MICROBIOLOGY
INDONESIA
ISSN 1978-3477, eISSN 2087-8575
Vol.9, No.3, September 2015, p 106-112

Available online at
http://jurnal.permi.or.id/index.php/mioline
DOI: 10.5454/mi.9.3.2

Biosorption of Lithium Using Biofilm Matrix of Natural Microbial Consortium

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This study examined the biosorption of lithium using biofilm matrices of natural microbial consortiums collected from Lake Biwa, Japan. The characterization of the biofilm polymer as a suggested binding site of biofilm was also revealed in this study. The followings were observed as results of this study: 1) biofilm has both negatively and positively charged sites; 2) lithium adsorption by biofilm matrix is a physicochemical process mainly promoted by the electrostatic interaction between the ion and the charged sites of biofilm polymers; 3) the adsorbing lithium ion promote the desorption of ions from biofilms through ion exchange mechanism; 4) biofilms components changed seasonally and seems to affect the ability of biofilm to adsorb ions. According the results of this study, natural biofilm may become a promising biosorbent in the biosorption of lithium ion.

Key words: biofilm, biofilm polymer, biosorption, lithium ion

Studi ini mengkaji biosorpsi ion lithium dengan menggunakan matriks biofilm dari konsorsium mikroba yang tumbuh alami di danau Biwa, Jepang. Karakteristik polimer biofilm yang diduga menjadi area pelekatan ion juga diungkapkan dalam studi ini. Berikut ini adalah hasil-hasil yang didapat studi ini: 1) biofilm memiliki area bermuatan listrik positif dan negatif; 2) adsorpsi lithium oleh matriks biofilm adalah proses fisika-kimia sebagai hasil interaksi elektrostatis antara ion lithium dan muatan listrik di polimer biofilm; 3) penyerapan ion lithium mengakibatkan pelepasan ion dari biofilm melalui mekanisme pertukaran ion; 4) komposisi biofilm berubah sesuai musim dan tampak mempengaruhi kemampuan biofilm untuk menyerap ion. Berdasarkan hasil dari studi ini, biofilm alami bisa menjadi biosorben yang menjanjikan dalam proses biosorpsi ion lithium.

Kata kunci: biofilm, biosorpsi, ion lithium, polimer biofilm

Biofilm is a predominant habitat of microbe and ubiquities in aquatic environments. Biofilm is formed when bacteria and other microorganisms attach on a surface and then replicate with producing extracellular polymers (Costerton *et al*. 1995). Biofilm has high sorption capacities and low production cost that attracted attention of many specialists in the field of water treatment (Chubar *et al*. 2008; Gadd 2009). However, the study concerning the utilization of biofilm formed in natural environment in development of water purification technologies such as biosorption of pollutant ions has been rarely conducted. The recent works mainly focus on the adsorption of heavy metal ions to bacteria or biofilm formed under artificial condition (Almaguer-Cantú *et al*. 2011; Fang *et al*. 2010; Joo *et al*. 2010; Pérez Silva *et al*. 2009; Quintelas *et al*. 2009; Vijayaraghavan and Yun 2008).

Heavy metal ions have been one of the biggest water contamination problems (Chubar *et al*. 2008; Volesky 2007). One of the heavy metals that recently become a serious pollutant is lithium. Lithium is utilized as an important compound of various things such as high-performance grease, heat-resistant ceramics, flux for welding, batteries, pharmaceuticals and nuclear fusion furnace (Kaneko and Takahashi 1990; Miyai *et al*. 1978; Dang and Steinberg 1978; Harlty *et al*. 1978). Therefore, the cleaning up of lithium contamination in the environment is an important subject particularly in the development of environmentally and economically water purification technology in order to support sustainable development (Tsuruta 2005).

