The European Journal of Mineral Processing and Environmental Protection
Vol.3, No.1, 1303-0868, 2003, pp. 75-87

ejmp&ep

Biosorption mechanism for anionic metal species with waste crab shells

H. Niu, B. Volesky*

Department of Chemical Engineering, McGill University
3610 University Street, MONTREAL, Canada H3A 2B2

Received 31 May 2002; accepted 13 January 2003

ABSTRACT

Biosorption of anionic metal species such as gold-cyanide, chromate and anionic vanadate species by acid-washed *Ucides* shells (AWUS) mainly involved anions binding on the positively charged amide groups of the AWUS. The binding force may relate to electrostatic attraction. No significant reduction of the selected metals was observed in the biosorption systems with AWUS. All selected metals were observed bound on AWUS at their respective original valent states, i.e. Au(I), Cr(VI) and V(V). Anionic metal species biosorption takes place through a combination of ion-exchange and adsorption. In the case of vanadate, the binding mechanism might involve other mechanism(s) in addition to the above ones.

Keywords: Biosorption; Anionic metal complexes; Crab shells; Chitin-containing materials; Chemical speciation

1. INTRODUCTION

Previous results showed that acid washed *Ucides* shells (AWUS) have a promising potential for binding anionic metal species such as gold-cyanide, chromate and vanadate (Niu and Volesky, 2001). For further work it is useful to elucidate the mechanism involved in the sorption by this biomaterial. The knowledge of the metal sequestering mechanism would assist in the manipulation of the biosorbent in order to optimize its performance. It could also lead to exploring the possibility of eventually using simple analogous materials, either synthetic or natural, for metal sequestering. While the biosorption mechanism has not been completely clarified as yet, metal binding in biosorption has been attributed to a number of different sequestration mechanisms such as adsorption, ion exchange and micro-precipitation (Volesky, 1990). Adsorption or ion exchange can be the result of two types of binding forces: a chemical force, a physical force, and the combination of both. Different specific sequestering mechanisms can be involved in binding metals from solution on different biomaterials.

It has been established that metal speciation in the solution and the functional groups on the biosorbent are both relevant to the metal binding mechanism. Infrared spectroscopy has been proven to provide a powerful tool for studying biological molecules and its application in obtaining structural and bonding information on complex and large molecules has been very useful (Nakamoto, 1997). This work deals mainly with elucidation of the binding mechanism of selected anionic metal complexes such as chromate (CrO_4^{2-}), vanadate (VO_4^{3-}) and gold-cyanide ($Au(CN)_2^-$) on the AWUS material.

* Corresponding author. E-mail: boya.volesky@mcgill.ca

2. MATERIALS AND METHODS

2.1. Biosorbent preparation

Raw waste crab shells of *Ucides cordatus* were obtained sundried from a food processing plant in Paraiba, Piaui, Brazil. Crushing of shells in a grinder and sieving gave particles within useful size ranges between 1 to 3.35mm for isotherms and 0.5 to 0.85mm for the FTIR analytical studies. They were washed with 1N HCl (55g dry shells/l HCl) for 6hrs to remove minerals and then rinsed with distilled water until the wash-solution pH stabilized at pH ~4.0. The residual material was dried at 55°C and represented approximately 23% of the original sized crushed shells by weight. It contained 53±4 (%) chitin, 43±3 (%) protein, 0.2±0.1 (%) ash and about 4±0.3 (%) of moisture and other matters. The extracted chitin had 78±10 (%) N-acetylation degree (i.e. the percentage of acetylated amino groups in chitin).

2.2. Soaking of AWUS

In the present work, AWUS sorbent was prepared by first washing the raw *Ucides cordatus* shells with 1N HCl, then rinsing with deionized water until pH stabilized at pH ~4.0, and finally drying at 50-55°C. In order to ascertain the biosorption mechanism, the experiments were performed as follows:

First, 40mg AWUS particles ranging in the size of 1-3.35mm in diameter was equilibrated with 20ml deionized water. The final solution pH was noted as pH 3.9.

Second, the AWUS material was transferred from the deionized water to 20ml 0.1M NaCl solution. In this step, the equilibrium solution pH was observed to rise to pH 4.7. If this pH change is caused by the exchange of Cl^- and OH^- on the protonated amino groups, the released OH^- was calculated to be 0.05mmol/g AWUS.

Third, the amount of the protonated amino groups on fresh AWUS initially occupied by Cl^- during the preparation of AWUS was determined by mixing 0.04g fresh AWUS with NaOH solution at pH 11. The total released Cl^- (analyzed by ion chromatography) was 0.06mmol/g AWUS. All these quantitative data were useful for assessing the mechanism of metal uptake by the biosorbent.

2.3. Solution preparation

Solutions of anionic metal complexes Au, Cr, and V were prepared by respectively dissolving solid $NaAu(CN)_2$, CrO_3, and Na_3VO_4 in distilled water. Ionic strength of solutions was adjusted by adding 0.01M or 0.1M NaCl. 0.1M or 0.5M HCl and NaOH were used for pH adjustment. All reagents were ACS reagent grade quality.

2.4. Equilibrium sorption experiments

Approximately 40±2mg of dried AWUS material was mixed with 20±0.2ml of solution containing metal species of interest in 150ml Erlenmeyer flasks. The pH of the solutions before and during the sorption experiments was controlled with HCl or NaOH. The solution was mixed and left to equilibrate for 24h, confirmed as sufficient time for establishing sorption equilibrium at room temperature. Sorption samples were run in duplicates, sometimes triplicates, with a blank undergoing the same treatment, and the data presented are the average values. The standard deviation of the equilibrium solution pH values for all data points was less than 0.1. Uptake of metal was determined from the difference in metal concentrations between the initial and final solutions using the following mass balance:

$$q^M = q_i^M + ([M]_0 - [M])\, v/w \qquad (1)$$

where:

q^M : equilibrium metal uptake (mmol/g).

q_i^M : metal initially loaded on the biosorbent (mmol/g). As neither of the selected acid

washed biosorbents in this study initially contained the selected metals such as Au, Cr and V (Remacle, 1990; Schiewer, 1996a; Troy and Koffler, 1969), $q_i^M = 0$.

