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Lead (Pb^{2+}) Removal from Wastewater by the Cyanobacterium *Calothrix marchica*

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ABSTRACT

Pb^{2+} removal ability of the living-freshwater cyanobacterium *C. marchica* was studied in batch experiments. The result showed that adsorption of Pb^{2+} by *C. marchica* reached equilibrium within 60 min. The amount of Pb^{2+} adsorbed (q_{eq}) increased when cyanobacterial age increased. At lower biomass concentration cyanobacterium showed higher q_{eq} than that at high biomass concentration. Elevated temperature increased Pb^{2+} adsorbed by *C. marchica*. The Langmuir adsorption isotherm fitted the results better than the Freundlich isotherm and, thus, was more suitable to describe Pb^{2+} adsorption by *C. marchica*. *C. marchica* had Pb^{2+} binding capacity (q_{max}) of 74.04 mg g^{-1}, and indicators of adsorption capacity (K_f) of 18.01. Pb^{2+} removal under light and dark conditions was not significantly different.

Key words: lead (Pb^{2+}), *Calothrix marchica*, adsorption isotherm

INTRODUCTION

Heavy metal released from agricultural and industrial processes into natural water can cause serious effects to the environment. Lead (Pb^{2+}) is one of the toxic heavy metals commonly found in aquatic environments. Its contamination in the environment has greatly increased as a result of major anthropogenic emission and improper waste disposal (Fourest and Roux, 1992). Therefore, Pb removal from wastewater has been examined extensively. Various techniques have been developed to remove Pb from the wastewater, and one of the most common methods is adsorption, with activated carbon being the most widely used adsorbent for this purpose. However, this can be expensive and there has been considerable interest in the use of other adsorbent materials, particularly biosorbents such as cyanobacteria (Wang *et al*., 1998), algae (Gupta *et al*., 2001), yeast (Volesky *et al*., 1993), and aquatic plants (Miranda and Ilangovan, 1996).

Cyanobacteria are one of the biomaterials that have high potential for removing heavy metals from wastewater (Inthorn *et al*., 2002). They can sequester heavy metal ions in a short period of time through adsorption and absorption mechanisms (Bajguz, 2000). Preliminary screening results from 43 strains of cyanobacteria showed that *Calothrix marchica* had a high Pb^{2+} removal ability (Inthorn *et al*., 2002). In this study, the removal ability of this cyanobacterium was

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further explored and examined under various conditions. Accordingly, the objective of this study was to know the effects of exposure time, cyanobacterium age, cyanobacterium biomass, temperature, light condition, and initial Pb^{2+} concentration on Pb^{2+} removal ability of *C. marchica*.

MATERIALS AND METHODS

Preparation of cyanobacterial biomass

Calothrix marchica (TISTR8109) was obtained from the Thailand Institute of Scientific and Technological Research (TISTR). Stock culture was grown at pH 7 in Medium-18 (Inthorn *et al*., 2001) under the continuous illumination of 400 μE m^{-2} s^{-1} at 25°C. The pH was adjusted to 7 and the medium was autoclaved before use. Upon harvesting, the cyanobacterium was washed three times with Milli-Q water and separated by centrifugation at 1,000 × *g* for 5 minutes at 4°C. These cells were used in the experiments thereafter. All the experiments were conducted in triplicate.

Determination of equilibrium time

To study the optimum exposure time (equilibrium time for all concentrations of Pb^{2+}) for Pb^{2+} adsorption, 0.3 g wet weight of 14-day old cells of *C. marchica* were added in 300 ml of 1 mg l^{-1} Pb solution in a 500 ml polypropylene beaker. The control set for this experiment was 300 ml of 1 mg l^{-1} Pb solution without cyanobacterium. The pH solution was adjusted to 4. The samples were shaken at 120 rpm for 120 min on a shaker at 25°C. Concentration of Pb^{2+} in each sample was then determined at 0, 1, 2, 3, 4, 5, 7, 10, 15, 20, 30, 45, 60, 90, and 120 min. Cyanobacterial cells were then separated by filtration with 0.2 mm pore size and 47 mm-diameter cellulose nitrate filter membrane, and were dried at 105°C for 24 h. The dry weights of cyanobacterial cells in each sample were determined. The left over supernatant was immediately preserved by adjusting the pH to 4 with 0.03 N HNO_3, and kept at 4°C. The residual Pb^{2+} was measured from the supernatant within 3 days after acid preservation.