In this study, the polymer characteristics of biofilms collected from the surface of the stone in a Lake Biwa as one of the natural biofilm were clarified. The biofilms formed on stones were used for following considerations: 1) stone is the substrate that exist nearly in all aquatic environments, 2) the condition of stone as biofilm substrate is relatively stable during seasonal changes, and 3) to collect sufficient amounts of biofilms for the experiments. The utilization of this natural biofilm for biosorption of lithium was also investigated. The purpose of the use of the natural biofilm is to improve the application possibility in the real aquatic ecosystem. Moreover, the comparison of lithium adsorption capacity of biofilms formed in

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different seasons was also conducted.

MATERIALS AND METHODS

Sampling Site and Sample Preparation. The samples used in this study were collected from the shore of the southern basin (Akanoiwan) of Lake Biwa. This lake is the largest lake in Japan located in the central part of Japanese Archipelago (Lat. 35° 15' N, Long. 136° 05' E). Samples of the biofilm were collected in winter (November 2009) and spring (April 2010) as the previous study founded that the biofilm thickness reach the maximum amount in these seasons (Tsuchiya *et al.* 2009). Stones were taken from the depth of 70-100 cm and brought back to the laboratory in a plastic container filled with nearby lake water; the container was maintained at 4 °C. The biofilms on the surfaces of the stones (ca. 100 stones in each sampling) were removed using a toothbrush and suspended in sterilized distilled water. The biofilm pellets were prepared by centrifuging (8 000 ×*g* at 4 °C for 10 min) the biofilm suspensions.

Dry Weight of Biofilms. The pellet of biofilms (approximately 1 wet-g) were taken and then dehydrated for 3 d until the weight was stable to give a dry weight (Sissons *et al.* 2000).

Electrophoretic Mobility. Biofilm was washed three times with 10 mM NaCl aqueous solution by centrifugation (8 000 ×*g* at 4 °C for 10 min), and the supernatant was discarded. The obtained biofilm pellet (ca. 0.03 g) was suspended in 1 mL of 10 mM NaCl aqueous solution. The suspension was mixed vigorously with a vortex for 5 min, then sonicated (2510J-MT, Yamato Scientific, Tokyo, Japan; 42 kHz, 125 W) for 10 min, followed by the vortex for 10 s. The obtained suspension was mixed with 10 mM of PBS at a ratio of 1:19 and used to analyze the electric charge of the biofilm polymer. The electrophoretic mobility (EPM) of the biofilm was measured on a ZETASIZER Nano-Z (Malvern Instruments, Ltd., Worcestershire, England) in phosphate-buffered saline (PBS) varying in pH values from 2.0 to 9.0. The pH of the buffer was adjusted with 20 mM of HCl or NaOH. The pHs of PBS before and after the addition of biofilm and after measurement of EPM were recorded.

Acid-base titration. Biofilms were washed six times with distilled water by centrifugation. One gram of the pellet of biofilm was placed in a plastic cup, and distilled water was added to give a total weight of 40 g. Then, 10 mM of HCl or 10 mM of NaOH aqueous solution was titrated onto the samples using an automatic titration machine (DL50, Mettler, Toledo, OH, USA). The pH changes were recorded and analyzed. Distilled waters (40 gram) were also subjected to titration. From the results, the adsorbed amounts of H^+ and OH^- by biofilms were calculated (Freifelder 1985) as follows:

$$C(H^+)=(10^{-pH(dw)}-10^{pH(bf)})v/(w) \quad (1)$$

Where, $C(H^+)$ is the uptake capacity of H^+ by unit weight of biofilm (μmol wet-g^{-1}), pH(dw) and pH(bf) is the pH value of distilled water and biofilm suspension when HCl solution was added, respectively, v is the volume of sample solution (L) and w is the wet-weight of biofilm (gram). When NaOH solution was added, the uptake capacity of OH^- ion, $C(OH^-)$, by unit weight of biofilm (μmol wet-g^{-1}), was given by the similar equation as follows:

$$C(OH^-)=(10^{-pH(bf)}-10^{pH(dw)})v/(w) \quad (2)$$

by the above equations the uptake capacity of H^+ and OH^- ions by biofilm was calculated.