$[M]_0$: initial metal concentration (mM)
[M] : equilibrium metal concentration (mM)
w : the dry net biosorbent weight (g).
v : the working volume of the adsorption sample (L).

2.5. Desorption experiments

Chromate was selected to examine metal desorption from the AWUS. Compared to the other anionic metal species selected for studies in the present work it is the strongest oxidant. AWUS material was first loaded with Cr from the chromate solution (9.6mM Cr) at the optimum pH 2.0. Around 40mg Cr–loaded AWUS material, bearing 0.54mmol Cr/g, was mixed with 5ml or 10ml of NaOH eluant solution for 24 hours, whereby the ratio of solid (mg) to liquid (ml) (S/L) was about 8 or 4, respectively. The percentage of Cr recovery, represented by the ratio of the amount of Cr released during desorption to the Cr amount loaded on AWUS in equilibrium sorption uptake, was calculated for each desorption experiment.

2.6. Analyses

The total metal concentration in solution was determined with an atomic (emission) spectrometer (sequential inductively coupled plasma AS, Thermo Jarrell Ash, Trace Scan).

Hexavalent chromium was determined according to the standard method (Eaton *et al.*, 1995) by measuring absorbance of the purple complex of Cr(VI) with 1,5-diphenylcarbohydrazide at 540nm by UV-VIS spectrophotometer. The concentration of other forms of chromium was determined as the difference between the total concentration of chromium obtained by ICP-AES and the concentration of Cr (VI).

Cl^- was analyzed using ion chromatography (Dionex, DX100) with a Dionex AS12A column. The retention time for Cl^- was 4.13 minutes.

FTIR analysis was used to investigate the main functional groups involved in metal biosorption. The metal-loaded biosorbent samples were prepared by contacting of 40mg batches of biosorbent particles (0.5-0.85mm) with 20ml of metal solution at the optimum pH for maximum metal uptake. The metal-laden biosorbent particles were then collected by filtration and washed with distilled water and finally freeze-dried. The blank biomaterials were treated the same way except for the absence of metals. Solid $NaAu(CN)_2$, CrO_3, and Na_3VO_4 samples were also prepared for spectra comparison. Disks of 100mg KBr containing 1% of finely ground powder of each sample were prepared less than 24 hours before analyzing. Infrared spectra of samples were recorded on a Michelson 100 FTIR spectrophotometer.

3. RESULTS AND DISCUSSION

3.1. Anionic metal biosorption and functional groups

3.1.1. Chromate biosorption

It has been established that pH has a profound effect on biosorption of metals, both cationic and anionic species. Cr adsorption isotherms obtained with chromate (CrO_4^{2-}) solution at pH of 2.0 to 3.6 were previously discussed (Niu and Volesky, 2002). With the pH decreasing from 3.6 to 2.0, the Cr uptake increased up to 0.54mmol/g at the Cr equilibrium concentration of 7.7mM.

The diagram for chromate speciation in Figure 1 (Baes and Mesmer, 1976a; Greenwood and Earnshaw, 1985a) shows that the main forms of Cr (VI) species in solution at pH lower than 6 are chromate ($HCrO_4^-$) and dichromate ($Cr_2O_7^{2-}$).

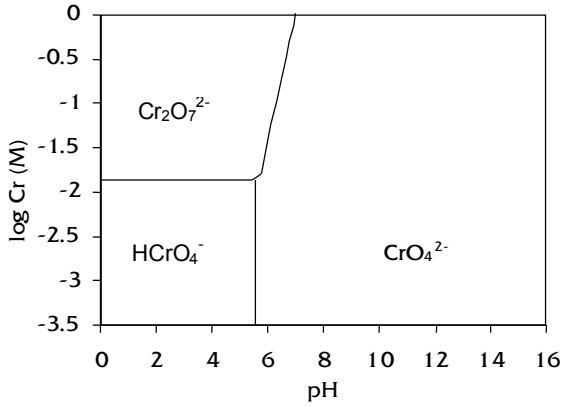

Figure 1. Cr(VI) speciation in solution at 25°C (Baes and Mesmer, 1976a; Greenwood and Earnshaw, 1985a)

AWUS material mainly consists of chitin and proteins containing weak-base groups such as amide or amine. When the solution pH is lowered from pH 4.5 to 2.0, negatively charged protein carboxyl groups and neutral weak-base amino groups on AWUS become protonated (Niu and Volesky, 2002), offering positive binding sites for anionic chromate or dichromate that also become less repelled by the decreasing overall negative AWUS charge. Under this condition, chromate or dichromate could be effectively bound onto positively charged sites on AWUS.

Chromate was the strongest oxidant among the selected anionic metal complexes of (CrO_4^{2-}), vanadate (VO_4^{3-}), and gold-cyanide ($Au(CN)_2^-$). The speciation of chromate in the biosorption system with AWUS could reflect the reducibility of the AWUS material.

