUDY OF BIOSORPTION AND BIOREDUCTION OF HEXAVALENT CHROMIUM BY UNMODIFIED SAWDUST

by

NG ZHUNG GIA

Thesis submitted in fulfillment of the requirements
for the degree of
Master of Science

December 2013

ACKNOWLEDGEMENTS

First and foremost, my appreciation goes to my supervisor, Prof Lim Poh Eng for his guidance and patience. It is impossible for me to finish my Master study without his assistance. Besides, my gratitude is also extended to Dr Seng Chye Eng and Prof Norita Mohamed for their concern all the time.

Secondly, I thank my family, especially my parents, for their strong support.

Also, I am thankful for the assistance from my colleagues and staff of School of Chemical Science, USM. Their experiences and advices during my study benefited me very much.

Last but not least, I wish to express my gratitude for the financial assistances from USM Fellowship and MyBrain15.

TABLE OF CONTENTS

Page

CHAPTER 1: INTRODUCTION

Page

CHAPTER 2: MATERIALS AND METHODS

CHAPTER 3: RESULTS AND DISCUSSION

CHAPTER 4: CONCLUSIONS

Appendix

LISTS OF TABLES

LIST OF FIGURES

KAJIAN KINETIK BAGI BIO-PENJERAPAN DAN BIO-PENURUNAN KROMIUM HEKSAVALEN OLEH SERBUK KAYU TAK UBAHSUAI

ABSTRAK

Kesan kepekatan awal Cr(VI), pH awal larutan dan dos serbuk kayu tak ubahsuai terhadap bio-penjerapan-bio-penurunan Cr(VI) telah masing-masing dinilai dalam linkungan kepekatan awal Cr(VI) 250 - 1500 μM, lingkungan pH 2.0 - 4.0 dan lingkugan dos serbuk kayu tak ubahsuai 2 - 4 g L^{-1}. Kesan fenol dan Zn(II) pula telah dinilai pada nisbah molar Cr(VI): fenol dan Cr(VI):Zn(II) sejumlah 1:0, 2:1, 1:1, 1:3 dan 1:5 masing-masing. Suatu eksperimen rujukan telah dijalankan pada keadaan: kepekatan awal Cr(VI) 500 μM, pH awal 2.0, dos serbuk kayu tak ubahsuai 4 g L^{-1}, kelajuan penggoncangan 250 rpm dan suhu pada 25 (±1) °C untuk menunjukkan bahawa penyingkiran Cr(VI) oleh serbuk kayu tak ubahsuai melibatkan proses bio-penjerapan dan bio-penurunan.

Keputusan menunjukkan bahawa kadar penyingkiran Cr(VI) menurun mengikut peningkatan kepekatan awal Cr(VI) akibat kehabisan tapak aktif pada bio-penjerap. Peningkatan pH awal larutan menyebabkan penurunan kadar penyingkiran Cr(VI) kerana pengurangan darjah protonasi bio-penjerap. Penurunan penyingkiran Cr(VI) juga didapati dengan pengurangan dos serbuk kayu tak ubahsuai. Kehadiran Zn(II) dengan kepekatan yang meningkat didapati melambatkan kadar penyingkiran Cr(VI) yang diakibatkan oleh persaingan antara H^+ dengan Zn(II) untuk dijerap atas bio-penjerap. Sebaliknya, kehadiran fenol dengan peningkatan kepekatan menunjukkan kesan yang dapat diabaikan terhadap penyingkiran Cr(VI).

Satu mekanisme telah dibangun untuk menghuraikan proses bio-penjerapan dan bio-penurunan Cr(VI)oleh serbuk kayu tak ubahsuai. Berdasarkan mekanisme itu, suatu model kinetik telah diterbitkan dan model ini mencadangkan bahawa kadar penyingkiran Cr(VI) masing-masing bertertib pertama terhadap kepekatan Cr(VI), H^+ dan sebatian organik dalam bio-penjerap yang mengakibatkan penurunan Cr(VI).

KINETIC STUDY OF BIOSORPTION AND BIOREDUCTION OF HEXAVALENT CHROMIUM BY UNMODIFIED SAWDUST

ABSTRACT

The effects of initial Cr(VI) concentration, initial pH and sawdust dosage on Cr(VI) biosorption-bioreduction were evaluated at the initial Cr(VI) concentration range of 250 -1500 μM, pH range of 2.0 - 4.0 and dosage range of 2 – 4 g L^{-1}, respectively. The effects of phenol and Zn(II) were evaluated at the Cr(VI):phenol and Cr(VI):Zn(II) molar ratios of 1:0, 2:1, 1:1, 1:3 and 1:5. A control experiment was conducted under the conditions: initial Cr(VI) concentration of 500 μM, initial pH of 2.0, sawdust dosage of 4 g L^{-1}, shaking speed of 250 rpm and temperature of 25 (±1) ^{o}C to show that Cr(VI) removal by sawdust involved biosorption and bioreduction.

The results showed that Cr(VI) removal rate decreased with the increase of initial Cr(VI) concentration due to the exhaustion of active sites on the biosorbent. Increasing initial solution pH resulted in slower Cr(VI) removal rate because of the decrease of the degree of protonation for biosorbent. Cr(VI) removal rate decreased with the decrease of unmodified sawdust dosage. The presence of Zn(II) in increasing concentration was observed to increasingly retard Cr(VI) removal rate due to competition with H^+ to be adsorbed onto biosorbent. In contrast, the presence of phenol in increasing concentration showed negligible effect on Cr(VI) removal.

