

# Utilization of Diatoms to Collect Metallic Ions

by

Masanao KUNUGI,<sup>\*1</sup> Takahiro SEKIGUCHI,<sup>\*2</sup> Hiroaki ONIZAWA<sup>\*1</sup> and Itaru JIMBO<sup>\*3</sup>

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## Abstract

Microalgae have the capability to collect metallic ions from aqueous solutions due to their negatively charged surface. A number of studies concerned with bioremediation have already been published in which heavy metals could be collected and a contaminated water system was able to be cleaned by biological measures. In the present study, diatoms are utilized to collect metallic ions such as silver and other ions from an aqueous solution. Collecting processes are adsorption of ions on the diatoms surfaces and taking the ions inside the diatoms bodies through the cell membrane. Analyses were performed with the observation of the dried specimen using Wet-SEM and EPMA. Experimental results revealed that these processes depend both on illumination and temperature. These processes seem to be closely related to photosynthesis. Discussion also includes seasonal variations in the yields of the metals by the diatoms. It will be of great help if these diatoms can be utilized in the cesium contaminated areas after the Fukushima nuclear disaster.

**Keywords:** Diatoms, Bioremediation, Cesium contamination, Adsorption

## 1. Introduction

Microalgae have a capability to collect metallic ions because of their negatively charged surface. Therefore, it is worthwhile to attempt the utilization of microalgae in order to remove and recover positively charged metallic ions dissolved in the aqueous solutions.<sup>1)</sup> One can utilize this process to recover metallic ions at a very low cost even if the reaction is very slow. It may also be possible to apply this process to bioremediation against the pollutions of several heavy metals.

An extensive work has been deployed in the biosorption research area with varieties of bacteria and fungi.<sup>2)</sup> On the other hand, a relatively small number of publications have been made for the microalgae. In the research field of the microalgae, blue-green and green algae have been received more attention. However, the detailed information on diatoms is not clear for few studies have been accomplished.

The purpose of this study is to develop the basic understanding on the collecting tendency of diatoms toward the metallic ions. An effort is made to observe the adsorption to the cell surface and the absorption inside the cell for the aqueous solution containing metallic ions such as silver, lead, chromium and cesium.

## 2. Experimental

### 2.1 Diatoms

Diatoms are easily found in our public water area. The water containing microalgae was obtained in a pond of the city park near by our university. The obtained raw water was filtrated and the filtrates were washed with distilled water to remove dirt and unnecessary organic matters. From the microscopic observation, the collected microalgae are found to be a mixture of *Pseudostaurosira brevistriata* and *Staurosira construens*.<sup>3)</sup> Fig. 1 shows the collected microalgae and their details. Fig. 2 shows that the observed microalgae contains Si and O as its characteristic, which proves that the microalgae is identified as diatom.



Fig. 1 Collected diatoms and the detailed image.

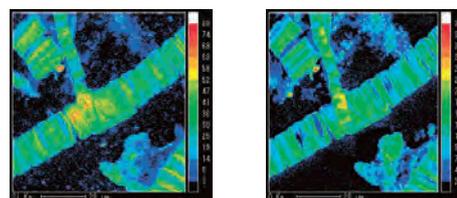


Fig. 2 Mapping images of Si (left) and O (right).

\*1 Graduate Student, Course of Metallurgical Engineering  
\*2 Undergraduate Student, Department of Materials Science  
\*3 Professor, Department of Materials Science

**2.2 NaOH treatment<sup>4)</sup>**

NaOH solution was adopted, where a 5 ml of 0.1 mol NaOH solution was mixed with a 5 ml of a diatom containing sample (the content of diatoms of 20 ml sample was ca. 310 mg as dry weight). The mixture was stirred and rinsed with distilled water until pH of the sample solution reached 7. In the experiment, the pretreated samples were compared with the corresponding samples without the NaOH treatment.

**2.3 Experimental procedure**

Experiments were accomplished in the seasons of summer and winter with varieties of conditions; temperature, duration, illumination, etc. A 20 ml sample diatoms was mixed with an 80 ml solution to be tested. Analyses are made with an optical microscope, Wet-SEM and EPMA. The experimental conditions for silver addition are summarized in Table 1. Conditions for lead are summarized in Table 2.