3.1.1.1. The Effect of AWUS on Cr speciation in solution

Cr-loaded AWUS particles (1-3.35mm in diameter) were prepared by contacting 40mg AWUS with 9.12 mM chromate solution at pH 2.1. In this solution chromate theoretically exists as $HCrO_4^-$ and $Cr_2O_7^{2-}$ (Greenwood and Earnshaw, 1985b). However, it was necessary to establish the actual chromate speciation which could be different due to the presence of AWUS in the system. While the total Cr concentration was determined by the ICP-ASE and the Cr(VI) concentration by the UV Spectrophotometer, another form of Cr could be determined by the concentration difference between the two.

Table 1
Determination of chromate speciation in a biosorption system (pH 2.1)

	$[Cr, _{total}]$* (mM)	[Cr(VI)]* (mM)	[Cr(VI)/$[Cr, _{total}]$ (%)	q^{Cr} (mmol/g)**
Control solution	9.12±0.11	7.69±0.10	84.3±0.1	-
Biosorption sample	7.93±0.13	6.70±0.11	84.5±0.1	0.59±0.06

* Equilibrium concentration.
** The uptake of chromium in all forms (mmol/g).

Table 1 summarizes the concentration of different Cr ionic species in the equilibrium biosorption system as well as in the control solution (without biosorbent). The results showed that in the control solution up to 84.3% of the total Cr concentration was there as Cr(VI), indicating that Cr (VI) could be reduced to other forms even in deionized water at pH 2.1. This represents a departure from the ideal thermodynamic calculations. However, the percentage of the reduced part is minor (15.7%). As known, Cr (VI) is the highest oxidation state of Cr. While there are other possible states of Cr such as Cr (V), Cr (IV), and Cr (II), the most stable oxidation state is Cr (III) (Greenwood and Earnshaw, 1985a). Under acidic conditions, chromate is apparently reducible most possibly to Cr(III). In the biosorption system, the percentage of Cr (VI) in the solution was also 84.5%, the same as in the control solution. This was different from what

was observed during chromate adsorption by *Sargassum* biomass where the concentration ratio of Cr(VI) to Cr_{total} in the solution decreased greatly with the addition of the biomass to the chromate solution (Kratochvil, 1997). For the present system with AWUS biosorbent it could be concluded that AWUS is not significantly active in reducing chromate. However, the form of Cr bound on the crab shells was determined through the FTIR analysis.

3.1.1.2. FTIR analysis of chromate binding

FTIR analysis was conducted to investigate the metal form(s) sequestered on AWUS and the involvement of main functional chemical groups in metal biosorption. Metal-loaded biosorbent samples (0.5–0.85mm in diameter) contained 0.79mmolCr/g. The results of FTIR analyses of solid chromium trioxide, blank AWUS and Cr-loaded AWUS are shown in Table 2 and Figures 2a and b.

Table 2
Cr biosorption on AWUS: Summary of FTIR spectral data (cm^{-1})

Bond vibration	Chromate (ν_3)	amide II	ν (C=O)
CrO_3	954	-	-
Blank AWUS	-	1450	1739
Cr-loaded AWUS	952	-	-

The spectrum of chromium trioxide (Cr(VI)) (Table 2) showed a characteristic peak at $954cm^{-1}$ ascribed to chromate (ν_3) vibration (Gadsden, 1975a). This peak appeared on the spectrum of Cr-loaded AWUS indicating that chromate was bound on the shells. As the characteristic peaks of dichromate are also located between 800 and $950cm^{-1}$ (Gadsden, 1975a), it is not possible to distinguish the form of chromate from that of dichromate bound on the shells. However, this result indicated that there was Cr(VI) bound on the shells.

Furthermore, the spectrum of blank AWUS displayed peaks at 1450 and $1739cm^{-1}$ (Table 2) which can be ascribed to the vibration of amide II (Morrison, 1987; Schrader, 1995) and ν (C=O) of the carboxyl group (Nakamoto, 1997), respectively. The peak of amide II was invisible on the spectrum of Cr-loaded AWUS, indicating the amide group involvement in Cr adsorption. The major organic substances in AWUS material are chitin and protein. The determined N-acetylation degree of extracted chitin was 78%, indicating that 78% of N in the AWUS chitin was in the amide form. In addition, amide is also a crucial characteristic group of proteins. The amide II peak of either chitin or protein is located around $1450–1580cm^{-1}$ (Morrison, 1987; Roberts, 1992d; Schrader, 1995). Therefore, the change of amide II peak could be the contribution of either chitin amide or protein amide. As the Cr adsorption was performed at pH 2 at which the amide groups could theoretically be fully protonated (Roberts, 1992a) and available for anionic chromate binding, the peak of amide II shifted and was invisible, probably overlapped by the bigger peaks next to it.

Lastly, the peak of ν (C=O) of the carboxyl group on AWUS disappeared on the spectrum of Cr-loaded AWUS. Carboxyl group is a weak-acid group that could not directly contribute to anionic chromate binding. However, the previous chromate speciation analysis indicated that there was around 15% of Cr(III) existing in the solution. Kratochvil confirmed that Cr(III) could be bonded to the carboxyl group at pH 2 (Kratochvil, 1997). Cr(III) in the present system could also be bonded onto the AWUS resulting in the shift of the ν (C=O) peak, possibly overlapped by the bigger peak next to it.

In summary, there is a strong indication that AWUS cannot significantly reduce chromate even though chromate is a strong oxidant. Cr(VI) compounds either as chromate or dichromate were found bound on AWUS. Amide groups were involved in binding of anionic chromate. The carboxyl group on the protein may be responsible for the small amount of Cr(III) binding.