Effect of cyanobacterial age on Pb^{2+} removal ability

Cyanobacterial cells at the three growth phases were harvested, i.e., 4-day old (lag phase), 10-day old (early log phase), and 14-day old (mid log phase). 0.15 g wet weight of cyanobacterium from each growth stage was added in to 150 ml of 2 mg l^{-1} Pb solution in a 250 ml polypropylene flask at pH 4. The control set for this was 150 ml of 2 mg l^{-1} Pb solution without cyanobacterium. The flasks were shaken at 120 rpm on a shaker at 25°C for 60 min. Cyanobacterial cells were then separated and concentrations of Pb^{2+} in the solution were then determined.

The metal adsorption (q_{eq}) in mg g^{-1} was calculated from the initial concentration (C_i, mg l^{-1}) and the final concentration (C_{eq}, mg l^{-1}) of the Pb^{2+} according to Eq. 1:

$$q_{eq} = V(C_i - C_{eq})/M \qquad \text{(Eq. 1)}$$

where V is the liquid sample volume (ml) and M is the biomass dry weight (g).

Effect of cyanobacterial biomass on Pb^{2+} removal ability

0.05 and 0.1 g wet weight (0.005 and 0.01 g dry wt) of 14-day old cells were added in to 100 ml of 10 mg Pb l^{-1} solution in a 250 ml polypropylene flask at pH 4. The control set for this experiment was 100 ml of 10 mg Pb l^{-1} solution without cyanobacterium. The flask was shaken at 120 rpm on a shaker at 25°C for 60 min. Cyanobacterial cells were then separated and concentrations of Pb^{2+} in the solution were determined.

Effect of temperature on Pb^{2+} removal ability

0.15 g wet weight (0.015 g dry wt) of 14-day old cells were added in to 150 ml of 10 mg Pb l^{-1} solution in a 250 ml polypropylene flask at pH 4. The control set for this was 150 ml of 10 mg Pb l^{-1} solution without cyanobacterium. The flasks were shaken at at 120 rpm under temperatures of 0, 4, 25, 35 and 45°C for 90 min. Residual Pb^{2+} concentrations were then measured and compared among these temperatures.

Adsorption isotherm

0.3 g wet weight (0.03 g dry wt) of 14-day old cells was added into 300 ml Milli-Q water with various Pb^{2+} concentrations, ranging from 1 to 120 mg l^{-1}. They were shaken at 120 rpm on a shaker at 25°C for 60 min. From preliminary tests, the systems attained equilibrium within 60 min. Subsequently, cyanobacterial cells were harvested and the residual Pb^{2+} concentrations were determined. The adsorption characteristics of *C. marchica* were described by using the Langmuir (Eq. 2) and the Freundlich (Eq.3) adsorption isotherms (Volesky and Holan, 1995):

$$q_{eq} = q_{max} b C_{eq} / 1 + b C_{eq} \qquad \text{(Eq.2)}$$

where q_{eq} is the amount of monolayer coverage of solute adsorbed per unit weight of adsorbent (mg g^{-1} of algae) at equilibrium, q_{max} is the maximum monolayer coverage for sorbate uptake under the given conditions (mg g^{-1}), b is a coefficient related to the affinity between the sorbent and sorbate (l mg^{-1}), C_{eq} is the equilibrium concentration of solute (mg l^{-1}). q_{max} and b can be determined from a linear plot of $C_{eq}\ q_{eq}^{-1}$ vs. C_{eq} or $1\ q_{eq}^{-1}$ vs. $1\ C_{eq}^{-1}$

$$q_{eq} = K_f C_{eq}^{1/n} \qquad \text{(Eq.3)}$$

where K_f and $1/n$ are the constant characteristics of the system. K_f and n are indicators of adsorption capacity and adsorption intensity, respectively.

