



# Adsorption of Heavy Metal Ions onto Green Microalga *Chlamydomonas reinhardtii*

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Microalga *Chlamydomonas reinhardtii* was used for adsorption of Pb(II), Fe(III) and Ni(II) ions. FT-IR analysis and pH titration of the algal biomass revealed the presence of amino, carboxyl and sulfonate groups, which were responsible for adsorption of the metal ions. The optimum pH for adsorption of Pb(II), Fe(III) and Ni(II) ions were found to be 5.0, 4.0 and 7.0, respectively. The adsorption equilibrium was established in about 200 min for Pb(II) and Fe(III) ions and 120 min for Ni(II) ion. The maximum adsorption capacities of the microalga for Pb(II), Fe(III) and Ni(II) ions were 1.47, 1.13 and 0.870 mmol/g-biomass, respectively. The order in metal affinity for the algal biomass was Fe(III) > Pb(II) > Ni(II). The adsorption equilibrium was well described by the Langmuir adsorption isotherm. *C.reinhardtii* could be considered as one of the suitable biosorbents for the removal of metal ions in waste water treatment because of the relatively high adsorption capacities. The biosorbents could be reused five times in consecutive adsorption/desorption cycles for Fe(III) ion with 25% loss in the adsorption capacity..

## 1. Introduction

The release of heavy metals from industries into the environment has resulted in many problems for both human health and aquatic ecosystems (Tuzun *et al*, 2005; Inthorn *et al*, 1996). Microorganism plays an important role in self purification of water and soil environment as decomposers in ecosystem. Microbial technologies, such as activated sludge or oxidation pond processes, have been extensively applied to the treatment of industrial and domestic sewages to break down organic wastes. Another type of the treatment is based on their abilities to remove specific substances such as phosphates, nitrates and heavy metals.

Common treatments to remove heavy metal ions from contaminated water are mostly based on solvent extraction, precipitation, evaporation, and adsorption on ion exchange resins. Precipitation processes have several disadvantages, and the other technologies are expensive in case that metal ions are present at low concentrations in the wastewater. In order to develop economic, practical and efficient techniques, fungal algae and other microbes have been used to remove and recover metallic element. Microalgae possess a high metal-binding capacity (Ramelow *et al*, 1992; Holan and Volesky, 1994) through the cell wall playing an important role in the binding (Crist *et al*, 1992). This is due to the presence of various functional groups such as carboxyl, amino, sulphate, and hydroxyl groups, which can act as binding sites for metals. The main mechanisms of binding include ionic interactions and complex formation between metal cations and ligands on the surface of microalgae (Yun *et al*, 2001). However, in many biosorption processes, several metal binding mechanisms are said to take place

simultaneously and so it is very difficult to distinguish between the mechanisms (Lacher and Smith, 2002).

This study adopts a systematic and kinetic approach by studying the adsorption of Pb(II), Fe(III) and Ni(II) ions to dried biomass of the green microalgae, *Chlamydomonas reinhardtii*.

## 2. Experimental

### 2.1 Biomass

*Chlamydomonas reinhardtii* used in this study was purchased from the IAM culture collection of the university of Tokyo molecular cell biology laboratory. *C.reinhardtii* has two flagellums, and an oval unicellular green microalga of about 10  $\mu\text{m}$ . *C.reinhardtii* was cultured for 200 hours, and then centrifuged for 15 minutes at 9000 rpm in a high-speed cooling centrifugal separator (Tommy GRX-220). The collected algal biomass by the centrifuge was put in a beaker, and it was rinsed with distilled water. Afterwards, they were dried for 24 h in a dry sterilizer at 90 °C (Yamato science SH-62). The dry biomass was ground and sieved to a particle size of 212-300  $\mu\text{m}$ , this was used for all experiments.

### 2.2 FT-IR spectroscopy

FT-IR spectra of native and metal-ion loaded *C. reinhardtii* were measured using a spectrophotometer (Shimadzu FT-IR 8400).

### 2.3 Potentiometric titration

#### 2.3.1 Protonation of biomass

To identify the functional groups presenting at the cell surface of the alga, pH titration experiment was carried out. The biomass of 0.05 g was protonated by coming into contact with 30  $\text{cm}^3$  of 0.1  $\text{mol/dm}^3$  HCl. This treatment ensures that all remaining ions such as Ca(II), Mg(II), Na(I) and K(I) can be removed from the algal surface. This was allowed for 3 h to attain equilibrium. The protonated biomass was dried in an oven at 90 °C for 24 h.

#### 2.3.2 Titration of biomass

0.02 g of the protonated biomass was dispersed in 30  $\text{cm}^3$  of 0.1  $\text{mol/m}^3$  NaCl solution in a flask. Titration was carried out by adding stepwise 0.05  $\text{cm}^3$  of 0.1  $\text{mol/m}^3$  NaOH solution to the flask. After addition of the titrant, the solution was allowed to attain equilibrium until the pH reading became stable.