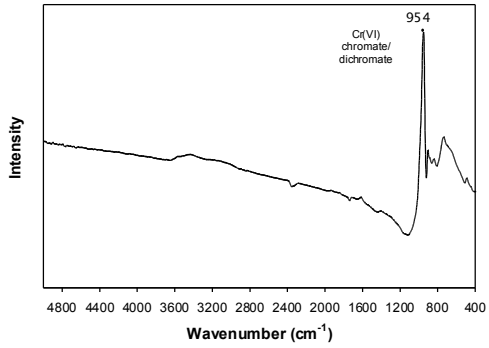

Figure 2a. FTIR spectrum of chromium trioxide (CrO_3)

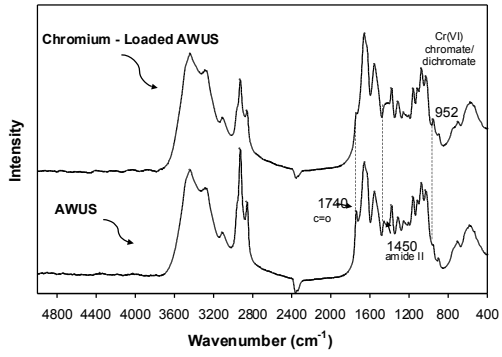

Figure 2b. FTIR spectra of blank and chromium-loaded AWUS

3.1.2. Vanadate biosorption

Vanadium uptake isotherms, determined and reported earlier (Niu and Volesky, 2002), were obtained using vanadate (VO_4^{3-}) solution at pH 1.5 to 4.5. The results showed that the V isotherm at pH 2.5 started off lower, then it rose dramatically, showing the maximum uptake of about 0.79mmolV/g which wass higher than both Au and Cr uptakes.

Vanadate (VO_4^{3-}), representing the multi-valent metal anion, appears in more complicated forms than chromate when it is in aqueous solution (Sillen and Mortell, 1964a; Pope and Dale, 1968; Kepert, 1973; Baes and Mesmer, 1976a; Greenwood and Earnshaw, 1985c; Gupta and Krishnamurthy, 1992; Larson, 1995). The distribution of V(V) species in aqueous solution depends on the solution pH and on the vanadium concentration as shown in Figure 3 (Greenwood and Earnshaw, 1985c). The species distribution relationship and equilibrium constants are well documented in the literature (Sillen and Mortell, 1964a; Baes and Mesmer, 1976a; Larson, 1995).

The established vanadate speciation shown in Figure 3, determines that at pH 2.5, VO_2^+ is predominant in the solution at low vanadium concentrations (less than ~1.7mmol V/l). The weak competition of VO_2^+ with the proton for the site may account for the lower V uptake observed in the low V concentration range. However, when the vanadium concentration is continuously increased, anionic vanadate $V_{10}O_{26}(OH)_2^{4-}$ and vanadium pentoxide (V_2O_5) form (Figure 3). Vanadium pentoxide is slightly soluble in water, making pale yellow solutions that sometimes include colloidal material (Baes and Mesmer, 1976a). The precipitation of V_2O_5 occurs between pH 1 and 3 at vanadium concentration over about 50mM (Gupta and Krishnamurthy, 1992), which is far beyond the V concentration range in this work (0-10mM). The colloid can develop erratic localized charges all over its surface from interactions with other ions in the solution (Silberberg, 1996).

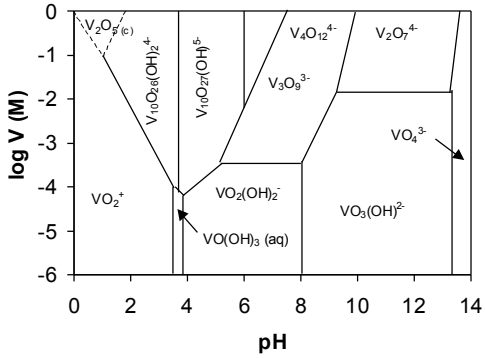

Figure 3. V(V) speciation in solution at 25°C

The dashed lines represent the solubility of V_2O_5 in terms of the V(V) concentration. The solid lines represent conditions under which the predominant species in adjacent regions contain equal amounts of V(V) (Greenwood and Earnshaw,1985c)

Correspondingly, when a vanadium colloidal particle or vanadate $V_{10}O_{26}(OH)_2^{4-}$ anion is sorbed, many orders of magnitude of higher amounts of vanadium are removed from solution.

In summary, vanadate binding could be attributed to anionic with some cationic vanadate binding, colloid sorption, and neutral molecule precipitation depending on the solution conditions.

Earlier studies (Niu and Volesky, 2002) revealed that the main effect of pH on anionic metal species binding consists of an increase in the number of positively charged sites available with decreasing pH and an increase in the amount of metal species with high affinity for the sites.

Previous results revealed that AWUS could not effectively reduce chromate. Compared to chromate, vanadate has lower oxidizability (Greenwood and Earnshaw, 1985d). V(V) could be reduced to V(IV), V(III) and V, however, in the presence of air, V(V) is the most stable oxidized state of vanadium in aqueous solution (Gupta and Krishnamurthy, 1992). Logically speaking, a significant reduction of vanadate is not expected in the present similar biosorption system. The form of vanadium bound on AWUS was also examined by the FTIR analysis. The V-loaded AWUS material (0.5–0.85mm in diameter) contained 1.5mmol/g taken up from the solution initially containing 7.18mM V (vanadate) at pH 2.5.

3.1.2.1. FTIR analysis of vanadate binding

The FTIR spectra of sodium orthovanadate, blank AWUS and V-loaded AWUS are shown in Table 3 and Figures 4a and b. The spectrum of sodium orthovanadate displayed a peak at 837cm^{-1}. This peak is characteristic for the vanadate (ν_3) vibration (Gadsden, 1975b), which appeared on the spectrum of V-loaded AWUS at 833cm^{-1}. In addition, there was another new peak at 758cm^{-1} that appeared on the spectrum of V-loaded shells. It could also be ascribed to the vanadate vibration usually located at around 700–900cm^{-1} (Gadsden, 1975b). All these new peaks observed for the V-loaded shells confirmed that vanadate (V(V)) was bound.