Effect of initial Pb^{2+} concentration and light intensity on Pb^{2+} removal ability

Cyanobacterial cells were placed in a polypropylene beaker and kept under light (400 µE m^{-2} s^{-1}) or dark (wrap the beaker with aluminum foil) for 3 hours before the experiment. 0.3 g wet weight (0.03 g dry wt) of 14-day old cells were added into 300 ml Milli-Q water with various Pb^{2+} concentrations. Pb^{2+} concentration range of 2-10 mg l^{-1} was used. The control for this experiment was 300 ml of 2-10 mg l^{-1} Pb solution without cyanobacterium. They were shaken at 120 rpm on a shaker at 25°C under light or dark for 60 min. Subsequently, cyanobacterial cells were harvested and the residual Pb^{2+} concentrations were determined.

Dry weight determination

Cyanobacterial cells from all experiments were filtered through a pre-weighed cellulose nitrate filter membrane with 0.2 µm pore size. Prior to use, all the filter papers were dried at 105°C for 24 h and weights were recorded. The filter paper with cells on it was dried at 105°C for 24 h, dry weight was determined after cooling to room temperature in a desiccator.

Chemical analysis

Pb^{2+} concentration was analyzed using an atomic absorption spectrophotometer (GBC Avanta, Australia). The detection limit was 20 ng $ml^{-1}$for graphite furnace method.

Statistical analysis

All the experiments were conducted in three replicates. Significant differences were determined using analysis of variance (ANOVA). Ninety-five percent confidence (probability limit of $p<0.05$) was considered as significant.

RESULTS AND DISCUSSION

Determination of equilibrium time

Pb^{2+} removal as a function of time of *C.*

marchica is shown in Figure 1. The percent removal was calculated from the amounts of Pb^{2+} removed from the solution. The Pb^{2+} removed by *C. marchica* reached 42.2 % within 10 min. After that Pb^{2+} sorption continued until equilibrium point was reached at 60 min with 61.62 % of Pb^{2+} removal. Two main mechanisms for the removal of heavy metals from solutions by biomaterials were reported: physico-chemical interaction (e.g., adsorption) and metabolic-dependent processes (e.g., carrier-mediated transport) (Ting *et al*., 1991). The former was a rapid process while the latter was relatively slow through metabolic processes. From the removal characteristics in Figure 1, it seemed that both two steps were involved. The adsorption was fast in the first step (before 10 min), possibly due to abundance of binding sites on cyanobacterial surface which were ready to bind to Pb ions. After that (10-30 min) the binding sites were possibly nearly full and only a few of binding sites were free to bind to Pb ions, which resulted in slowing down the sorption rate. The second step of removal was observed between 30-60 min. This was likely due to transport of Pb^{2+} into the cells or to the binding sites located in the inner part of the colony of *C. marchica*.

In the green alga, *Dunaliella tertiolecta*, Santana *et al*. (1995) reported that Pb^{2+} adsorbed rapidly at the first step and followed by the slow sorption step until equilibrium was reached. Santana *et al*. (1995) explained that at the first step Pb^{2+} was bound on cell surface and this took place rather fast. It was followed by Pb^{2+} uptake by the living cells, which took place more slowly, and might be controlled by the diffusion process through the cell wall or regulated by intracellular metabolic process.