### 2.4 Adsorption of metals

$\text{PbCl}_2$ ,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$  and  $\text{NiCl}_2 \cdot 7\text{H}_2\text{O}$  were used as the metal sources. The adsorption of Pb(II), Fe(III) and Ni(II) ions onto the algal biomass was investigated at 30 °C in batch experiments. The solution was brought to the desired pH (1.0-7.0) for each metal ion by adding 0.1  $\text{mol/dm}^3$  HCl and  $\text{CH}_3\text{COONa}$  solutions. The effect of initial metal concentration on the adsorption rate was studied at pH 5.0 for Pb(II), pH 4.0 for Fe(III), and pH 7.0 for Ni(II) ions. Adsorption of the metal in metal-coexisting system was also studied using metal solutions containing Fe(III) and Ni(II) ions by similar procedure to that described above. These experiments were performed using a solution containing each metal ion with a concentration of 0.33  $\text{mmol/dm}^3$ .

The amount of metal ions adsorbed per unit biosorbent was obtained by using the following equation,

$$q_e = \frac{(C_0 - C_e)V}{M} \quad (1)$$

Where  $q_e$  is the amount of metal ions adsorbed onto the unit quantity of the biomass ( $\text{mmol/g}$ -biomass),  $C_0$  and  $C_e$  are the metal concentration at initial stage and at equilibrium, respectively,  $V$  is the volume of the aqueous solution, and  $M$  is the amount of biomass used.

### 2.5 Desorption of metals

In order to examine the reusability of the biosorbents, consecutive adsorption-desorption experiment was repeated five times for Fe(III) ion using an identical algal biomass. Desorption of Fe(III) ion was performed by using 50  $\text{cm}^3$  of 0.5  $\text{mol/m}^3$  HCl solution. The algal biomass loaded with Fe(III) ion was placed in the desorbing medium and was stirred at 300 rpm for 6 h at 30 °C. After every cycle of the

adsorption-desorption, the algal biomass was rinsed with distilled water and reconditioned for adsorption in the succeeding cycle.

### 3. Results and discussion

#### 3.1 FT-IR spectra of biomass

The functional groups responsible for adsorption of the metal ions onto *C. reinhardtii* are estimated by FT-IR spectra. The FT-IR spectrum of Ni(II)-treated algal biomass was also measured. The FT-IR spectrum of the algal biomass indicated the presence of amino, carboxylic and sulfonate groups. These ionizable functional groups are thought to interact with the metal ions.

#### 3.2 Potentiometric titration

Figure 1 shows the potentiometric titration curves obtained from the addition of NaOH solution. Acidic groups of the biomass and their  $pK_a$  values are evaluated by identifying the inflection points of the titration curves. Figure 2 shows the first derivative plots of the pH titration data of Figure 1. The first derivative plots consist of  $dpH/dV$  versus the midpoint of successive quantity of NaOH added ( $V$ ). The corresponding  $pK_a$  values are estimated by using both the first derivative plots and the original titration curve. It was shown from Figure 2 that *C. reinhardtii* possessed the acidic functionalities at the algal surface. Sulfonate groups usually contribute to metal binding at low pH and their typical  $pK_a$  values are in the range 1.0-2.5 (Sheng *et al*, 2004). Apparent  $pK_a$  values in this range were not detected by the titration, but the presence of sulfonate groups at the algal surface was confirmed by FT-IR analysis mentioned above. The acidity of hydroxyl groups in algal cell wall (polysaccharides) are considerably weaker than that of carboxyl groups and may interact with cations only at higher pH. Therefore, only surface hydroxyl groups play a significant role in the metal binding at very high pH values (Davis *et al*, 2003). Algal proteins have been known to interact with metal ions particularly at pH values between 6-9, and the protonated amino groups have a  $pK_a$  value of around 8 (Percival and McDowell, 1967).

#### 3.3 Effect of pH on metal adsorption

The pH of solution is an important parameter affecting metal adsorption by algal biomass. Therefore, the optimum pH for metal uptake is related to the  $pK_a$  of these functionalities. The solution chemistry of the metal complexes formed must also be considered since the speciation of metals in solution is dependent on pH (Sheng *et al*, 2004).

Figure 3 shows the relationship between the metal uptake and the equilibrium pH. It is clearly seen that higher pH values lead to higher metal uptake for Pb(II), Fe(III) and Ni(II) ions. The optimum adsorption pH was determined as pH 5.0 for Pb(II), 4.0 for Fe(III) and 7.0 for Ni(II) ions, respectively. Above these pH's, insoluble metal hydroxide was seen to precipitate out. At a low pH, Pb(II), Fe(III), Ni(II) and hydrogen ions may compete in each other for binding ligands at the cell wall. As the pH increases,  $H^+$  ions in the solution become fewer. This means that there is less competition for binding sites, thus this leads to more enhanced adsorption of the metals. At pH 1.0-2.0, few metal uptakes was observed. This may be due to the presence of sulfonate groups that are dissociated at this pH range. At pH 3.5-5.0, carboxyl groups in the algae generate a negatively charged surface and electrostatic interactions between the surface and cationic species can be responsible for the metal adsorption.