Furthermore, the peak at 1550 cm^{-1} (amide II vibration) observed for the spectrum of blank AW shells shifted to 1538 cm^{-1} for V-loaded AWUS, once again confirming that amide groups on the shells were involved V binding. The weak shift of less than 15cm^{-1} was attributed to electrostatic attraction (Mooiman and Miller, 1986).

As the present biosorption system was at pH 2.5 whereby vanadate could exist as cationic VO_2^+, anionic forms of vanadate and neutral colloidal vanadium pentoxide (V_2O_5) in the aqueous solution (Baes and Mesmer, 1976b; Greenwood and Earnshaw, 1985c; Gupta and Krishnamurthy, 1992; Kepert, 1973; Larson, 1995; Pope and Dale, 1968; Sillen and Mortell, 1964a), different adsorption mechanisms could contribute to the overall V uptake. Consequently, it cannot be concluded that vanadium adsorption involves only anionic vanadate binding. However, the above results did confirm that vanadate (V) was bound on the shells and

that the amide groups on the shells were involved in the binding through electrostatic attraction.

Table 3
V biosorption on AWUS: Summary of FTIR spectral data (cm^{-1})

Bond vibration	vanadate	amide II
Solid $NaVO_4$	837	-
Blank AWUS	-	1550
V-loaded AWUS	833, 758	1538

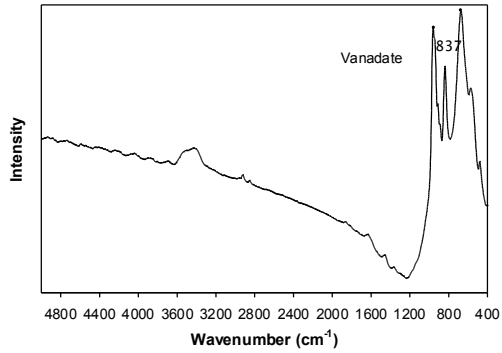

Figure 4a. FTIR spectrum of sodium orthovanadate (Na_3VO_4)

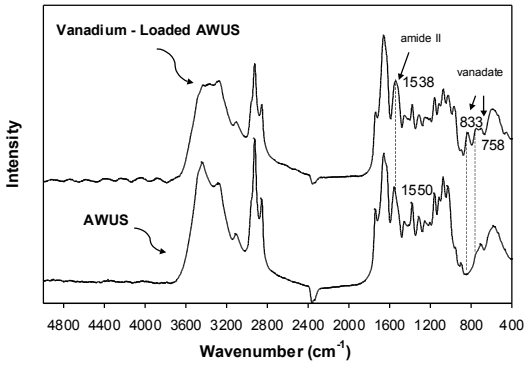

Figure 4b. FTIR spectra of blank and vanadium-loaded AWUS

3.1.3. Gold-cyanide biosorption

Au adsorption isotherms for $Au(CN)_2^-$ sorbed by AWUS at equilibrium pH of 2.4 to 4.5 were presented (Niu and Volesky, 2002). The Au uptake increased with decreasing pH from 4.5 to 3.4. The Au uptake at pH 3.4 was 0.17mmol/g at the Au equilibrium concentration of 2.2mM. However, at the lower pH of 2.4 the Au uptake was lower. Furthermore, during the adsorption process the sorption system pH had an increasing tendency. This was also observed with chromate adsorption by *Sargassum* or peat moss (Sharma and Forster, 1993; Kratochvil *et al.*, 1998) and explained to be resulting from the weak-base groups on the biomaterials picking up protons from the solution so as to bind anionic metal species. When Giles (Giles and Hassan, 1958; Giles *et al.*, 1958) examined dye sorption by chitin at pH 1.8 - 4.7, he postulated that the weak-base functional group in chitin under these conditions was amide.

Of the metal complexes studied, the gold-cyanide complex ($Au(CN)_2^-$) is the most stable one, with the dissociation constant of Au from the cyanide complex being $10^{-38.9}$ (Marsden and House, 1993). $Au(CN)_2^-$ adsorption by *Sargassum* biomass confirmed that gold-cyanide is such a stable complex that it could not be reduced by *Sargassum* which was capable of significant chromate reduction (Niu and Volesky, 1999). As AWUS could not appreciably reduce chromate

at all, it was not expectable that it would reduce Au from the gold-cyanide complex. As a result, in the tested pH values, pH 3.4 appears to be the optimum with the sufficient binding sites available for $Au(CN)_2^-$ uptake without excessive Cl^- interference.

3.1.3.1. FTIR analysis of gold-cyanide binding

In order to confirm the form of $Au(CN)_2^-$ on the AWUS, the FTIR analysis was performed. The Au-loaded sample was prepared by mixing 20ml 3.44mM Au ($Au(CN)_2^-$) with 40mg AWUS particles (0.5–0.85mm in diameter) at pH 3.2. The Au uptake (loading) was 0.2mmolAu/gAWUS.

The FTIR spectrum (Figures 5a and b) of Au-loaded AW shells confirmed that $Au(CN)_2^-$ (characteristic peak at 2150cm^{-1}) was bound on the AWUS (Nakamoto, 1986). If Au was reduced from $Au(CN)_2^-$, there would have to be free CN^- released which could also compete for the sites. However, there was no peak observed for free CN^- on the shells, usually located at 2080cm^{-1} (Nakamoto, 1986).

In summary, no significant redox action occurred during biosorption of the selected anionic metal species. The metal complex binding was mainly on the amide groups of AWUS. The binding force can be considered weak indicating thus electrostatic attraction.