The pH change of Pb^{2+} solution during cyanobacterium exposure is shown in Figure 2. The pH increased after cyanobacterium was added to Pb^{2+} solution. pH of Pb^{2+} solution in the control set (no cyanobacterium added) was not changed (pH 4). After 120 min, pH of Pb^{2+} solution with *C. marchica* was 5.33. While the adsorption was proceeded, the pH of the solution rapidly increased within a few minutes. It was shown that when Pb^{2+} was removed from the solution the pH increased, because the OH^- was released from

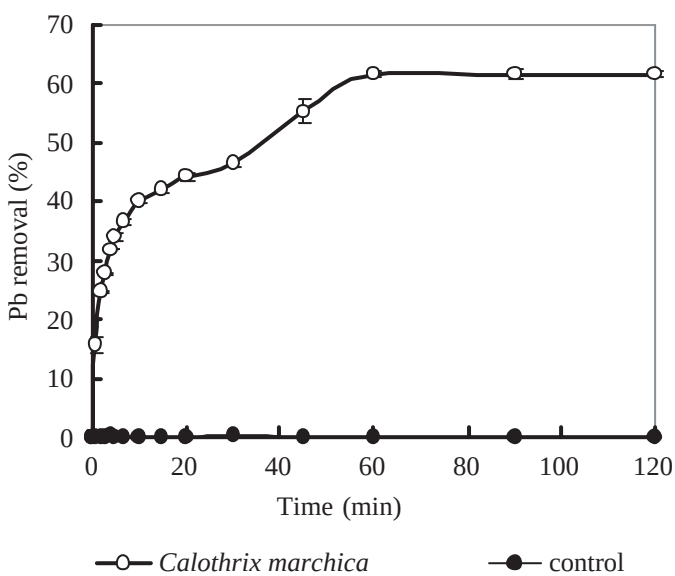

Figure 1 Time-course removal of Pb^{2+} by cells of cyanobacterium *C. marchica*.

$PbOH^+$, or $Pb(OH)_2$ (formed in the solution) when Pb^{2+} was adsorbed. From these results, could can be concluded that it was appropriate to set 60 min as the equilibrium time for Pb^{2+} removal by *C. marchica*, because no significant adsorption could be seen beyond this.

Effect of cyanobacterium age on Pb^{2+} removal

The growth stage of cyanobacterial cell affects heavy metal removal through availability and characteristics of binding sites that differ and change along the growth phase. In this study the results of Pb^{2+} adsorbed by *C. marchica* showed that when cyanobacterial age increased, the amount of Pb^{2+} adsorbed (q_{eq}) increased (Table 1). Cyanobacterial ages of 4 and 10 days showed no significant difference in their Pb^{2+} removal ability. A 14-day old cell had higher Pb^{2+} removal ability (11.64 mg Pb g^{-1} dry weight) than that of 4 and 10 days. Cyanobacterial cell could adsorb Pb^{2+} on to functional groups present on the cell surface. 4-day and 10-day old cyanobacterium might have similar numbers of binding site. The 14-day cyanobacterium, which showed higher Pb^{2+} removal ability might contain more binding sites on their cell surface.

Normally several functional groups on the microbial surface can interact with metals and play a major role in heavy metal removal (Ledin, 2000). These functional groups commonly exist on polysaccharides and some proteins, which cover the cell surface. Polysaccharides and

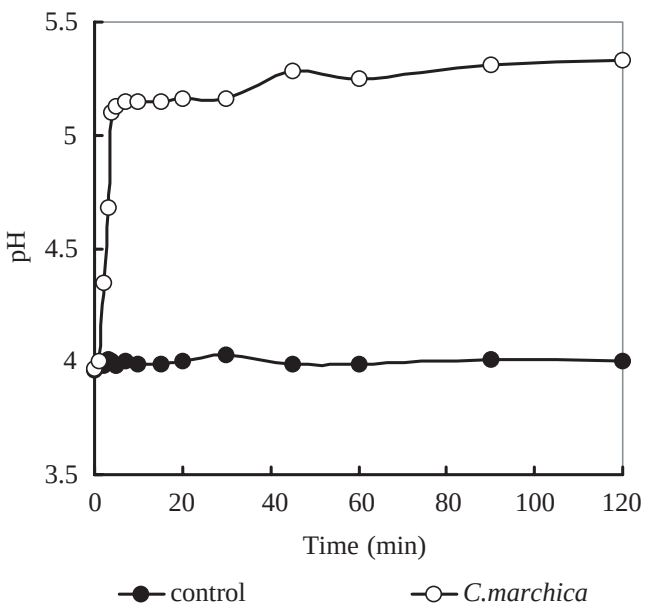

Figure 2 Changes of pH during Pb^{2+} removal by cells of *C. marchica*.