A mechanism was developed to describe the biosorption and bioreduction of Cr(VI) by sawdust. Based on the mechanism, a kinetic model was derived and it suggests that

Cr(VI) removal rate is first-order with respect to the concentrations of Cr(VI), H^+ and organic compounds in biosorbent participating in Cr(VI) reduction, respectively.

CHAPTER 1

INTRODUCTION

1.1. Chromium

Chromium (Cr) has an atomic number of 24 and a nucleon number of 51.9961. It has 6 isotopes. In the world, it is the 21st abundant element. Owing to its magnificent effect in dealing with corrosive agent, Cr is used widely in the production of stainless steel. It is also used in the industries of leather tanning, dye and pigment, wood preservation and refractory. In the periodic table of elements, it is one of the transition metals and hence exhibits various oxidation states, ranging from zero-valent (Cr^0), divalent (Cr^{2+}), to trivalent (Cr^{3+}), tetravalent (Cr^{4+}), pentavalent (Cr^{5+}) and hexavalent (Cr^{6+}). Divalent chromium is stable only in anaerobic condition. Both tetravalent and pentavalent chromium are the intermediates in the reactions involving Cr(VI) reduction and/or Cr(III) oxidation. Trivalent and hexavalent chromium are the most stable, and hence can exist in environment. Also, both species have many forms in solution at varying pH.

1.1.1. Trivalent chromium, Cr(III)

Cr(III) is the naturally occurring species of Cr in the environment. It is present in soil, rocks, air and water. Hence, human race is exposed to Cr naturally via breathing and drinking Cr containing water. Besides, cigarette smoke and food are also the sources of Cr (Assem and Zhu, 2007). Exposure to Cr(III) is not a health issue to human due to low toxicity of Cr(III). The International Agency for Research on Cancer (IARC), a part of

World Health Organization, has listed Cr(III) compounds as “not classifiable as to its carcinogenicity to humans”. It is poorly absorbed by human body as only about 0.5 – 1% of dietary Cr(III) is absorbed (Assem and Zhu, 2007). Absorbed Cr(III) strengthens insulin in regulating blood glucose level in body (Sharma et al., 2011) and the absence of Cr(III) results in the ineffective transportation of glucose into cells and a rise in the blood glucose level. However, the deficiency of Cr(III) is rare as human obtains it easily via daily intake of fruits, vegetables, meat, cheese, etc. Trace amount of Cr(III) in body is also needed for the metabolisms of fat, lipid, protein and carbohydrates (Chirwa and Wang, 2000). However, chronic exposure of Cr(III) still results in weight loss, anaemia, liver dysfunction and renal failure.

Environmentally, the pH of natural water bodies is higher than pH 4, which favours the precipitation of Cr(III) as $Cr(OH)_3$. Besides, the Cr(III) species is also easily absorbed by solids in water, which makes it immobilized. In addition, Cr(III) species is thermodynamically stable in the natural environment. Other forms of Cr has a tendency to be converted into Cr(III) under that condition (Barnhart, 1997).

1.1.2. Hexavalent chromium, Cr(VI)

In contrast to Cr(III), Cr(VI) is the most notorious species among all Cr species. Cr(VI) does not occur naturally in the environment. It is produced due to anthropogenic activities, such as the combustion of fuels and industrial wastes. This species of Cr is toxic to human with its toxicity 100 times higher than that of Cr(III) (Chirwa and Wang, 2000). The major routes of exposure to Cr(VI) are by inhalation, ingestion, skin and eye exposure (Assem and Zhu, 2007). Unlike Cr(III), Cr(VI) is more readily absorbed. It is unstable in human body and can be reduced. Some believe that the reduction of Cr(VI) causes the damage to our body by releasing free radicals. The exposure to Cr(VI) can also result in liver damage, internal hemorrhage, respiratory ulcer, dermatitis, etc (Xu et al., 2005). As there are many studies revealing the carcinogenicity of Cr(VI), IARC has grouped it into group 1, which means that it is carcinogenic to human.

In the environment, Cr(VI) behaves differently from Cr(III) in water. It is considered as a totally soluble compounds in nature, unless in contact with lead. Therefore, it can travel for a great distance. Due to its mobility and toxicity, the discharge of Cr(VI) containing industrial waste into natural water bodies contributes to the environmental contamination. Since water bodies are contaminated, the drinking water becomes a potential source of Cr(VI). Hence, removing Cr(VI) from water is of great concern and interest.

1.1.3. Speciation of Cr(VI) and Cr(III)

Fig. 1.1 shows the distribution of Cr(VI) species in an aqueous medium. At different ranges of pH, Cr(VI) species transform from one form to the other. There are as many as 5 species of Cr(VI), namely H_2CrO_4, $HCrO_4^-$, CrO_4^{2-}, $HCr_2O_7^-$ and $Cr_2O_7^{2-}$ from pH 0 to pH 14. All species are soluble. At pH 0, the dominant species of Cr(VI) are H_2CrO_4 and $HCrO_4^-$. The H_2CrO_4 species starts to decrease and finally disappears at around pH 2. Meanwhile, $HCrO_4^-$ predominates at around pH 2 and becomes the most abundant