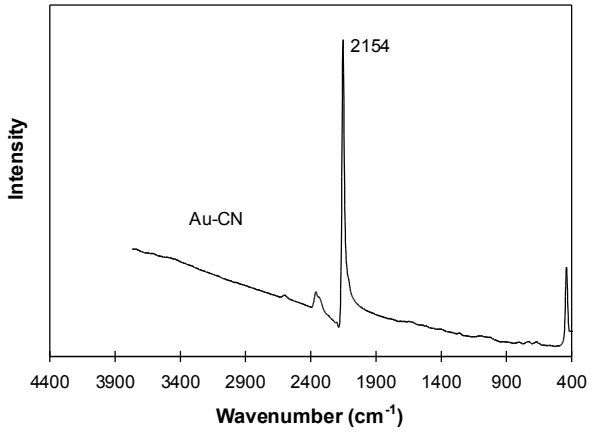

Figure 5a. FTIR spectrum of $NaAu(CN)_2$

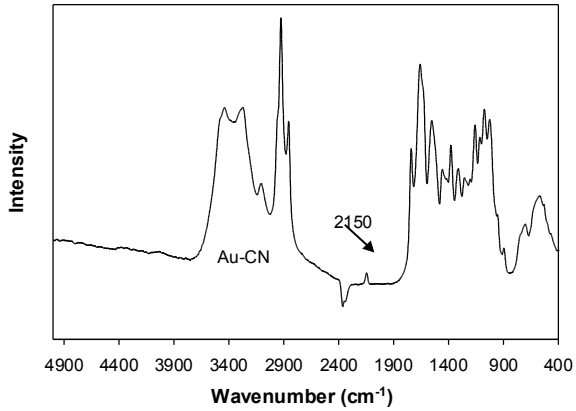

Figure 5b. FTIR spectrum of Au-loaded AWUS

3.2. Determination of electrostatic attraction

The predominant binding force of electrostatic attraction for anionic metal species biosorption by AWUS can be postulated based on the following results. The weak shifts of the functional amide peak observed in the FTIR analysis (above) indicated that the anionic metal species biosorption involves electrostatic attraction. The strong increase of the selected metal uptakes with increasing solution pH within the range from 4.0 to 2.0 (Niu and Volesky, 2001) is another indication. Similar pH effect phenomena were found for anionic dye adsorption by chitin from pH 6.5-2.5 (Giles and Hassan, 1958; Giles *et al.*, 1958) and for chromate uptake by chitosan from pH 8.0-4.0 (Roberts, 1992b) which were attributed to electrostatic attraction of anionic species to the positively charged groups of amide or amine. Ion adsorption by electrostatic attraction is significantly affected by the enhanced ionic strength in the solution (Davis and Leckie, 1980). Previous results concerning the ionic strength effects (Niu and Volesky, 2001) showed a strong dependence of metal uptakes on solution ionic strength, confirming that the anionic metal species adsorption by AWUS is through electrostatic attraction.

Furthermore, chromium desorption from Cr-loaded AWUS by NaOH solution showing that all of the loaded Cr (0.54mmol/g) was eluted from the AWUS into a small amount (10ml) of eluant solution at pH 10.6 (solid [mg] to liquid [ml] ratio of 8). However, when Cr was eluted into only 5ml of eluant solution, a new sorption equilibrium was apparently established and about 4% of deposited Cr remained in the sorbent. At pH 10.6, chromate exists as CrO_4^{2-} in the aqueous solution (Greenwood and Earnshaw, 1985a; Greenwood and Earnshaw, 1985c; Gupta and Krishnamurthy, 1992; Sillen and Mortell, 1964b) and Cr elution does not only result from the drastically reduced number of positively charged weak-base groups but also from the competition of OH^-, HCO_3^- or CO_3^{2-} in the solution. Even though the elution experiments in this work were only cursory, the successful elution of bound Cr by the simple increase of solution pH confirmed that the anionic metal species adsorption was through electrostatic attraction. If chemisorption were occurring, simple pH adjustment could not accomplish the elution of the bound metals, such as was observed for cationic metal adsorption by *Sargassum,* where Cd and Cu were covalently bonded on the biomass (Aldor *et al.*, 1995; Figueira *et al.*, 1999).

Vanadate binding might involve other binding forces besides electrostatic attraction as colloidal V_2O_5 was present in the solution making this biosorption case more complicated.

3.3. Combination of binding mechanisms

The analyses in the above sections provided information on the forms of loaded metals and AWUS functional groups as well as on the binding force. However, they could not determine the nature of the metal binding, i.e. adsorption, ion exchange or others. In the context of this work, the term “adsorption” refers to the binding of a solute to free sites that had not been previously occupied by other ions. If the sites are initially occupied by other ions and if these ions are released upon the binding of the new ion, then the term “ion exchange” is used to describe the phenomenon (Schiewer, 1996b). Possibly the best proof of the exchange mechanism is the change in pH of the sorption system when the sorbent is transferred from water to a salt (NaCl) solution (Nachod, 1949a). The addition of salt (NaCl) should have little effect when the uptake mechanism is adsorption: $BNH + HCl \leftrightarrow BNH_2^+ Cl^-$ (2)

However, in the exchange reaction $BNH_3^+ OH^- + Cl^- \leftrightarrow BNH_2^+ Cl^- + OH^-$ (3)
the addition of NaCl will drive the equilibrium to the right and raise the pH value.

Strong-base resin was confirmed to be involved in ion exchange that contained appreciable amounts of the ionized hydroxide, $RNH_3^+ OH^-$ in a neutral and alkaline condition. However, with weak-base resins, no such significant exchange of chloride for hydroxyl was observed (Nachod, 1949b).