Table 1 Lead adsorbed (q_{eq}) by *C. marchica* at different ages.

Cyanobacterial age (day)	q_{eq} (mg Pb g^{-1} dry wt)
4	2.53 ± 0.03^a
10	2.56 ± 0.01^a
14	11.64 ± 0.95^b

The same superscript letters in each column denote no significant difference with 95 % confidence limit ($p<0.05$).

proteins are produced in microbial cells and released to the outside of cells (Geesey and Jang, 1990). Thus, when cyanobacterial age increased, the amounts of polysaccharides and proteins on the cell surface were also assumed to increase, resulting in the existence of more functional groups. Hence, Pb^{2+} removal increased with cell age.

Several studies on metal removal with cell age were reported. One-day-old cells of *Thiothrix* accumulated considerably less Ni or Zn than 2-5 day-old cells (Shuttlewort and Unz, 1993). However, Inthorn *et al*. (1996) reported that Cd adsorption ability by the filamentous cyanobacterium *Tolypothrix* was not significantly different on the cultivating time of 3-day, 1-week, and 3-week cultures.

Effect of cyanobacterial biomass on Pb^{2+} removal

Table 2 shows the percent Pb^{2+} removal and the amount of Pb^{2+} adsorbed (q_{eq}) by *C. marchica* using different cell mass. The percent of Pb^{2+} removal using 0.5 and 1.0 g wet weight l^{-1} was not significantly different, but its q_{eq} values was significantly higher when using 0.5 g wet weight l^{-1} cell mass than when using 1.0 g wet weight l^{-1} cell mass.

Lower biomass resulted in different Pb^{2+} removal (q_{eq}) efficiency even though percent removal was not significantly different between the biomass of 0.5 and 1.0 g wet weight l^{-1}. It seemed that 0.5 g of biomass had similar numbers of binding site as that of 1.0 g biomass, because they showed nearly similar percent removal. Actually 1.0 g biomass should have more binding sites than 0.5 g biomass and should have higher percent removal. However, high cell density may reduce the available binding sites than that at lower cell density, because cells are attached to one another and subsequently they aggregate. Although they had higher biomass but not all binding sites were made available to Pb^{2+} (Itoh *et al*., 1975). This was possibly attributed to the electrostatic interactions of the binding sites at the cell surface as suggested by Ledin (2000). Therefore, lowering the cell surface area can reduce its effective biosorption area (Aksu and Kutsal, 1990).

Similar results were reported in other algae, cyanobacteria and other microbial systems. The decreased accumulation of Pb^{2+} with an increase in biomass concentration was found in the marine bacterium *Pseudomonas atlantica* (Lion and Rochlin, 1989); in the cyanobacterium *Oscillatoria anguistissima* (Ahuja *et al*., 1997), the fungus *Aspergillus carbonarius* for Cu, Co and Cr (Al-Ashes and Duvnjak, 1995); the cyanobacteria, *Oscillatoria* sp. for Zn (Ahuja, *et al*., 1999b); and Cd accumulation by *Tolypothrix tenuis* (Inthorn *et al*., 1996).

Effect of temperature on Pb^{2+} removal

To understand the effects of temperature of Pb^{2+} solution on Pb^{2+} adsorption on to the cell surface, the experiment was carried out at 5 different temperatures, between 0 and 45°C. The results of this study are shown in Table 3. Among the temperatures between 0 and 35°C, the amount of Pb^{2+} adsorbed (q_{eq}) was not significantly different (42.01-55.98 mg Pb g^{-1} dry wt). But at 45°C, the amount of adsorbed Pb^{2+} was significantly higher than at 0-35°C (77.96 mg Pb g^{-1} dry wt). Therefore, the amount of Pb^{2+}

Table 2 Lead adsorbed (q_{eq}) by *C. marchica* with different biomass.