Table 1 Experimental condition for Ag solution

Season	Winter			
Location	Indoor		Roof	
NaOH treatment	○	×	○	×
Illuminance (lux)	0~1500		0~110000	
Temperature (°C)	5~12		3~10	
Duration	1, 3, 5 Weeks			

Season	Summer			
Location	Indoor		Roof	
NaOH treatment	○	×	○	×
Illuminance (lux)	0~1500		0~110000	
Temperature (°C)	12~21		15~23	
Duration	8 hours / 2 Weeks			

Table 2 Experimental condition for Pb solution

Location	Roof							
Concentration	0.3ppm		3 ppm		30 ppm		50 ppm	
NaOH treatment	○	×	○	×	○	×	○	×
Illuminance (lux)	0~110000							
Temperature (°C)	5~16							
Duration	3, 10, 17 days							

**3. Results and Discussion**

**3.1 Ag collection**

The initial concentration of silver was 1000 mg per liter. As seen in Table 1, the experiments were conducted both in winter and summer with different locations, illuminance, temperatures and durations.

The pictures in Fig. 3 are the examples of observation on silver solutions which were not treated with NaOH solution.

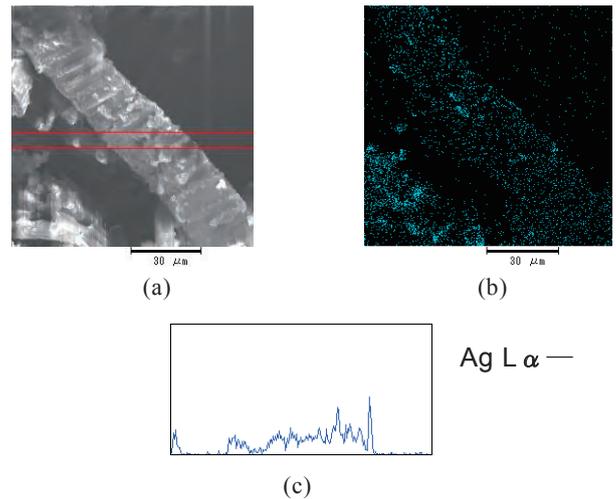


Fig. 3 Observation for Ag collection at pH=7 without NaOH treatment; (a) Secondary Electron Image, (b) Mapping image of Ag, and (c) Result of linear analysis.

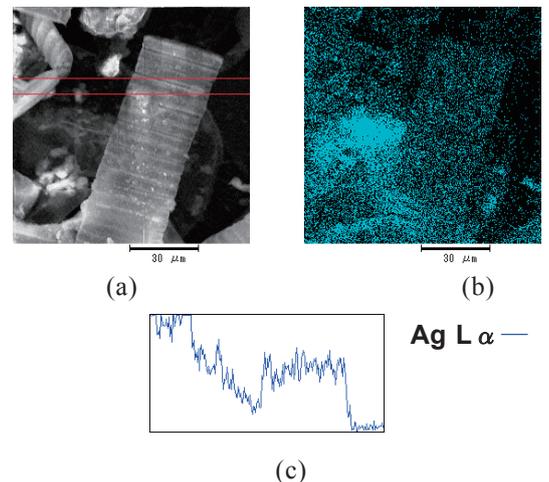


Fig. 4 Observation for Ag collection at pH=7 with NaOH treatment; (a) Secondary Electron Image, (b) Mapping image of Ag, and (c) Result of linear analysis

Table 3 Results of Ag recovery in winter and summer; Effect of NaOH treatment is compared. (unit: mass%)

Duration (Winter)	Untreated		Pretreated	
	Indoor	Roof	Indoor	Roof
1 week	3.21	3.61	2.43	3.54
3 weeks	3.53	3.36	2.30	3.77
5 weeks	1.60	2.30	2.75	4.73

Duration (Summer)	Untreated		Pretreated	
	Indoor	Roof	Indoor	Roof
8 hours	1.60	4.73	1.91	2.82
2 weeks	1.71	8.31	2.82	7.84

The authors tested different pH conditions to find out the optimal pH. From these series of experiment, it was found that more silver was observed on the surface for

pH 7, whereas no significant change was observed for pH 1. Accordingly, as a conclusion, extremely acidic condition as pH equals to 1 seems not to be good for diatoms. The solutions adjusted at pH 7 seem suitable for collecting the metal. The pictures in Fig. 4 are the similar examples which were pretreated with NaOH. As can be seen here again, substantial amount of silver is observed on the surface of the diatoms.

Table 3 summarizes the resultant silver collected with diatoms during the experiment, where the untreated and pretreated samples with NaOH solution are compared. These analytical results by EPMA give the calculated amount against 0.1 gram of dried diatoms. It can be said from the table that even in winter, diatoms can collect as much as about 3% of silver to their dry weight for a week or so. In summer time, the recovery is substantially high because their enhanced activity of photosynthesis.<sup>5)</sup> It can also be stated that if the activity of diatoms is not enough like in winter time, the increase in duration does not affect the increase in the recovery.