Biosorption experiment. The procedures of the biosorption experiment in this study were modified from previous works (Vijayaraghavan and Yun 2008 and Gadd 2009). Biofilms were washed six times with 5 mM phosphate-buffered saline (PBS) of pH 7 by centrifugation. The biofilm pellets were stored at -40 °C until ion adsorptions analyses were conducted. 1 wet-g of the biofilm pellet was resuspended in 50 mL of 5 mM PBS of pH 7. The suspension was mixed vigorously with a vortex for 5 min, and then sonicated for 10 min, followed by the vortex for 30 s. Then, 5.0 mL of 20 mM of solution of reagent grade LiCl, prepared by dilute the chemical compound (Wako Pure Chemical Industries, Osaka, Japan) in 5 mM PBS of pH 7, was added to the suspension. The temperature of the suspension was maintained at 25 °C using a water thermostat, and mixed well using magnetic stirrer.

The aliquots of the suspension were taken after 1 min-300 min, and then centrifuged (15 000 ×*g* at 4 °C for 1 min) to separate the supernatant and the pellet. The ion concentration in the solution was measured using a capillary electrophoresis method (CAPI-3300, Otsuka electronics, Osaka, Japan). The adsorbed amount of ion to biofilm was calculated from the difference between ion concentration in the supernatant and in the control (only PBS and ion). The desorbed ion (different species from added ion) from biofilm due to addition of ion was also investigated. As a control of investigation of desorbed ion, same amount of biofilm was resuspended in 50 mL of 5 mM PBS of pH 7, and then was treated with the same treatment described above but without ion addition. The adsorption experiment was also conducted using one dry-g of strong acidic resin (SP-650M; Toyopearl, Tokyo, Japan) and weak acidic resin (CM-650M; Toyopearl).

RESULTS

Electrophoretic Mobility. The EPM of biofilm was shown as a function of pH (Fig 1). At pH 7, the biofilm showed negative EPM values, whereas these values decreased at smaller pH values, especially with greater extent around pH 4. At pH 2, the biofilm showed positive EPM value. The pH values of the buffer solution shifted to a greater value from its original value when biofilm samples were added to the buffer solution; e.g., the original pH values of 4.0 and 5.0 shifted to 5.6 and 5.9, respectively after adding biofilm samples.

Acid-base Titration. Acid base titrations to the biofilm and distilled water were conducted (Fig 2). The acid-base titration curve of biofilm was different from that of distilled water (as a background solution). From the results of acid-base titration (Fig 2), the uptake capacities of H^+ and OH^- by biofilm were calculated (Fig 3). The maximum difference of pHs in the acid-base titration curve between biofilm and distilled water were at around pH 4 for HCl addition and at around pH 11 for NaOH addition. Similarly, the maximum uptake capacity of biofilm appeared at pH 3 - 4 for proton adsorption and pH 10 - 11 for hydroxyl ion adsorption (Fig 3).

Biosorption of Lithium. The time course of lithium ion biosorption using biofilm was shown in Fig 4. The adsorption process was very fast as the adsorption amount reached, within 1 min, to as much value as observed in the later stage. In order to evaluate the potential of biofilm as an adsorbent in biosorption of lithium ion, the adsorbed amount of ions to biofilms was compared with strongly and weakly cation exchange resins as one of the common adsorbents in the adsorption of heavy metal ions such lithium (Table 1). The adsorbed amount of ions was greater to biofilm than those to resins for a dry gram of these substrates. Moreover, various kinds of ions were suggested to be desorbed from biofilms or cation exchange resins by adsorbing ions (Table 2).

The time course of adsorption of lithium to biofilm sampled in spring was also studied (Fig 5). The result was compared with the adsorption to biofilm sampled in winter. The adsorbed amount of ions to the biofilms sampled in winter (85.3 µmol dry-g^{-1}) was greater compared with those of the biofilms sampled in spring (19.4 µmol dry-g^{-1}).