Cell mass (g wet wt l^{-1})	% removal	q_{eq} (mg Pb g^{-1} dry wt)
0.5	62.27 ± 0.29^{a}	82.15 ± 0.77^{a}
1	63.16 ± 0.43^{a}	39.38 ± 1.26^{b}

The same superscript letters in each column denotes no significant difference with 95% confidence limit ($p<0.05$).

adsorbed by *C. marchica* was not affected by temperature at 0-35°C, but the adsorption could be stimulated at higher temperature.

The reactions increase with increasing temperature is a endothermic reaction and the rate of reaction decreases with temperature implies a exothermic reaction (Steinfeld *et al*., 1989). For *C. marchica*, there might be another explanation for the increased Pb^{2+} adsorption at high temperature; because high temperature probably caused its colony to break down, thus resulted in the increasing surface area and Pb^{2+} adsorption ability. The result of this study suggested that the Pb^{2+} adsorption by *C. marchica* was endothermic, because the adsorption increased with temperature. However, in this study adsorption isotherm was not studied at different temperatures. Therefore, clear interpretation for the effects of temperature on Pb^{2+} adsorption by this cyanobacterium could not be concluded using traditional way of calculating enthalpy to interpret endothermic or exothermic reactions.

Increases in metal adsorption capacity with temperature were also reported. Examples are the adsorption capacity of various heavy metals by *Chlorella vulgaris* (Aksu and Kutsal, 1990), the adsorption of Cu by *Oscillatoria* (Ahuja *et al*., 1997), the accumulation of U by *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa* (Standberg *et al*., 1981), and the accumulation of Pb by *Dunaliella tertiolecta* (Santana-Casiano *et al*., 1995). As suggested by Al-Ashes and Duvnjak (1995) too high temperatures would kill the cells. In such cases, both increasing and decreasing Pb^{2+} adsorption could be the result, depending on removal mechanisms. At high temperature, the consequence is destruction of the cell membranes, thereby exposing intracellular components as well as surface binding sites. In conclusion, it seemed that Pb^{2+} sorption of *C. marchica* tended to increase at high temperature more than 35°C. Thus, its Pb^{2+} adsorption might involve some of the physical processes in addition to physico-chemical interactions as discussed above and reported by Ahuja *et al*. (1999a).

Adsorption isotherm

The Freundlich and Langmuir isotherms were employed to study Pb^{2+} adsorption characteristics. The results are presented in Table 4. The Langmuir adsorption isotherm had a higher correlation coefficient (r) than the Freundlich isotherm. Therefore, in this study the mechanisms involved in Pb^{2+} removal by *C. marchica* were discussed based on the Langmuir isotherm parameters. The maximum Pb^{2+} uptake calculated according to the Langmuir isotherm (q_{max}) was 74.04 mg g^{-1} of dry weight of algae (Table 4). This was much higher than those by other types of cyanobacteria. *C. marchica* showed a higher q_{max} than *Phormidium* (13.60 mg Pb g^{-1} dry wt) (Wang *et al*., 1998). However, the Pb^{2+} uptake capacity of living *C. marchica* was lower than the dried brown algae (242.42-304.58 mg Pb g^{-1} dry wt) (Matheickal and Yu, 1999). This was probably due to the fact that brown algae cell surface contained acidic sugars (such as alginic acid), which showed the high capability in cation adsorption (Percival and McDowell, 1967).

Table 3 Lead adsorbed (q_{eq}) by *C. marchica* with different temperature.

Temperature (°C)	q_{eq} (mg Pb g^{-1} dry wt)
0	42.01 ± 0.41^{a}
4	52.98 ± 2.86^{b}
25	49.88 ± 2.65^{ab}
35	55.97 ± 4.06^{b}
45	77.96 ± 6.08^{c}

The same superscript letters in each column denotes no significant difference with 95% confidence limit ($p<0.05$).