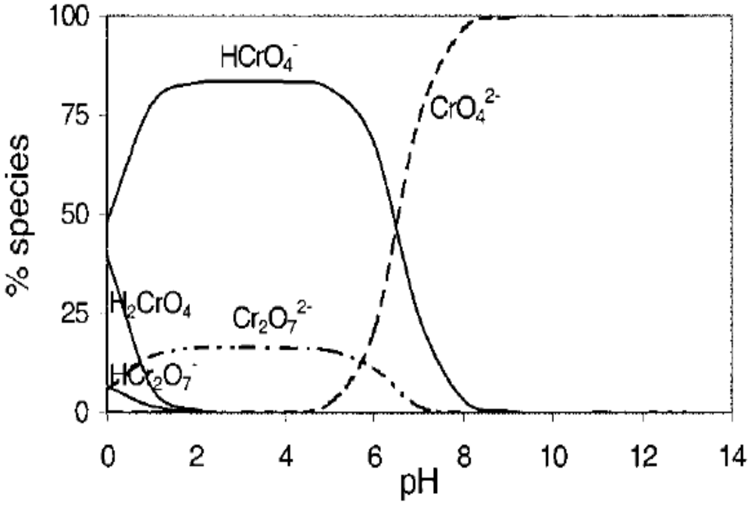

Figure 1.1: The distribution of Cr(VI) species in an aqueous medium with a total Cr(VI) concentration of 7.69 x 10^{-3} M at a temperature of 25 °C (Cabatingan et al., 2001)

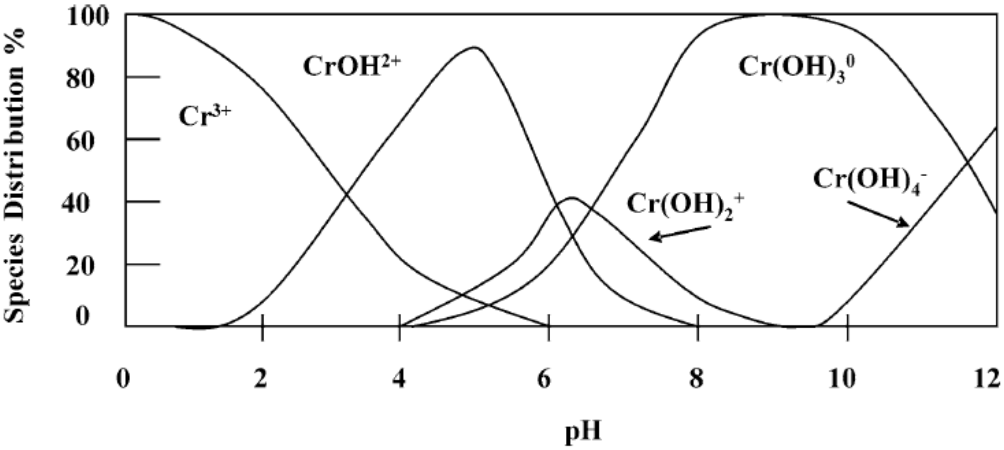

Figure 1.2: The distribution of Cr(III) species as a function of pH (Bradl, 2004)

species and also maintains at a percentage of around 80% in the medium. This is accompanied by the existence of $HCr_2O_7^-$ at a percentage of 20%. This shows that only 2 species occur from around pH 2 to around pH 5. Starting at around pH 5, both $HCr_2O_7^-$ and $HCrO_4^-$ start to decrease and are converted into another species, CrO_4^{2-}. At around pH 8, CrO_4^{2-} is the only species that is stable in the alkaline condition.

Fig. 1.2 shows the distribution of Cr(III) at varying pH condition. The species of Cr(III) found are Cr^{3+}, $CrOH^{2+}$, $Cr(OH)_2^+$, $Cr(OH)_3^0$ and $Cr(OH)_4^-$. Starting from pH 0, the only species is Cr^{3+}. This species gradually decreases and at pH 6, it converts totally to other species and cannot be found anymore beyond pH 6. At around pH 1, $CrOH^{2+}$ starts to increase and achieves its highest amount of around 90%, at around pH 5. After that, this species decreases and totally converts into other species at pH 8. Meanwhile, another species, $Cr(OH)_2^+$ starts to build up at around pH 4. It attains a percentage of 40% in solution at around pH 6 and after that, it decreases in amount and finally converts into other species at around pH 9. Starting from pH 4, $Cr(OH)_3$ exists, This is a known insoluble species of Cr(III), especially at alkaline condition. All Cr(III) species converts into this species at around pH 9. At higher pH, the OH^- ions bind to insoluble $Cr(OH)_3$ and resulting in the existence of $Cr(OH)_4^-$, which is a soluble species.

1.2. Removal methods of Cr(VI)

Many methods have been developed and utilized to remove Cr(VI) from industrial wastewaters. The following sections describe some conventional treatment methods for Cr(VI) removal.

1.2.1. Conventional Cr(VI) treatment

The conventional method to remove Cr(VI) from industrial wastewater is by reducing Cr(VI) to Cr(III) and precipitating the latter species using hydroxide, sulfide, carbonate or phosphate. A review by Barrera-Díaz et al. (2012) mentioned that there are two conventional methods to regulate Cr concentration in water. One of the treatment methods employs sulfur dioxide gas or sodium bisulfate in an acid solution as a reducing agent, followed by precipitating Cr(III) produced using sodium or calcium hydroxide. Theoretically, 3 kg of sodium bisulfate together with 2 to 3 kg of sulfuric acid can reduce 1 kg of Cr(VI). The other treatment method involves the reduction of Cr(VI) by using Fe (II) in acidic solution. After the reduction, Cr(III) is precipitated using alkali. The reducing agent is either ferrous sulfate or sodium sulfite. A solid waste of ferric hydroxide is produced if ferrous sulfate is used. Sulfur dioxide, which is toxic, odorous and volatile, is produced if sodium sulfite is used. In general, both conventional treatments employ vast quantity of chemicals. In addition, these popular methods generate sludge which becomes a secondary solid waste (Miretzky and Cirelli, 2010). Also, these methods are ineffective if Cr(VI) concentration is too low (Wang and Chen, 2009).