DISCUSSION

Electric Charges and Functional Groups of Biofilm. A biofilm suspension was put in an electric field, and the electrophoretic velocity was measured under various pH conditions. The electrophoretic mobility (EPM) was normalized by dividing with the electric field strength to obtain the EPM values (Fig 1).

Table 1 Adsorbed of lithium to biofilm and cation exchange resins

Adsorbent	Absorbed Li^+ (µmol dry-g^{-1})
Biofilm (winter)	85.3
Strongly cation exchange resin	18.2
Weakly cation exchange resin	33.2

Table 2 Adsorbed and desorbed amount of ions to and from biofilm

Absorbed amount of Li^+ (µmol dry-g^{-1})	Desorbed ions	
	Ion	Amount (µmol dry-g^{-1})
85.3	K^+	10.012
	Ca^{2+}	14.6
	Mg^{2+}	33.01
	Na^+	42.8

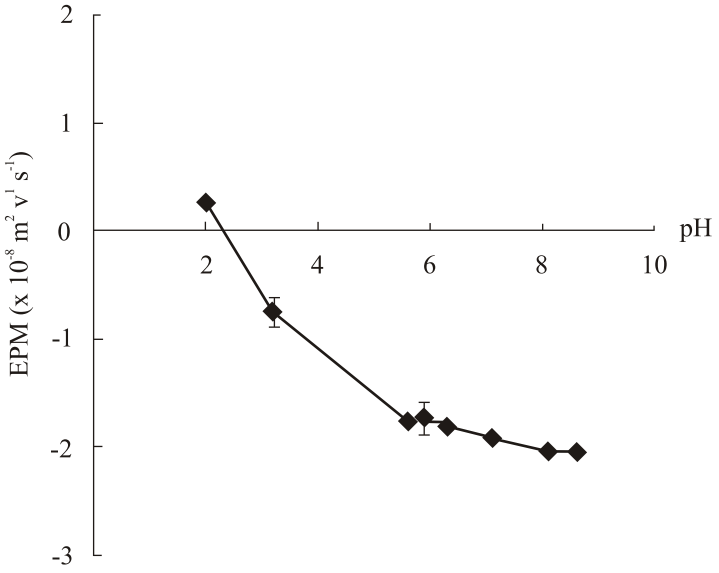


Fig 1 EPM of biofilm measured in various pH.

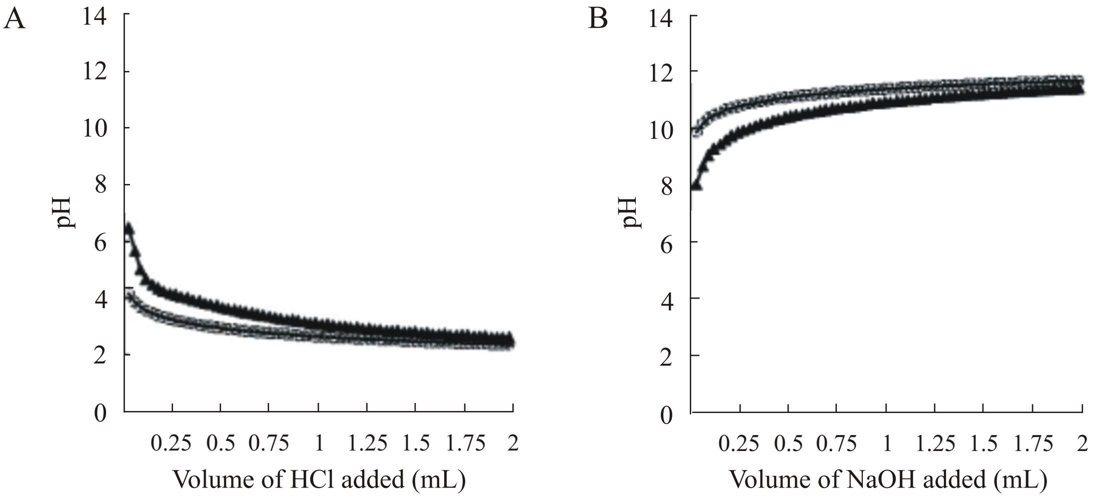


Fig 2 (A) Acid titration to biofilm and distilled water; (B) Base titration to biofilm and distilled water. ◇ : distilled water and ▲ : biofilm.