3.4. Soaking of AWUS

The sum of released OH^- and Cl^- was 0.1mmol/g as determined during the AWUS soaking (Materials and Methods). The total amount of protonated amino sites initially present on AWUS should be no higher than this sum. If the sites for OH^- and Cl^- binding could also be reached by anionic metal species such as $Au(CN)_2^-$ and Cr(VI) ($HCrO_4^-$ or $Cr_2O_7^{2-}$ under the present biosorption conditions), based on the obtained maximum uptakes of Au and Cr being 0.17mmol/g and 0.54mmol/g with the same size of the AWUS particles (Niu and Volesky, 2001), then the above amount of pre-occupied sites only takes 55% of the total capacity in case of Au binding and 32% in case of chromate binding. In addition, only part of the functional amide groups in AWUS are fully protonated when stabilized in deionized water at pH ~4.0 during the preparation of AWUS as the conjugate acid dissociation constant (p*Ka*) of chitin amide is less than 3.5 (Roberts, 1992c). While all the amine groups on AWUS could be fully protonated initially as the conjugate acid dissociation constants (p*Ka*) of chitin and protein amine are 6.5-10 (Buffle, 1988; Roberts, 1992a), the available capacity of amine is questionable because the metal uptakes obtained at pH 4.5 were very low (for example, the Au uptake was only 0.07mmol/g (Niu and Volesky, 2001)). In addition, FTIR analysis did not show the involvement of amine groups in the biosorption. Therefore in the gold-cyanide and chromate biosorption system, the binding mechanism appears to be a combination of ion-exchange and adsorption.

In the case of vanadate binding, the observed vanadium uptake of 0.79mmol/g (Niu and Volesky, 2001) was by far more than what could be expected based on previously calculated pre-occupied sites. As cationic VO_2^+, anionic forms of vanadate and neutral colloidal vanadium pentoxide (V_2O_5) are all present in the aqueous solution within the pH range of 1.5-4.5 and less than 10mM V employed in the present biosorption experiments (Greenwood and Earnshaw, 1985c; Gupta and Krishnamurthy, 1992; Kepert, 1973; Larson, 1995; Pope and Dale, 1968; Sillen and Mortell, 1964a), vanadium uptake may involve other mechanism(s) in addition to ion exchange and adsorption. It is a recognized fact that a combination of several mechanisms, each functioning independently, can contribute to the overall metal uptake in the biosorption system (Volesky, 1990).

4. CONCLUSIONS

Biosorption of anionic metal species such as gold-cyanide, chromate and anionic vanadate species by AWUS mainly involved anions binding on the positively charged amide groups of the AWUS. The binding force is likely due to electrostatic attraction.

As there was no alteration of the anionic species present in the solution, it could be postulated that no significant reduction of the selected metals was observed in these biosorption systems with AWUS. All selected metals were observed bound on AWUS at their respective original valent states, i.e. Au(I), Cr(VI) and V(V).

Biosorption of anionic metal species examined takes place through a combination of ion-exchange and adsorption. In the case of vanadate, the binding mechanism most likely involves yet another one in addition to the above-mentioned fundamental binding mechanisms. This hypothesis, as yet unconfirmed, is based on the presence of neutral colloidal vanadium pentoxide (V_2O_5) in the solution within the pH (pH 1.5-3.0) and concentration (less than 10mM V) ranges used in this study. Further and more specific study would be required to ascertain more precisely the fate of the V_2O_5 species that, upon its binding, would contribute a higher amount of immobilized vanadium. Indication of this actually taking place has been noted in this work.

ACKNOWLEDGEMENTS

Special thanks to Felipe Aristizabal and Amy Tin-Pui Lam for their valuable contribution to the experimental work.

NOMENCLATURE

q^M : equilibrium metal uptake (mmol/g).
q_i^M : metal initially loaded on the biosorbent (mmol/g).
$[M]_0$: initial metal concentration (mM).
[M] : equilibrium metal concentration (mM).
w : the dry net biosorbent weight (g).
v : the working volume of the adsorption sample (L).

REFERENCES

Aldor, I., Fourest, E., Volesky, B., Desorption of cadmium from algal biosorbent. Can. J. Chem. Eng., 1995, **73**, 516-522.

Baes, C.F.J., Mesmer, R.E., The Hydrolysis of Cations, 1976a, Wiley-Interscience, John Wiley & Sons, New York, 220 pp.

Baes, C.F.J., Mesmer, R.E., The Hydrolysis of Cations, 1976b, Wiley-Interscience, John Wiley & Sons, New York, pp.208-210.

Buffle, J., Complexation Reactions in Aquatic Systems: An Analytical Approach. 1988, Ellis Horwood Ltd., Chichester, UK, pp.195-303.

Davis, J.A. and Leckie, J.O., Surface ionization and complexation at the oxide-water interface: 3. Adsorption of anions. J. Colloid Interface Sci., 1980, **74**, 32-43.

Eaton, A.D., Clesceri, L.S., Greenberg, A.E., Standard Methods for the Examination of Water and Wastewater, 1995, American Public Health Association (APHA), AWWA, WPCF, Washington, DC, Ch.4, 23 pp.

Figueira, M.M., Volesky, B., Mathieu, H.J., Instrumental analysis study of iron species biosorption by *Sargassum* biomass. Environ. Sci. Technol., 1999, **33** (11), 1840-1846.

Gadsden, J.A., Infrared Spectra of Minerals and Related Inorganic Compounds, 1975a, Butterworth, NY, 29 pp.

Gadsden, J.A., Infrared Spectra of Minerals and Related Inorganic Compounds, 1975b, Butterworth, NY, 26 pp.