This pH dependence of the metal adsorption demonstrates that the algae can potentially be developed as multi-use adsorption materials. As seen in Figure 3, the metal adsorption is reduced at lower pH thus facilitating removal of the metal ions by simple pH adjustment.

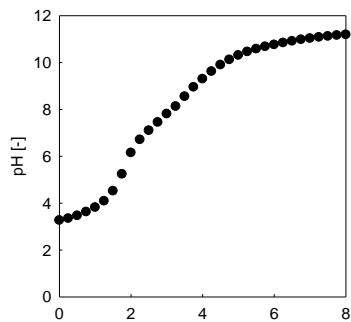


Figure 1 : pH titration curve of *C. reinhardtii*

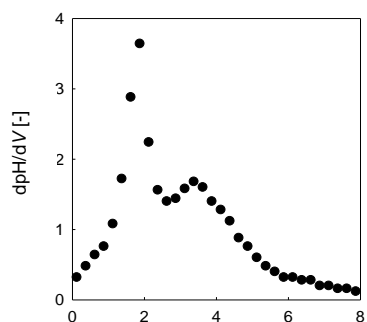


Figure 2 : pH titration curve of *C. reinhardtii*

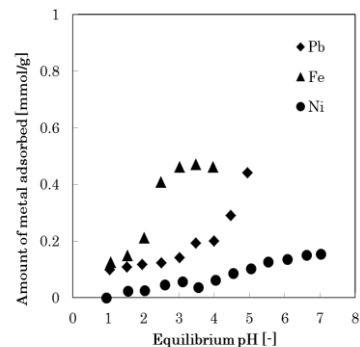


Figure 3 : Effect of pH on metal adsorption

Algal biomass dose : 0.5g/dm<sup>3</sup>  
 Initial concentration of metal ion : 1 mmol/dm<sup>3</sup>, Temperature : 303K,  
 Stirring speed : 300rpm

### 3.4 Langmuir isotherms

The experimental results are shown in Figure 4. Langmuir adsorption isotherm is a widespread-used model for describing metal adsorption to a sorbent. Langmuir equation relates the coverage of molecules on solid surface to the concentration in solution contacting the solid surface. This isotherm based on three assumptions, namely (1) adsorption is limited to monolayer coverage, (2) all surface sites are alike and can accommodate only one adsorbed atom, and (3) ability of molecule to be adsorbed on a given site is independent of its neighbouring sites occupied. By considering these assumptions, the Langmuir equation can be derived as follows,

$$q_e = \frac{q_{\max} K_L C_e}{1 + K_L C_e} \quad (2)$$

Where  $q_{\max}$  is the maximum adsorption capacity of biomass,  $C_e$  is the equilibrium concentration of metal ions in solution and  $K_L$  is the Langmuir adsorption equilibrium constant. The Langmuir adsorption equilibrium constant and the maximum adsorption capacity calculated from the isotherms and the correlation coefficients ( $R^2$ ) are presented in Table 1. From these results, it is found that the algal biomass have a high adsorption capacity for Pb(II) and high binding affinity for Fe(III) ion.

### 3.5 Kinetic study of metal adsorption

The kinetics of metal adsorption onto the algal biomass was examined from measuring the initial adsorption rates. Time course of adsorption of the metals was measured varying the pH and the initial metal ion concentration in the solution. The initial adsorption rate,  $R_0$ , was calculated from the initial slope at  $t=0$  of the curved line presenting the time course of adsorption of the metals.

Figure 5 shows the relationship between  $R_0$  for Pb(II) and hydrogen-ion concentration in log scale. And Figure 6 shows the relationship between  $R_0$  and initial metal ion concentration of Pb(II) in log scale. The similar result was obtained for Fe(III) and Ni(II) adsorption. It is assumed that the adsorption is expressed by the following equation.



Where  $M^{n+}$  means each metal ion (Pb(II), Fe(III) and Ni(II)).

Reaction orders with respect to hydrogen ion and metal ion are summarized in Table 2. In the case of Fe(III) ion, the reaction is not proportional to the metal concentration. It may be due to the influence of diffusion since the adsorption rate of Fe(III) ion is the fastest among the metals.