1.2.2. Electrochemical reduction of Cr(VI)

The electrochemical reduction of Cr(VI) is another technique found promising in reducing Cr(VI) (Gao et al., 2005; Ölmez, 2009; Barrera-Díaz et al., 2012). The popular electrode used is Fe.

With Fe as the anode, the reactions are as follows:

Anodic reaction: $Fe(s) \rightarrow Fe^{2+}(aq) + 2e$ (1.1)

Cathodic reaction: $2H_2O + 2e \rightarrow 2OH^{-}(aq) + H_2(g)$ (1.2)

The Fe^{2+} ions released from the anode reduce Cr(VI) to Cr(III) instantly in the solution.

$$6Fe^{2+} + Cr_2O_7^{2-} + 14H^{+} \rightarrow 6Fe^{3+}\ 2Cr^{3+} + 7H_2O \tag{1.3}$$

The Cr(III) species is precipitated by OH^{-} produced from cathode. The overall reaction that governs the process is:

$$Cr_2O_7^{2-} + 6Fe^{2+} + 14H^{+} \rightarrow 2Cr^{3+} + 6Fe^{3+} + 7H_2O \tag{1.4}$$

Aluminium (Al) is another popular element used as an anode. In this context, Cr(VI) is reduced to Cr(III) and there is an amount of Cr(VI) found adsorbed on the $Al(OH)_3$, the sludge formed in this process. This suggests that adsorption also plays a role in this process.

This method, however, suffers from certain disadvantages: (i) the requirement of external energy for operation, (ii) the usage of large amount of chemicals and (iii) the generation of sludges, namely $Fe(OH)_3$ and $Al(OH)_3$.

1.2.3. Other treatment methods

A number of methods have in fact been developed and they promise eco-friendliness, low-cost and great efficiency. Ion exchange, adsorption using activated carbon and reverse osmosis are among the methods that have drawn the attention of researchers and

Table 1.1 Conventional metal removal techniques (Volesky, 2001)

Method	Advantage	Disadvantage
Electrochemical treatment	metal recovery	for high concentration expensive
Reverse osmosis	pure product	high pressure membrane scaling expensive
Ion exchange	effective pure effluent metal recovery	sensitive to particle expensive
Adsorption	conventional sorbents	expensive (activated carbon)
Evaporation	pure product	energy extensive expensive resulting sludges

scientists. These techniques are promising in Cr removal but are still expensive due to high operational costs and require a lot of chemicals. The advantages and disadvantages are shown in Table 1.1. Hence, there is a need in developing a better and eco-friendly technique to reduce the cost and minimize the production of secondary waste.

1.3. Biosorption

The technique of biosorption has become the attention of scientists as it offers promising result while it is low-cost and eco-friendly. Biosorption is the process of removing metals, metalloid species, compounds and particulates from solution using biological materials, especially microbial biomass (Gadd, 1993). The latter is invariably known as biosorbent. Early attempt of biosorption was done during 1930s (Volesky and Holan, 1995). This technology was brought to the foreground of scientific interest during 1970s after the awareness of the environment motivated research (Tsezos, 2001). Most researchers were using microbial biomasses, for examples, algae, moss and fungi and bacteria as the raw materials for biosorption (Ting and Teo, 1994; Rapoport and Muter, 1995; Sağ and Kutsal, 1997; Yin et al., 1999; Göksungur et al., 2005; Pagnanelli et al., 2009; Ramrakhiani et al., 2011). In their research, they found that the microbial biomasses were able to remove many kinds of heavy metal ions, such as, Fe(III), Cu(II), Zn(II), Pb(II), Cd(II) and Cr(VI). In addition, these microbial biomasses have been the interest of researchers because of their ready availability as industrial and laboratory by-products (Ramrakhiani et al., 2011) and their abundance in nature (e.g. seaweed) (Volesky, 2001) which underscore the attributes of low-cost and eco-friendliness. The mushrooming research pertaining to biosorption was in its native form until 1980s, during which the approaches of immobilizing microbial biomass were developed, tested, patented and proposed (Tsezos, 2001). Also, some researchers studied the difference between the microbial biomass and the corresponding modified or derived biosorbents (Aderhold et al., 1996). However, relatively fewer work were done using abundant natural materials, for instance, sawdust, rice husk and bark (Kratochvil and Volesky, 1998). Since 2000s, more researchers have been putting effort in using agricultural wastes as their biosorbents because the culturing of microbial biomass requires the use of chemicals, which increases the cost (Sud et al., 2008).

1.3.1. Biosorbents

There have been many materials that are used for biosorption. Elementally, the biosorbents are categorized into, but not limited to, two groups, namely microbial biomasses and agricultural wastes.

1.3.1.1. Microbial biomasses

In principle, materials that are living, or derived from living organisms can be considered as microbial biomasses. The commonly used microbial biomasses are fungi, algae and bacteria. Dead or living microbial biomass makes the biosorption different (Veglio and Beolchini, 1997). In the case of living microbial biomass, the biosorption becomes metabolism dependent and heavy metals become toxicants to the living cells. The reaction is hence not instant as it takes time for living cells to respond over the presence of the toxicants. Besides, this metabolism dependent biosorption involves two mechanisms, namely transport across the cell membrane and precipitation. Cells need metabolically essential ions, such as Ca(II) and Mg(II). However, other divalent heavy metal ions, such as Pb(II) and Cu(II), if present, could also be transported across cell membrane. On the other hand, the defense system of living cells plays the role in the precipitation of heavy metals.