Giles, C.H., Hassan, A.S.A., Adsorption at organic surfaces. V - A study of the adsorption of dyes and other organic solutes by cellulose and chitin. J. Soc. Dyers Colour., 1958, **74**, 846-857.

Giles, C.H., Hassan, A.S.A., Subramanian, R.V.R., Adsorption at organic surfaces. IV - Adsorption of sulphonated azo dyes by chitin from aqueous solution. J. Soc. Dyers Colour., 1958, **74**, 681-688.

Greenwood, N.W., Earnshaw, A., Chemistry of the Elements, 1985a, Pergamon Press, NY, 1169 pp.

Greenwood, N.W., Earnshaw, A., Chemistry of the Elements, 1985b, Pergamon Press, NY, 1175 pp.

Greenwood, N.W., Earnshaw, A., Chemistry of the Elements, 1985c, Pergamon Press, NY, pp.1147-1148.

Greenwood, N.W., Earnshaw, A., Chemistry of the Elements, 1985d, Pergamon Press, NY, 891 pp.

Gupta, C.K., Krishnamurthy, N., Extractive Metallurgy of Vanadium, 1992, Elsevier, NY, 209 pp.

Kepert, D.L., Chap.51. Isopolyanions and heteropolyanions, Comprehensive Inorganic Chemistry, 1973, Pergamon Press, Oxford, UK, pp. 607-72.

Kratochvil, D., A Study of the metal biosorption process utilizing *Sargassum* seaweed biomass. PhD Thesis, McGill University, Montreal, 1997, 186 pp.

Kratochvil, D., Pimentel, P., Volesky, B., Removal of trivalent and hexavalent chromium by seaweed biosorbent. Environ. Sci. Technol. 1998, **32**, 2693-2698.

Larson, J.W., Thermochemistry of vanadium(5+) in aqueous solutions. J. Chem. Eng. Data, 1995, **40** (6), pp. 1276-1280.

Marsden, J., House, L., Chemistry of gold extraction, 1993, Ellis Horwood, Hartnoll, UK, 193 pp.

Mooiman, M.B., Miller, J.D., The chemistry of gold solvent extraction from cyanide solution using modified amines. Hydrometallurgy, 1986, **16**: 245-261.

Morrison, R.T., Organic Chemistry, 1987, Allyn and Bacon, Inc., Newton, 985 pp.

Nachod, F.C., Ion Exchange - Theory & Application, 1949a, Academic Press, NY, 65 pp.

Nachod, F.C., Ion Exchange - Theory & Application, 1949b, Academic Press, NY, 25 pp.

Nakamoto, K., Infrared and raman spectra of inorganic and coordination compounds, 1986, John Wiley & Sons, New York, NY, 231 pp.

Nakamoto, K., Infrared and Raman Spectra of Inorganic and Coordination Compounds - B. Application in Coordination, Organometallic, and Bioinorganic Chemistry, 1997, John Wiley & Sons, New York, 60 pp.

Niu, H., Volesky, B., Characteristics of gold biosorption from cyanide solution. J. Chem. Technol. Biotechnol., 1999, **74** (8), 778-784.

Niu, H., Volesky, B., Biosorption of anionic metal complexes. In: V.S.T. Ciminelli and J.O. Garcia (Eds.), Biohydrometallurgy: Fundamentals, Technology and Sustainable Development. Part B - Biosorption and Bioremediation. Elsevier Science B.V., Amsterdam, 2001, pp. 189-197.

Niu, H., Volesky, B., Characteristics of anionic metal species biosorption with waste crab shells. Hydrometallurgy, 2002, (special issue): in press.

Pope, M.T. and Dale, B.W., Isopoly-vanadates, -niobates and -tantalates. Quart. Rev., 1968, **22** (4), 527-548.

Remacle, J., The cell wall and metal binding. In: Biosorption of Heavy Metals, ed. B. Volesky, CRC Press, Inc., Boca Raton, FL, 1990, pp. 83-92.

Roberts, G.A.F., Chitin Chemistry, 1992a, Macmillan, London, UK, pp. 204-206.

Roberts, G.A.F., Chitin Chemistry, 1992b, Macmillan, London, UK, 213 pp.

Roberts, G.A.F., Chitin Chemistry, 1992c, Macmillan, London, UK, pp.203-206.

Roberts, G.A.F., Chitin Chemistry, 1992d, Macmillan, London, UK, 89 pp.

Schiewer, S., Multi-metal ion exchange in biosorption. PhD Thesis, McGill University, Montreal, Canada, 1996a.

Schiewer, S., Multi-metal ion exchange in biosorption. PhD Thesis, McGill University, Montreal, Canada, 1996b, 8 pp.

Schrader, B., Infrared and raman spectroscopy: methods and applications, 1995, VCH, Weinheim, 356 pp.

Sharma, D.C., Forster, C.F., Removal of hexavalent chromium using sphagnum moss peat. Water Res., 1993, **27**, 1201-1208.

Silberberg, M., The Chemical nature and charge, 1996, Mosby: St. Louis, MI, pp. 497-499.

Sillen, L.G., Mortell, A.E., Stability constants of metal-ion complexes: Special Publication 17, 1964a, The Chemical Society, London, UK, pp.84-86.

Sillen, L.G., Mortell, A.E., Stability constants of metal-ion complexes: Special Publication 17, 1964b, The Chemical Society, London, UK, 111 pp.

Troy, F.A., Koffler, H., The chemistry and molecular architecture of the cell walls of *Penicillium chrysogenum*. J. Biol. Chem., 1969, **244**, 5563-5576.

Volesky, B., Removal and recovery of heavy metals by biosorption. In: Biosorption of Heavy Metals, ed. B. Volesky, CRC Press, Boca Raton, FL, 1990, pp. 7-43.