For the treatment of NaOH solution, it seems that untreated diatoms showed better recovery of silver than pretreated ones within 3 weeks in the winter. Even in summer, the recovery on the roof shows more silver yields for the untreated samples than that for the pretreated ones. It is true that NaOH treatment can remove organic matters and cleans the surface of diatoms.<sup>4)</sup> However, at the same time, substantial damage should cause the decrease in the active population of diatoms, even if the perished diatoms still can adsorb metallic ions on their surfaces. It is concluded that NaOH treatment does not necessarily promote the collection of metallic ions in the solution.

Diluted silver solution and silver compounds were often used as disinfectants and microbiocides. However, at least for the experiments accomplished here, such an effect of silver seems to be minimal. It is said that the diatoms are very actively functioning to collect the ions. As a conclusion, the following summary is obtained:

1. Diatoms may be an effective biosorbent for silver, which activity depends on the extent of photosynthesis. This is governed by the illuminance and temperature.
2. Adsorption of silver by diatoms varies by season. However, the adsorption was observed even in winter.
3. The treatment with NaOH is not as effective for diatoms to adsorb silver as expected, especially in the conditions where photosynthesis is very active.

### 3.2 Pb collection

As the experimental condition for lead is summarized in Table 2, 4 different concentrations of Pb are tested under the active photosynthesis condition. Fig. 5 is the example of observation on lead solutions of 3 ppm Pb. At this concentration range, no significant change on the surface of diatoms is observed in the mapping image (b), whereas only a small trace of Pb is detected in the linear analysis (c). For the 30 ppm concentration, the observed images are almost the same, which are shown in Fig. 6.

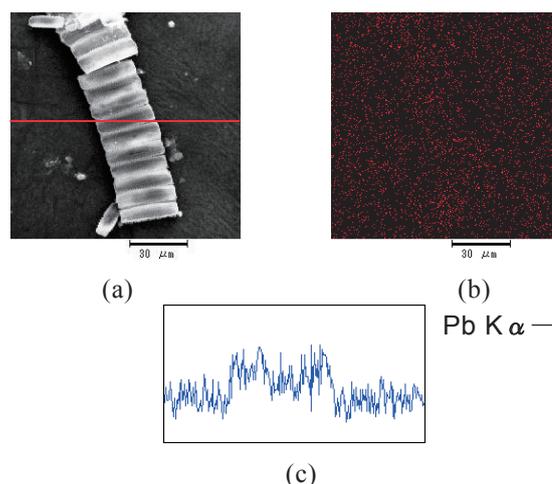


Fig. 5 Observation for Pb collection at 3 ppm Pb without NaOH treatment; (a) Secondary Electron Image, (b) Mapping image of Pb, and (c) Result of linear analysis.

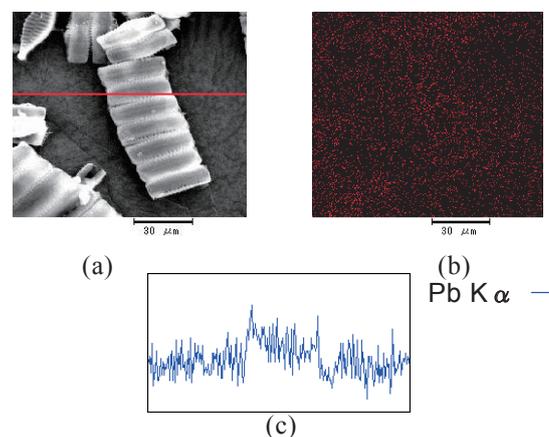


Fig. 6 Observation for Pb collection at 30 ppm Pb with NaOH treatment; (a) Secondary Electron Image, (b) Mapping image of Pb, and (c) Result of linear analysis.

Fig. 7 shows the experimental samples in the test tubes. From the left to the right; the original solution, 30 ppm without and with NaOH treatments and 50 ppm without and with the treatment in contact with lead solution.

Based on the water quality standards of Japan, the limitation of lead for public water area and underground water is under  $0.01 \text{ pm}^{(6)}$  and the lead concentrations studied in the present study are 3000 times and 5000 times higher. In the case of 50 ppm lead, diatoms stopped the photosynthesis and the color turned to be white. Pb is a toxic substance to creatures like other heavy metals at a certain dose level. Such high level of concentration of Pb does not seem to be suitable for diatoms to continue the recovery process.