The mechanism of biosorption of dead cells, however, always occurs at the surface of the cells since the metabolic transport of heavy metals is impossible. Primarily, there are four mechanisms in this category, namely physical adsorption, ion exchange, complexation and precipitation. However, the mechanisms of the biosorption are still not well understood because the reactions of heavy metals by microbial biomass are complicated (Volesky, 2001). This could be the reason that many researchers did not put effort in decoding the real mechanism of biosorption in their studies (Siegel et al., 1986; Aksu et al., 1999).

By comparing both living and dead microbial biomass, the latter is more outstanding since it offers lower cost and more rapid rate of uptake. Besides, the dead microbial biomass requires no external metabolic energy, not severely affected by temperature compared with living microbial biomass and can be regenerated easier (Vijayaraghavan and Yun, 2008).

1.3.1.2. Agricultural wastes

Countless agricultural wastes, such as banana skin (Park et al., 2008b), rice husk (Bansal et al., 2009a), sawdust (Vinodhini and Das, 2010), pine cone (Ucun et al., 2009), coconut coir (Gonzalez et al., 2008), leaves (Park et al., 2011), palm flower (Elangovan et al., 2008b), etc, have been used in the study of biosorption. Agricultural waste is favoured by researchers because of its abundance in nature and also it has more advantages, just the same as the dead microbial biomass, compared with the living microbial biomass. Agricultural waste can be considered as dead biomass and hence the mechanisms of biosorption involved are extracellular, such as ion exchange, complexation and physical adsorption.

Among all agricultural wastes, rice husk and sawdust are abundant in Malaysia. According to the statistics of Malaysia External Trade Development Corporation (MATRADE), Malaysia ranks as the eighth largest furniture exporter and contributes 3% to the value of the world furniture export in 2010. This confirms that sawdust can be obtained easily. Meanwhile, Department of Statistics Malaysia documented that the rice production of Malaysia in 2010 was more than 1.14 million tonnes, which indirectly affirms the abundance of rice husk.

(a) Rice husk

Rice husk is a kind of agricultural wastes produced by rice milling industry. The average length of rice husk is 4-5 mm and the bulk density is 96-160 kg m^{-3}. The components of rice husk are cellulose (43.3%), sugar (32-33%) and lignin (23%) (Bansal et al., 2009a).

Rice husk has been widely used in research to remove heavy metals. Bansal et al. (2009a) studied the biosorption of Cr(VI) using boiled rice husk and formaldehyde-treated rice husk and found that the efficiencies were 71.0% and 76.5%, respectively. Rice husk treated with 50% H_2SO_4 was found to remove 91.75% of Cr(VI) (Bansal et al., 2009b). Besides, rice husk was used to produce carbonaceous sorbent by heating 20 g of rice husk and heated to 175-180°C in 20 min. It was found to remove Zn(II) effectively in 2 h but around 120 h to remove Hg(II) by reducing it to Hg(I) (El-Shafey, 2010). However, there were very few biosorption studies that used raw rice husk.

(b) Sawdust

Sawdust is a by-product of timber industry and furniture industry. Many researchers have been using it for the removal of contaminants from water. It has been a favorable option because it is a lignocellulosic waste that comprises lignin and carbohydrates (cellulose and hemicelluloses). These compounds contain a number of functional groups, which are hydroxyl, carboxyl and phenolic groups (Akmar Zakaria et al., 2009) that contribute to biosorption. Due to the complexity and the abundance of functional groups in sawdust, it has been used widely in removing dyes (Dulman and Cucu-Man, 2009), oils (Shukla et al., 2002) and heavy metals (Yu et al., 2003; Semerjian, 2010).

As heavy metal pollution is one of the major environmental concerns, sawdust was used as a biosorbent by many researchers to remove heavy metals. Li et al. (2007) used sawdust to investigate the kinetics of biosorption of Pb(II), Cr(III) as well as Cu(II) and found that the adsorption rate of Pb(II) was the fastest, followed by Cu(II) and that of Cr(III) was the slowest. Sawdust from walnut was found to preferably adsorb Pb(II), than Cd(II) and Ni(II) (Bulut and Tez, 2007). Besides, sawdust was also modified to improve its heavy metal binding ability. Red pine sawdust was modified into base extracted sawdust and tartaric acid-modified sawdust and the percentage of Cr(VI) removal by tartaric acid-modified sawdust (87.7%) was higher than that of base extracted sawdust (70.6%) at the initial pH 3 (Gode et al., 2008). Oak sawdust was treated by HCI acid and was found to remove 84% of Cr(VI) at pH 3 (Argun et al., 2007). Holly sawdust under formaldehyde modification was found to remove Cr(VI) with its maximum adsorption capacity of 18.86 mg g^{-1} at pH 7 (Siboni et al., 2011). However, there are no reports on Cr(VI) bioreduction using sawdust but a number of reports on Cr(VI) biosorption (Argun et al., 2007; Gode et al., 2008; Gupta and Babu, 2009; Miretzky and Cirelli, 2010; Siboni et al., 2011; Hamed Mosavian et al., 2012).