Table 4 Langmuir and Freundlich parameters calculated from experimental data with initial Pb concentration 1-120 mg l^{-1}.

Isotherm	Parameters	Value
Langmiur	q_{max} (mg Pb g^{-1} dry wt.)	74.04
	b (l mg^{-1})	1.32
	r^2	0.87
Freundlich	K_f	18.01
	n	3.34
	r^2	0.85

Effect of initial Pb^{2+} concentration and light on Pb^{2+} removal

The effects of initial Pb^{2+} concentration and light are shown in Table 5 and Figure 3. Pb^{2+} adsorbed by *C. marchica* increased when Pb^{2+} concentration increased. The amount of Pb^{2+} adsorbed onto the cells (q_{eq}) was not significantly different between under light and dark conditions for all concentrations. Pb^{2+} adsorbed on *C. marchi*ca cells at higher initial concentration showed significantly higher than lower initial Pb^{2+} concentration. The highest Pb^{2+} adsorbed under light and dark were 41.30 and 42.61 mg Pb g^{-1} dry weight at the initial Pb^{2+} concentration of 10 mg l^{-1}.

At low initial Pb^{2+} concentration, adsorption of cyanobacterium reached its equilibrium point faster with higher removal efficiency than that at high Pb^{2+} concentration (Figure 3). This was possibly due to the relatively large amount of binding sites for Pb^{2+} on cell surfaces (low adsorbate:adsorbent ratio) at low concentration. On the other hand, at high Pb^{2+} concentration repulsive force could occur among Pb ions and, thus, resulting in a slower adsorption time than at low concentrations. There was no significant difference ($p<0.05$) in removal ability between under light and dark conditions. However, it seemed that Pb^{+2} adsorption under dark condition reached equilibrium slower than that under light condition.

Normally light can affect nutrient uptake indirectly through photosynthesis, which can provide energy for active transport and production of carbon skeletons necessary for incorporation of nutrient ions into larger molecules (e.g., amino acids and proteins) (Lewin, 1962). In addition, a decrease in photosynthetic activity in plants may lower the intracellular pH, which may decrease the non-metabolic absorption of metal (Gutknecht 1963). In this study *C. marchica* had slower Pb^{+2} adsorption rate under dark condition than under light condition. This was possibly due to no photosynthesis activity under dark condition and

Table 5 Lead adsorbed (q_{eq}) by *C. marchica* under light and dark conditions.

Initial Pb^{2+}	q_{eq} (mg Pb g^{-1} dry wt)	
(mg l^{-1})	Light	Dark
2	11.64 ± 0.95^{aA}	10.36 ± 0.20^{aA}
4	19.30 ± 0.35^{bA}	18.28 ± 0.99^{bA}
6	25.71 ± 0.37^{cA}	27.90 ± 0.59^{cA}
8	33.40 ± 2.48^{dA}	36.66 ± 1.82^{dA}
10	41.30 ± 3.37^{eA}	42.61 ± 1.94^{eA}

The same higher case letter of the same row, and the same lower case of the same column denotes no significant difference with 95 % confidence limit ($p<0.05$).

thus no energy for metal sorption processes. This indicated that Pb^{2+} adsorption by this cyanobacterium somehow depended on metabolic activities, which could be seen from the different adsorption characteristics between under light and dark conditions.