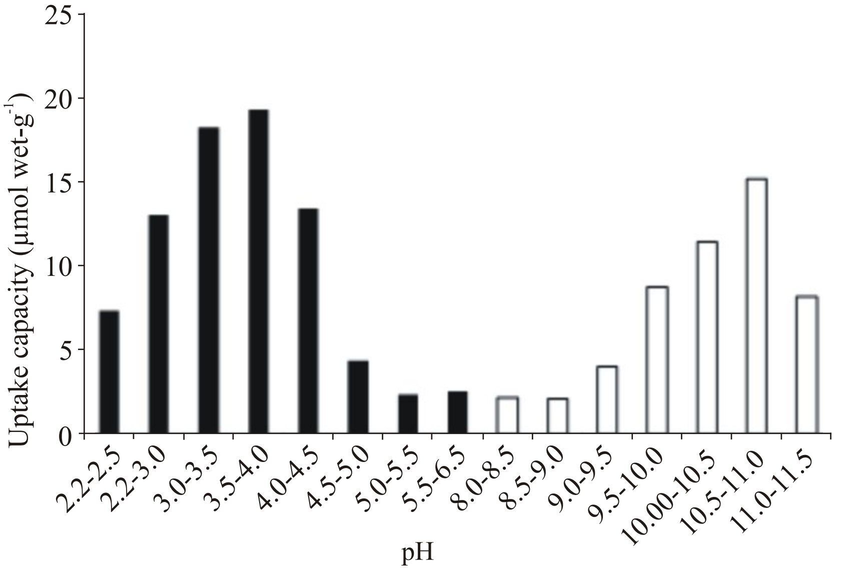


Fig 3 Uptake capicity of H$^+$ (black column) or OH$^-$ (white column) per unit weight of the biofilm.

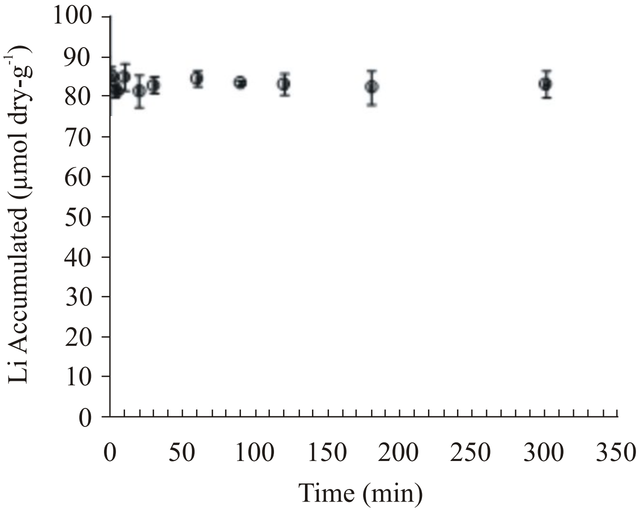


Fig 4 Lithium accumulation to winter biofilm. The experiments were repeated 3 times, independently (average values are shown). Bars represent the standard deviation.