1.3.2. Effects of various operational factors on biosorption

1.3.2.1. Initial concentrations of heavy metals

The study of the initial concentrations of heavy metals means varying the initial concentrations of the heavy metals while keeping the dosage of biosorbents, the solution pH and the temperature constant. Under these conditions, the heavy metal concentration at equilibrium and the heavy metal uptake by biosorbents can be determined. Basically, the higher the initial concentration of heavy metals, the larger will be the amount of heavy metals ions sequestered by biosorbents. Çetinkaya Dönmez et al. 1999 revealed that by increasing the initial concentration of Cu(II) from 25.0 to 250.0 mg L^{-1}, the uptake of Cu(II) by dried algae increased from 10.3 to 46.8 mg g^{-1}. In terms of removal, the percentage of the heavy metals removal decreased with the increase of initial concentration of heavy metals. Barkat et al. (2009) found that by increasing the initial concentration of Cr(VI) from 20 to 120 mg L^{-1}, the percentage of Cr(VI) adsorbed decreased from around 95% to around 80%.

1.3.2.2. Contact time

The contact time is the time required for the adsorption to achieve the equilibrium. The contact time varies from one case to another. The biosorption of heavy metals by different biosorbents involves different contact times. For instance, the biosorption of Cd (II) by *Rhizopus cohnii*, a type of fungi, took around 2 h to achieve equilibrium while only 55 min was required by non living algal biomass *Oedogonium* sp. (Gupta and Rastogi, 2008; Luo et al., 2010). The biosorption of heavy metals by the same kind of biosorbents may require different contact time too. Argun et al. (2007) reported that, using modified oak sawdust, the contact times required for Cu(II), Ni(II) and Cr(VI) to achieve equilibrium were 4, 8 and 8 h, respectively. Without adequate contact time for biosorbents and substances of interest to mix well, the equilibrium may not be attained. There are some researchers who did not report the contact time in their studies. Thus, the reliability of the results can be in doubt (Çetinkaya Dönmez et al., 1999; Nurbaş Nourbakhsh et al., 2002). The contact time of biosorption involving bioreduction also alters with varying pH condition. Park et al. (2005a) found that the contact time of Cr(VI) bioreduction was pH-dependent. The lower the pH, the shorter was the contact time required to reduce Cr(VI) completely. Besides, as long as the biosorbents remained in excess, all Cr(VI) can be reduced to Cr(III) with adequate time. Park et al. (2007a) found that no equilibrium was achieved.

1.3.2.3. Initial solution pH

Varying the initial pH in the study of biosorption changes the condition of the study dramatically. It effectively alters the nature of the active sites of the biosorbents, as well as the states of heavy metals. For instance, the species of Cr(VI) and Cr(III) are different at different pH values as shown in Fig. 1.1 and Fig. 1.2. Biosorbents are well known of containing various functional groups that are used to interact with heavy metals. The major functional groups are hydroxyl, carbonyl, carboxyl, phosphate, amide, amine and others. (Volesky, 2007). At lower initial solution pH, the active sites of biosorbents are protonated and hence repel cationic heavy metals. At higher initial solution pH, the solution becomes too alkaline and hydroxides of heavy metals are formed and precipitated prior to the biosorption process. In a recent study, it was shown that free Pb^{2+} and Cu^{2+} ions form precipitates due to higher concentrations of OH^- at pH higher than 3.0 and 5.0, respectively (Tunali et al., 2006). At pH higher than pH 2.5, Fe (III) ions form precipitates as well (Sağ and Kutsal, 1998). The investigation of the biosorption of Cd (III) ion, on the other hand, should not exceed pH 7 (Luo et al., 2010). There are some researchers who overlook this factor while studying the biosorption of Cu (Tong et al., 2011) and Pb (Say et al., 2001). This may yield unreliable results.

1.3.3. Effects of co-pollutants on biosorption

1.3.3.1. Cations, anions and oxyanions

The wastewater containing Cr(VI) also contains other heavy metals as pollutants and the method of biosorption for Cr(VI) removal must hence take this factor into consideration. There were researchers studying the effect of cations on Cr(VI) biosorption and their findings differed. Tang et al. (2003) studied the effect of Cu(II) on Cr(VI) biosorption and found that Cr(VI) biosorption was enhanced by 1.8 times in the presence of Cu(II). They attributed this phenomenon to the enhancement of the electrostatic forces between Cr(VI) and the surface adsorbing Cu(II). However, this study might not be comprehensive and rigorous as only single concentration of Cu(II) was studied. In contrast, Aksu et al. (2002) studied the effect of Ni(II) with concentrations ranging from 0 to 500 mg L^{-1} on Cr(VI) biosorption and found that Ni(II) exhibited inhibitory effect on Cr(VI) biosorption. The higher the concentration of Ni(II) in the system, the greater was the inhibitory effect of Ni(II) on Cr(VI) biosorption. They claimed that the partial competition between Ni(II) and Cr(VI) to be adsorbed on the biosorbent resulted in poorer Cr(VI) biosorption. However, Park et al. (2006) drew a different conclusion. They reported that Ni(II) showed insignificant effect on Cr(VI) biosorption after running two sets of experiments at two different initial Cr(VI) concentrations with Ni(II) concentrations ranging from 50 to 500 mg L^{-1}. Based on the results, they inferred that the surface sites on the biosorbent for Ni(II) and Cr(VI) biosorption were totally different. In other words, the biosorption of Cr(VI) and Ni(II) was independent of each other. This finding was substantiated by another study (Park et al., 2005c) which reported that Zn(II) and Cr(III) at the concentration of 500 mg L^{-1}, respectively, showed no effect on Cr(VI) biosorption. In light of the above contradictory findings, further study on the effect of heavy metal cations on Cr(VI) biosorption is warranted.