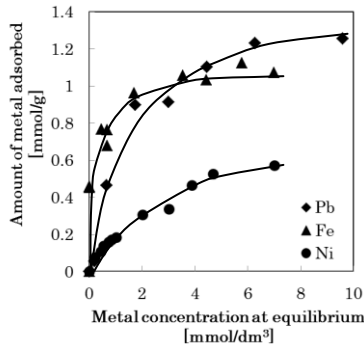


Figure 4 : Langmuir adsorption isotherms

Table 1: The Langmuir constants for adsorption of metals

Metal ions	pH	$q_{max}$ [mmol/g-biomass]	$K$ [dm <sup>3</sup> /mmol]	$R^2$ [-]
Pb(II)	5.0	1.47	0.682	0.982
Fe(III)	4.0	1.13	3.43	0.964
Ni(II)	7.0	0.870	0.277	0.983

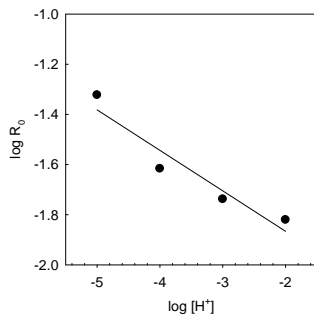


Figure 5 : Plots of the logarithm of initial rate

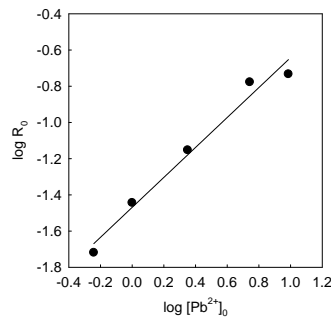


Figure 6 : Plots of the logarithm of initial rate

Table 2 : Order of reaction of metal adsorption

Metal ions	Reaction order of [H <sup>+</sup> ]	Reaction order of [M <sup>n+</sup> ]
Pb(II)	-0.121	0.827
Fe(III)	-0.205	0.368
Ni(II)	-0.259	0.975

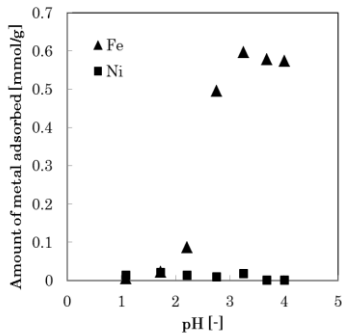


Figure 7 : Effect of pH on adsorption in metal-coexisting system

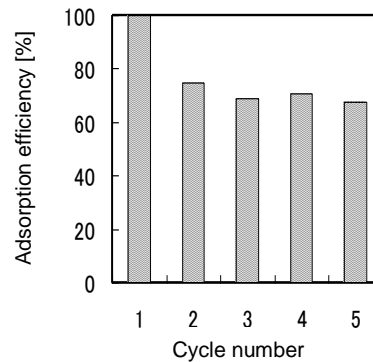


Figure 8 : Reuse of algal biomass

### 3.6 Effect of pH on metal adsorption in metal-coexisting system

Figure 7 shows the pH dependency of the metal adsorption in Fe(III) and Ni(II) coexistence system. Comparing to the adsorption experiment in single metal system, Fe(III) was selectively adsorbed in a high pH range, while Ni(II) was not adsorbed in the metal-coexisting system. The value of Langmuir

equilibrium constant  $K_L$  of Fe(III) at pH 4.0 in the single metal system is very high as 3.43. It is considered that this is a reason for high selectivity of Fe(III) in the metal-coexistence system.

### 3.7 Reusability of algal biomass

Figure 8 shows the adsorption efficiency when the algal biomass was reused. The cycle of adsorption-desorption was repeated five times. HCl solution of 0.5 mmol/dm<sup>3</sup> was used as the desorbing solution. Fe(III) ion of about 95 % was able to be desorbed from Fe(III)-loaded algal biomass by the desorbing solution. The first adsorption capacity is defined as 100 %. Approximately 75 % of the adsorption capacity was kept after five times in the adsorption-desorption cycles.

## 4. Conclusions

The microalga *Chlamydomonas reinhardtii* was used for biosorption of Pb(II), Fe(III) and Ni(II) ions. The FT-IR analysis and the pH titration of algal biomass revealed the presence of amino, carboxyl and sulfonate groups, which were responsible for biosorption of the metal ions.

The results of adsorption studies indicate that the biomass of *C. reinhardtii* is suitable for the removal and recovery of Pb(II), Fe(III) and Ni(II) ions. Biosorption of Pb(II), Fe(III) and Ni(II) ions onto the algal biomass was dependent on pH and the maximum amount of biosorption was obtained at pH 5.0 for Pb(II), pH 4.0 for Fe(III) and pH 7.0 for Ni(II) ions, respectively. The adsorptive sites at the algal biomass showed a higher affinity for Fe(III) ion compared with the other metal ions. The experimental data were well described by the Langmuir adsorption isotherm model. The algal biomass can be reused even after HCl treatment.

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