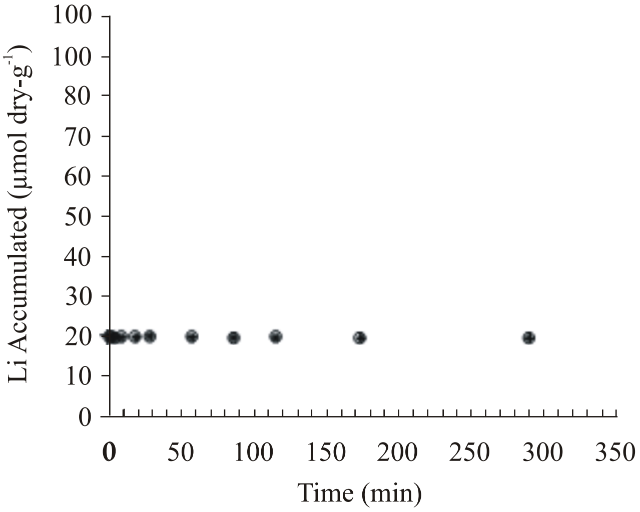


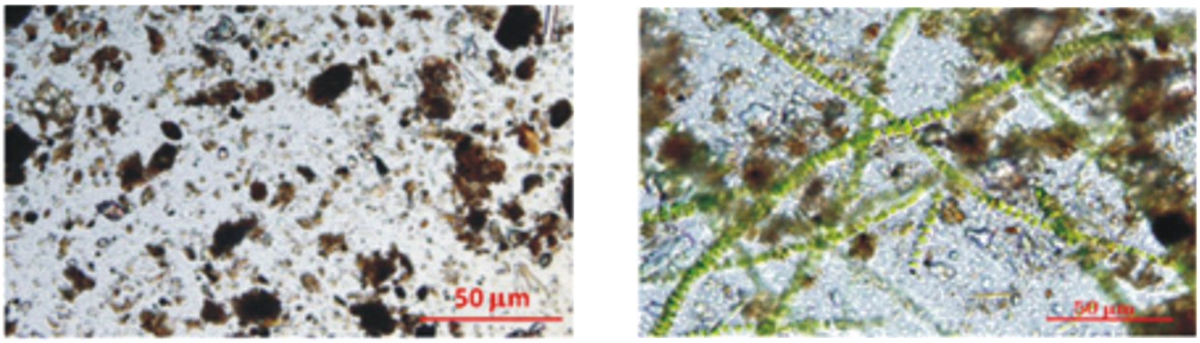
Fig 5 Lithium accumulation to spring biofilm. The experiments were repeated 3 times, independently (average values are shown). Bars represent the standard deviation.

The decrease of negative EPM values from pH 7 to smaller pHs particularly with greater extent around pH 4 seems to be due to the depression of ionization of functional groups, such as carboxylic group whose pKa is around pH 4, at the acidic condition (Freifelder 1985).

Positive EPM value at pH 2 indicates the existence of positively charged functional group, supposed to be amino group, in the biofilm matrix. The shifted of the original pH values after adding biofilm samples suggested the decreasing in the concentration of proton by adding the biofilm sample. It seems that proton was used to de-ionize acidic functional group in the biofilm. Carboxylic acid is the most probable as the functional group as discussed above and further confirmed by the buffer action as a result of acid-base titration (Freifelder 1985; Vijayaraghavan and Yun 2008).

The differences of acid-base titration curves of biofilm to that of distilled water (Fig 2) reveal the presence of ionizable functional groups associated with biofilm polymer. The results show that the maximum difference of pHs in the acid-base titration curve between biofilm and distilled water were at around pH 4 for HCl addition and at around pH 11 for NaOH addition. Similarly, the maximum uptake capacity of biofilm appeared at pH 3-4 for proton adsorption and pH 10-11 for hydroxyl ion adsorption (Fig 3). It seems that at around pH 4 and pH 11 there are some functional groups in biofilm polymer having ability to adsorb proton and hydroxide ions respectively. The peak at pH 4 may indicate the existence of carboxyl groups (pKa = ca. 4), and that at pH 11 the existence of amino groups (pKa = ca.11).