In terms of the effect of anions on Cr(VI) biosorption, Park et al. (2005c) studied the effects of several anions, i.e. Cl^-, NO_3^-, CO_3^{2-}, SO_4^{2-} and HPO_4^{2-} on Cr(VI) biosorption and found that the anions exerted the inhibitory effect in the order of $Cl^- < NO_3^- < CO_3^{2-} < SO_4^{2-} < HPO_4^{2-}$. The inhibition could be explained by the interruption of anions on the binding of Cr(VI) anions with the active sites of biosorbent. However, it should be noticed that the study was based on a single concentration of respective anions and therefore the result might not provide a clear picture.

The effect of oxyanion on Cr(VI) biosorption was rarely studied. Niu and Volesky (2006) studied the effect of V(V) on Cr(VI) biosorption and their result showed that the presence of V(V) severely affected the chromium uptake by acid-washed crab shells. At higher initial Cr(VI) concentration, viz. 5.3 mM, the uptake of Cr(VI) was only at 43% and this value slumped to 30% at the initial Cr(VI) concentration of 1.3 mM. As a conclusion, the effect was more prominent at low concentration of initial Cr(VI) concentration.

1.4. Mechanisms of Cr(VI) biosorption

Throughout the extensive studies by scientists and technologists, the mechanisms of biosorption have been found to involve ion exchange, complexation, coordination, adsorption, electrostatic interaction, chelation, bioreduction and microprecipitation. There is also a possibility for redox reaction to take part in biosorption as well. The combination of any of these may also occur in any case of biosorption (Kratochvil and Volesky, 1998a; Çetinkaya Dönmez et al., 1999; Volesky, 2001; Sud et al., 2008;Wang and Chen, 2009). However, the understanding of the mechanism is still not comprehensive and inconclusive.

Among all heavy metals that have been studied for biosorption, Cr exhibits a distinctly different behavior. The characteristics of having two stable ions, cation for Cr(III) and oxyanion for Cr(VI), in water, which was overlooked by a number of researchers in earlier studies, resulted in four proposed mechanisms to decode the behavior of the Cr(VI) removal by biosorption.

1.4.1. Anionic adsorption

The mechanism of anionic adsorption is generally accepted by a pool of researchers (Ajmal et al., 1996; Raji and Anirudhan, 1998; Agarwal et al., 2006; Sarin et al., 2006a; Sarin and Pant, 2006b ;Sawalha et al., 2006; Argun et al., 2007; Basha and Murthy, 2007; Uysal and Ar, 2007; Basha et al., 2008; Meena et al., 2008; Bansal et al., 2009a; Gupta and Babu, 2009; Jain et al., 2009; Hamed Mosavian et al., 2012). The researchers who agreed to this mechanism were using a common procedure of Cr analysis. They analysed either remaining Cr(VI) only or total Cr only in solution. As such, the researchers failed to identify the existence of Cr(III). As a consequence, they interpreted that the adsorption of Cr(VI) and biosorbents was due to electrostatic attraction between the negatively charged Cr(VI) and the positively-charged biosorbents. This false observation led them to conclude that anionic adsorption plays the role in the biosorption of Cr(VI).

1.4.2. Anionic and cationic adsorption

Daneshvar et al. (2002) assumed that at low pH (pH 2.0), H^+ combined with negatively charged organic functional groups of the surface of biosorbents. This enhanced the sorption between anionic Cr(VI) species and the functional groups. They claimed that the reduced form of Cr(VI), which is Cr(III), was chelated in solution. This minimized the repulsion between the cationic Cr(III) species and the positively charged surface, for instance, NH_3^+. Hence, both Cr(III) and Cr(VI) were sorbed onto biosorbents in their study. Hasan et al. (2008) advocated this mechanism too. They observed that the biosorbent after biosorption process in their study turned greenish. Based on this observation, they inferred that Cr(III) was bound to the biosorbent because Cr(III) is known commonly in green colour. Besides, due to the reason that biosorbent was positively charged at low pH, binding of negatively charged Cr(VI) by the biosorbent became permissible. Regardless of the different deductions that have been made, the two groups of researchers confirmed that the biosorption of Cr(VI) involved anionic and cationic adsorption, which occurred simultaneously.

1.4.3. Reduction and anionic adsorption

Cabatingan et al. (2001) assumed that Cr(VI) biosorption involves the processes of simultaneous biosorption and reduction of chromate. They developed a kinetic model to describe the mechanism.

During the sorption:

$$\mathrm{Cr(VI)(l)} + \mathrm{B(s)} \overset{\mathrm{k_s}}{\leftrightarrow} \mathrm{B-Cr(VI)(s)} \tag{1.5}$$

During the reduction:

$$\mathrm{Cr(VI)(l)} + \mathrm{B(s)} \overset{\mathrm{k_r}}{\rightarrow} \mathrm{Cr(III)(l)} \tag{1.6}$$

where B is the available active sites, k_s is the rate constant of sorption and k_r is the rate constant of reduction

The rate laws for the processes are:

$$\frac{\mathrm{d[Cr(VI)]}}{\mathrm{dt}} = -\mathrm{k_s[B][Cr(VI)]} - \mathrm{k_r[B][Cr(VI)]} \tag{1.7}$$

$$\frac{\mathrm{d[Cr(III)]}}{\mathrm{dt}} = \mathrm{k_r[B][Cr(VI)]} \tag{1.8}$$

$$\frac{\mathrm{d[B]}}{\mathrm{dt}} = -\frac{\mathrm{k_r}}{\mathrm{a}}\mathrm{[B][Cr(VI)]} \tag{1.9}$$

where a is the efficiency factor for the oxidation of B sites

However, they found Cr(III), instead of Cr(VI), desorbed from the biosorbents, which was against their expectation. They ascribed this phenomenon to the difficulty in maintaining Cr(VI) oxidation state during desorption process. They did not realize, that it could be the Cr(III) species, rather than Cr(VI), which were bound to the biosorbents.