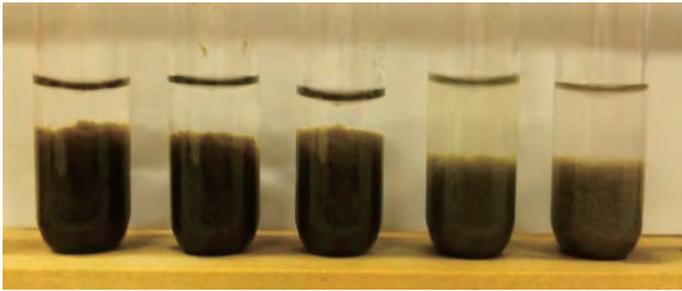


Fig. 7 Appearance of experimental samples: From left to right; the original solution, 30 ppm without and with NaOH treatments and 50 ppm without and with NaOH treatments.

Accordingly, the lead experiment is summarized in the following:

1. Lead can be adsorbed with diatoms in the concentration range up to 30 ppm which is 3000 times higher concentration than the water quality standards of Japan
2. Only a very small amount of lead was adsorbed on the diatoms surfaces.
3. The treatment with NaOH was not very effective for lead either.

### 3.3 Cr collection

Chromium collection was tested under a similar experimental condition as mentioned above; 10, 20, 40 ppm of Cr (VI) solutions for 3, 6 and 10 days of duration on the roof without NaOH treatment.

Fig. 8 is the example of observation on chromium solutions of 40 ppm Cr (VI) with 3 days duration. Chromium (VI) is one of a toxic substance at a certain level. However, metallic collection seems to be possible in this concentration range.

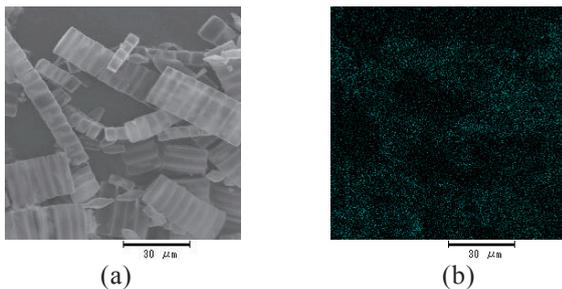


Fig. 8 Observation for Cr collection at 40 ppm Cr(VI) without NaOH treatment; (a) Secondary Electron Image, (b) Mapping image of Cr.

Fig. 9 shows the result of linear analysis, where chromium is detected in the range of coexisting Si and Fig. 10 summarizes the collection of chromium for the series of experiment. It can be said that the resultant collection of chromium shows a very little increase with the longer duration.

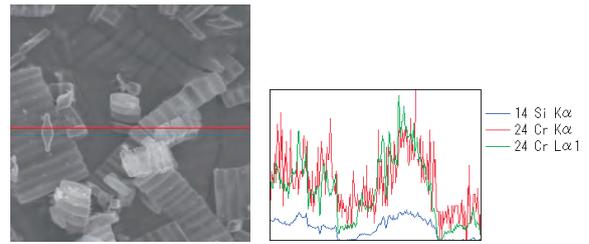


Fig. 9 Observation for Cr collection at 40 ppm Cr(VI); the linear analysis for Si and Cr.

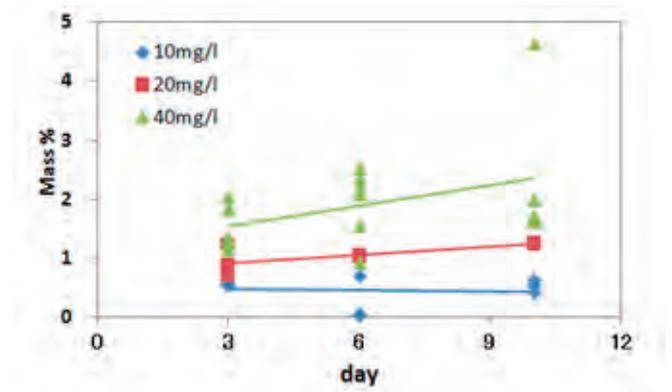


Fig. 10 The summary of Cr collection at 10, 20 and 40 ppm Cr (VI) for 3, 6 and 10 days duration; Cr contents plotted as a function of duration time.

About 1~2% of the mass could be adsorbed on the surface. In case when the diatoms can propagate under favorable conditions, available cell surface increases and then the adsorption will increase. In such a case, the mass ratio should not necessarily increase with increasing duration time. For the present, the collection phenomena shown in Fig. 10 are not sufficiently interpreted. The detailed mechanism on the collection should be clarified.

### 3.4 Cs collection

Cesium collection was also tested with the similar experimental condition as mentioned above is adopted for 40 ppm of Cs solutions for 21 days of duration. Fig. 11 shows the collection of cesium as a function of duration. It is said that the rate of cesium collection is significant in the first 7 days following a gradual increase for the rest of the duration time. It is seen that up to 3~4% of the mass could be adsorbed on the surface. The mapping images in Fig. 12 gives an idea where there are diatoms there is cesium.