The existences of the functional groups explain



A B

Fig 6 Microscopic images of biofilm matrix samples. (A) winter biofilm and (B) spring biofilm.

well the EPM change along with pH as shown in Fig 1. In case of greater amount of negative charge than positive charge in the biofilm, the biofilm would have a net negative charge. The negative EPM of biofilm at pH 7 corresponds to this case (greater amount of negative charge than positive charge). Thus, the biofilm matrix carries both positive and negative charge in the environment of lake water (pH value ca. 7) (Tsuchiya *et al.* 2009).

Biosorption of Lithium. The time course of lithium ion biosorption using biofilm was shown in Fig 4. The adsorption process was very fast as the adsorption amount reached, within 1 min, to as much value as observed in the later stage. This indicates that the lithium adsorption by biofilm is a physicochemical process where the electrostatic interaction between ions and the charged sites in biofilm matrixs was a driving force to adsorb ions to inside biofilm.

In order to evaluate the potential of biofilm as an adsorbent in biosorption of lithium ion, the adsorbed amount of ions to biofilms was compared with strongly and weakly cation exchange resins as one of the common adsorbents in the adsorption of heavy metal ions such lithium (Table 1). The adsorbed amount of ions was greater to biofilm than those to resins for a dry gram of these substrates. This indicates more charged sites in the biofilm or greater attractive force by biofilm matrixs compared to ion exchange resins. This result suggests that biofilm may become a promising adsorbent for the biosorption of pollutant ions such as lithium ions (Tsuruta 2005; Volesky 2007).

Various kinds of ions were desorbed from biofilms (Table 2). It seems that adsorbing lithium replaced the previously adsorbed ions that bound to the negatively charged site of the biofilms. The adsorbing ion seemed to promote desorption of ion from biofilms through ion exchange mechanisms.

Comparison to Spring Biofilm. The time course of adsorption of lithium to biofilm sampled in spring was also studied (Fig 5). The result was compared with the adsorption to biofilm sampled in winter. The adsorbed amount of ions to the biofilms sampled in winter (83.5 µmol dry-g^{-1}) was greater compared with those of the biofilms sampled in spring (19.4 µmol dry-g^{-1}). This indicated that more adsorption sites in biofilm sampled in winter compared with biofilms sampled in spring. This result is correlated well with the result of microscopic observation, that reveal the biofilms sampled in winter contained more polymer like substances (Fig 6), it seems that the adsorption sites of cation (negatively charge sites) was mostly exist in the polymer like substances. Thus, the biofilms sampled in winter (that have more polymers like substances) (Tsuchiya *et al.* 2009) could adsorb greater amount of ions than biofilms sampled in spring.

The difference of composition of biofilms indicates that the biofilms components change seasonally along with the change in the environmental factors, such as temperature and light intensity (Hiraki *et al.* 2009; Tsuchiya *et al.* 2009). This should be considered in the utilization of biofilm in the biosorption of pollutant ions. One of the characteristics of the natural biofilm matrix that important to be elaborate more is the microbial composition inside the biofilm matrix. Therefore, the microbial composition in the natural biofilm matrix is subjected in the further study.

In conluson, the followings were observed as results of the present study: 1) biofilms have both negatively and positively charged sites; 2) the lithium adsorption by biofilm is a physicochemical process where the electrostatic interaction between the ion and the charged sites in biofilm polymers is a main driving force; 3) the adsorbing ion promote the desorption of ions from biofilms through ion exchange mechanism; 4) the biofilm components change seasonally and seems to affect the ability of biofilm to adsorb ions. Based on the result of this study, biofilm may become a promising adsorbent for pollutant ions such lithium ions.

ACKNOWLEDGMENT

This study is part of research project supervised by Prof. Hisao Morisaki from Graduate School of Life Sciences, Ritsumeikan University. The authors are grateful to Dr. Yuki Tsuchiya from Faculty of Life Sciences, Ritsumeikan University for his suggestion in our discussion.

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