Similarly, Aoyama (2003) proposed that the biosorption of Cr(VI) involved two pathways, namely the reduction of Cr(VI) to Cr(III) and also the sorption of Cr(VI) by biosorbents. The author argued that Cr(III) is in its bulky form, $[Cr(H_2O_6)]^{3+}$, which severely hinders its sorption by the active sites of biosorbents. Hence, it was inferred that only Cr(VI) was sorbed onto biosorbents. In light of the above inference, the author concluded that the biosorption of Cr(VI) was a process of the combination of Cr(VI) reduction and the sorption of Cr(VI). Cr(III), as the reduced form of Cr(VI), remained in the solution.

1.4.4. Adsorption-coupled reduction

Dupont and Guillon (2003) investigated the species of Cr sorbed onto lignocellulosic substrate extracted from wheat bran. By using X-ray photoelectron spectroscopy, they found that Cr(III) sorbed onto the biosorbent. Park et al. (2007a) investigated the Cr species in sixteen biosorbents using X-ray photoelectron spectroscopy. Similar result as the fomer group of researchers was obtained. This indicated that no Cr(VI) was found sorbed onto biosorbents, which is of great contrast to the mechanisms described prior to this section. With this finding, they proposed the mechanism of adsorption-coupled reduction for Cr(VI). According to this mechanism, Cr(VI) is reduced and Cr(III) is adsorbed. For the proposed mechanism, there are two possibilities: (a) direct reduction and (b) indirect reduction. Direct reduction means that Cr(VI) species together with H^+ in solution come into contact with electron donor groups in biosorbents and were reduced. A portion of Cr(III) was released while another portion remained on biosorbents. For indirect reduction, Cr(VI) was bound by positively charged groups on biosorbent and subsequently reduced by adjacent electron donor groups. The Cr(III) produced is either bound to biosorbents due to complexation or released due to repulsion. Fig. 1.3 illustrates the mechanisms proposed by Park and his co-workers in year 2005. This mechanism has been widely accepted by other researchers (Daneshvar et al., 2002; Malkoc et al., 2006; Garg et al., 2007; Suksabye et al., 2007; Elangovan et al., 2008a; Gao et al., 2008; Krishnani et al., 2008; Park et al., 2008b; Park et al., 2008a; Prabhakaran et al., 2009; Suksabye et al., 2009; Poch and Villaescusa, 2010; Ye et al., 2010).

1.4.5. Similarities and differences among the proposed mechanisms

Unlike the mechanism of anionic adsorption, the existence of Cr(III) was predicted under the other three mechanisms, namely anionic and cationic adsorption, reduction and anionic adsorption as well as adsorption-coupled reduction. These three mechanisms therefore share a common premise - Cr(VI) is reduced by biosorbents.

However, there are differences among the three mechanisms. The mechanism of anionic and cationic adsorption suggests that both Cr(III) and Cr(VI) are adsorbed by biosorbents. The mechanism of reduction and anionic adsorption outlines that only Cr(VI) is adsorbed whereas Cr(III) is repelled. The mechanism of adsorption-coupled reduction, however, advocates that only Cr(III) is adsorbed onto biosorbents. Since the

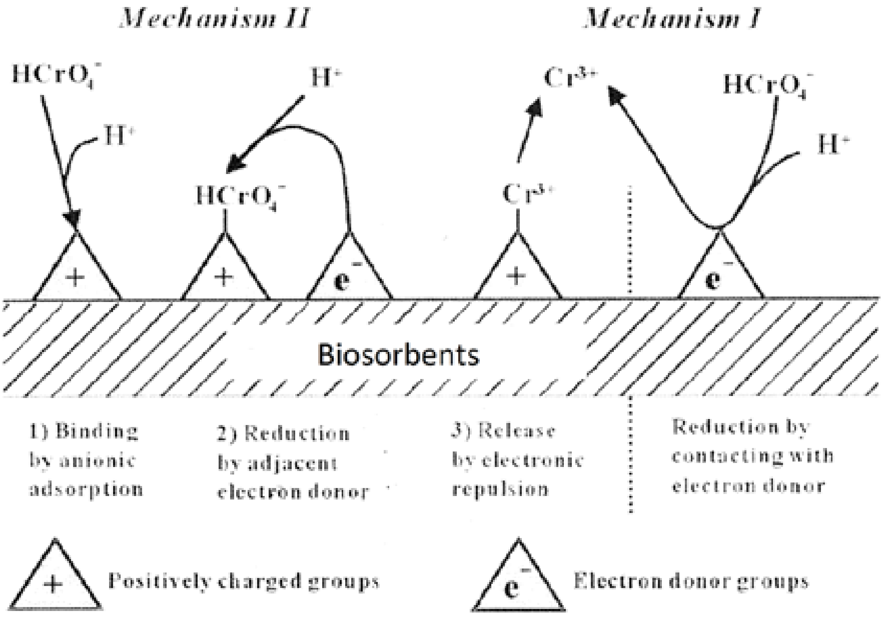

Fig. 1.3 Direct reduction (Mechanism I) and indirect reduction (Mechanism II) (Park et al., 2005a)