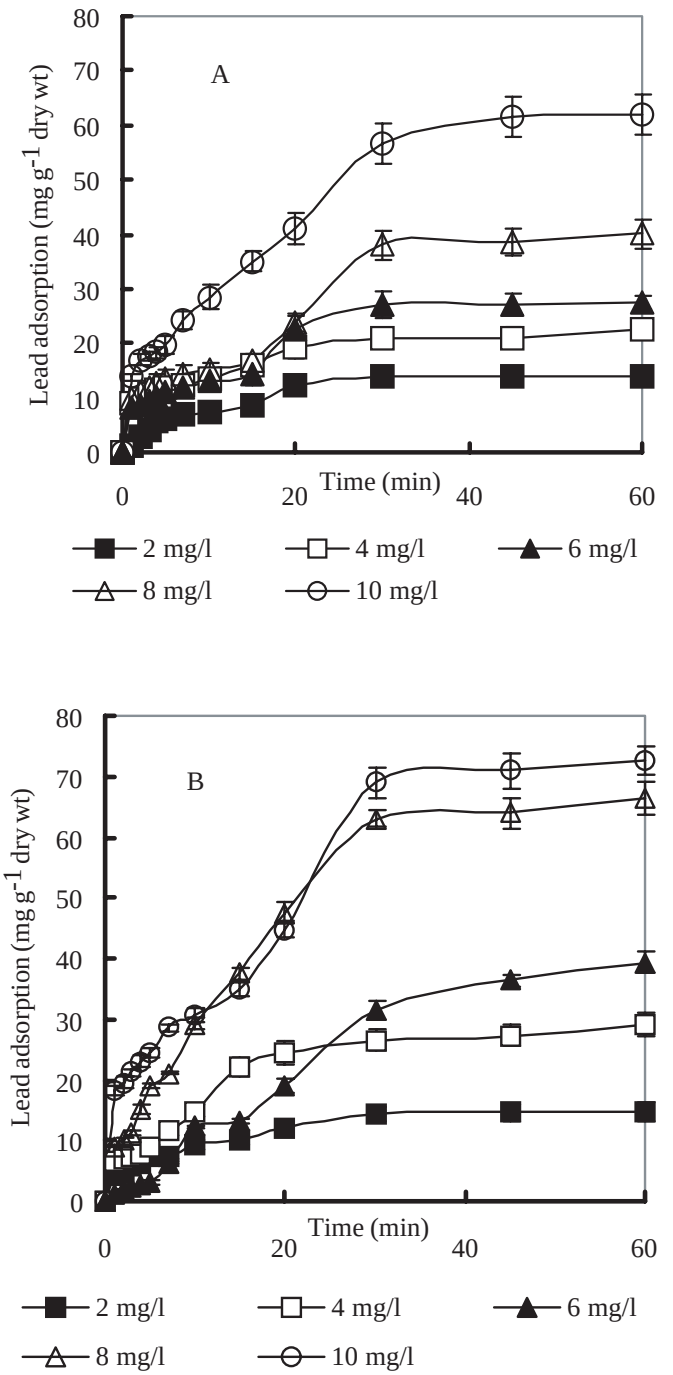

Figure 3 Pb^{2+} removal ability of *C. marchica* at various initial concentrations under light (A) and dark (B) conditions. Error bars represent ± S.D. of three replicates.

Some studies on the effects of light and dark conditions on metal uptake in seaweed were reported. In *Ulva lactuca* (green algae), dark exposure significantly inhibited the uptake of Cd and Zn, but the uptake of Cr and Se was relatively unaffected. In addition, metal (Cd, Zn, Cr, Se) uptake in *Gracilaria blodgettii* (red algae) was not significantly different between light and dark exposure (Wang and Dei, 1999).

CONCLUSIONS

C. marchica had potential to remove Pb^{2+} from wastewater. The Langmuir adsorption model could be used to describe the adsorption of Pb^{2+} by *C. marchica*. The maximum Pb^{2+} uptake (q_{max}) was 74.04 mg g^{-1} dry wt. The Pb^{2+} adsorption capacity of *C. marchica* was affected by growth and environmental conditions. At lower biomass concentration *C. marchica* showed higher adsorption efficiency (q_{eq}) than that at higher biomass concentration. Pb^{2+} removal under light and dark conditions were not different. Pb^{2+} adsorbed (q_{eq}) by *C. marchica* increased with the initial Pb^{2+} concentration. This suggested that *C. marchica* was suitable to use as the biosorbent to remove Pb^{2+} from wastewater under various environmental conditions.

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