This kind of adsorption with diatoms is relatively a slow process as usual biological activities are. However, it is noticeable to realize the possibility to take advantage of diatoms for bioremediation. Effect of radiation exposure on the activity of diatoms is not clear for the moment. At least, it will be make sense if this kind of diatoms can concentrate cesium in the environment by adsorbing the element on their negatively charged cells. It will be of great help if these

diatoms will be utilized in the cesium contaminated area after the Fukushima nuclear disaster.

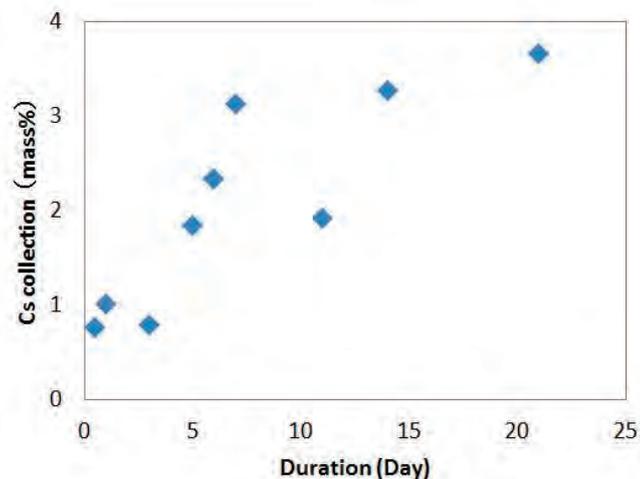


Fig. 11 Collected amount of cesium as a function of duration time

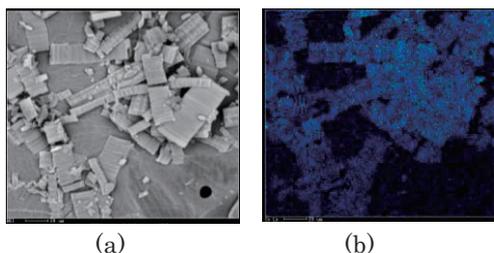


Fig. 12 Observation for Cs collection at 40 ppm Cs without NaOH treatment; (a) Secondary Electron Image, (b) Mapping image of Cs.

#### 4. Closing Remarks

Collection of metallic ions such as  $\text{Ag}^+$ ,  $\text{Pb}^{2+}$ ,  $\text{Cr}^{6+}$ ,  $\text{Cs}^+$  by utilizing diatoms seems very promising. At the present stage of the study, the following summary can be listed:

1. Silver can be collected with the diatoms. The activity of photosynthesis can enhance its recovery.
2. Lead can be collected with diatoms, but the effect was not as good as in the case of silver. The upper limit of Pb density should not exceed more than 30 ppm.
3. Chromium seems to be collected with diatoms, though the effect of diatoms may be limited.
4. Cesium can be collected with diatoms, which amount increases up to 3 to 4% of the applied diatoms.

According to the metal classification referred in the literature,<sup>2)</sup> metallic affinity for ligands can be classified

into three categories: Class A, Class B and borderline ions. It is remarked that cesium is a Class A element, and silver and lead belong to Class B, whereas Cr and Cd are borderline ions. According to the Hard and Soft Acid Base Principle (HSAB principle), hard ions which bind  $\text{F}^-$  strongly, such as  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  could form stable bond with  $\text{OH}^-$ ,  $\text{HPO}_4^{2-}$ ,  $\text{CO}_3^{2-}$ ,  $\text{R-COO}^-$  and  $=\text{C=O}$ , which are oxygen-containing ligands. This hard ion group is called Class A. On the contrast, Class B ions are called soft ions; heavy metal ions such as  $\text{Hg}^{2+}$  and  $\text{Pb}^{2+}$  form strong bond with  $\text{CN}^-$ ,  $\text{R-S}^-$ ,  $-\text{SH}^-$ ,  $\text{NH}_2^-$ , etc., which are groups containing nitrogen and sulfur atoms. It should be noticed that the hard ions show ionic nature of binding, whereas the soft ions binding exhibits more covalent degree.<sup>7,8,9)</sup>

As far as diatoms are concerned, the nature of adsorption of silver and lead ions may be similar, whereas that of Class A ion like cesium is considered to be more ionic. However, not only the adsorption but absorption into the cell should be considered from the results when active photosynthesis was occurring. Therefore, efforts should be made towards the detailed mechanism of absorption in the cell as well as adsorption on the surfaces.

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