Korean J. Chem. Eng., **31**(1), 68-73 (2014)
DOI: 10.1007/s11814-013-0184-7

pISSN: 0256-1115
eISSN: 1975-7220

On the reason why acid treatment of biomass enhances the biosorption capacity of cationic pollutants

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(*Received 29 May 2013 • accepted 22 September 2013*)

Abstract−The present work is aimed at understanding the effect of acid treatment and demonstrating the reason for its effect. For this, *Corynebacterium glutamicum* biomass was used as a model biomass. Two cationic (cadmium and Methylene Blue) and one anionic (Reactive Red 4) pollutants were used to evaluate the sorption capacity by the biomass. Isotherm experiments showed that acid treatment of the biomass increased the uptake of the cationic pollutants, but decreased that of the anionic pollutant. Through the results of FTIR and potentiometric titrations, it was found that carboxyl groups on the biomass increased after acid treatment. The carboxyl groups seem to be generated likely through hydrolysis of esters in the biomass under the acidic condition. Therefore, increase of the carboxyl groups provided the binding sites for cationic pollutants, whereas it may interfere with the binding of anionic pollutants.

Keywords: Acid Treatment, Biomass, Cadmium, Methylene Blue, Reactive Red 4

INTRODUCTION

Hazardous metals, such as chromium, copper, lead, cadmium, and mercury, are very harmful and significantly toxic to human beings and ecological environments [1]. Likewise, presence of dyes in effluent discharged from textile industries is visible and undesirable [2]. Some commercial dyes can be carcinogenic and mutagenic for aquatic life. However, removing dyes from aqueous solutions is tricky because dyes are recalcitrant organic molecules, resistant to aerobic digestion and stable to light, heat and oxidizing agents [3]. Therefore, toxic pollutants like heavy metals and dyes should be removed from aqueous media.

Biosorption has been considered as a promising technique for removal of toxic pollutants such as heavy metals and synthetic dyestuffs from aqueous or waste solutions [4-6]. Biosorption has several competitive advantages such as cost-effectiveness, high efficiency, and good sorption performance [7]. Biosorption utilizes the ability of dead or living biomass to remove pollutants in aqueous media. In particular, the use of dead biomass is preferred because of the flexibility to environmental conditions and toxicant concentrations. Moreover, dead biomasses do not require the supply of nutrients [8]. Various types of biomass including bacteria, fungi, algae, and yeast have been used as biosorbents to remove toxic pollutants [9]. However, considering the cost-effectiveness, industrial waste biomasses, such as *Corynebacterium glutamicum*, *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Streptoverticillum* sp., can be the most practical raw materials for biosorbents.

The sorption capacity of biological materials can be enhanced through a simple pretreatment with mineral acids, alkali and calcium chloride [10-12]. In general, acid treatment has been widely used to enhance sorption capacity of biomass. Acid-treated biomasses have shown a higher metal uptake compared to untreated biomasses [13-15]. However, very little is known about the reason. Knowing the reason is very important because it can maximize the effect of acid treatment.

The main purpose of the present work is to evaluate the effect of acid treatment of biomass and to elucidate the reason for this effect. The waste biomass of *C. glutamicum* was used for the biosorption of two cationic pollutants such as cadmium and Methylene Blue (MB) and an anionic pollutant Reactive Red 4 (RR4). Isotherm experiments for Cd(II), MB and RR4 were examined and compared. Potentiometric titration, FTIR and SEM analyses were conducted to determine the major changes in biomass functional groups.

MATERIALS AND METHODS

1. Materials

Cadmium nitrate tetrahydrate ($Cd(NO_3)_2 \cdot 4H_2O$, 98.0%) was purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). Basic dye MB (color index number: 52015, maximum absorption wavelength: 666 nm, dye content: 82%) and reactive dye RR4 (color index number: 18105, maximum absorption wavelength: 517 nm, dye content: 50%) were obtained from Sigma-Aldrich Korea Ltd. (Yongin, Korea). MB has a positive charge in aqueous solution; whereas, RR4 has four sulfonate groups which have negative charges in aqueous solution. All of the other reagents used in this work were of analytical

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grade.

2. Preparation of Water-washed Biomass and Acid-treated Biomass

The fermentation wastes of *C. glutamicum* biomass were obtained from a lysine fermentation industry (BASF-Korea, Gunsan, Korea) as dried powder form. The acid-treated biomass (ATB) was prepared by treating the raw biomass with 1 M HNO_3 solution for 24 h. The ATB was washed with deionized water several times and then dried at 60 °C for 48 h in an oven. On the other hand, the water-washed biomass (WWB) was prepared by washing the raw biomass with deionized distilled water. Other procedures were same as the preparation of ATB. The ATB and WWB were stored in a desiccator prior to use as biosorbents in the sorption experiments. The particle size of ATB and WWB was less than 0.25 mm.

3. Isotherm Experiments

To compare the sorption capacity and affinity of the ATB and WWB, the biosorption isotherms of Cd(II), MB and RR4 were examined in different pH solutions, respectively. The isotherm experiments were conducted with 0.4 g of the biomass in 80 mL of working solution volume. The initial concentrations were altered in the range of 0-562 mg/L (Cd(II)), 0-1,500 mg/L (RR4), and 0-2,000 mg/L (MB), resulting in different final concentrations after the sorption equilibrium had been achieved. Following the addition of biomass into the sorbate-containing solutions, the suspension was stirred at 160 rpm and 25 °C for 24 h in a shaker, which was enough to reach sorption equilibrium. The solution pH was also adjusted at the desired value using 0.1 M NaOH and 0.1 M HNO_3. Thereafter, the final pH was measured and the samples were obtained through solid-liquid separation by centrifugation. The supernatant portion was used to analyze the residual sorbate concentration after appropriate dilution.

The dissolved Cd(II) concentration of samples was analyzed using anodic stripping voltammetry (TEA-3000V, MTI Instruments, Perth, Australia). The RR4 and MB concentrations of samples were measured by UV-Vis spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan) at 517 and 666 nm, respectively, where the maximum absorption peak existed. To compensate the change of the working volume (up to 5%) by the addition of NaOH or HNO_3 solutions, the uptake (q) was calculated from the mass balance as follows:

$$q = \frac{V_i C_i - V_f C_f}{M} \quad (1)$$

where, C_i and C_f are the initial and final sorbate concentrations, respectively. V_i and V_f are the initial and final volumes, respectively. M stands for the weight of biomass used.

The isotherm experimental data were fitted using the Langmuir model as follows:

$$\text{Langmuir model: } q_e = \frac{q_{max} b C_e}{1 + b C_e} \quad (2)$$

where, q_e is the amount of sorbed sorbate (mg/g), C_e is the equilibrium sorbate concentration (mg/L), q_{max} is the maximum uptake (mg/g) and b is the affinity constant (L/mg). The isotherm data modeling was estimated by non-linear regression using SigmaPlot software (version 10.0, SPSS, USA).

4. FTIR and SEM Analyses

Fourier transform infrared spectroscopy (FTIR) was used to identify the change of the functional groups on the biomass. FTIR spectra of ATB and WWB were obtained with a Fourier transform infrared spectrometer (FT/IR-300E, Jasco).

The surface morphologies of ATB and WWB were examined by scanning electron microscopy (SEM, JSM-6400, JEOL, Japan) at magnification 10Kx. WWB and ATB were mounted on a stainless steel stab, respectively, with a double-stick tape and coated under vacuum with a thin layer of gold to improve the electron conduc-

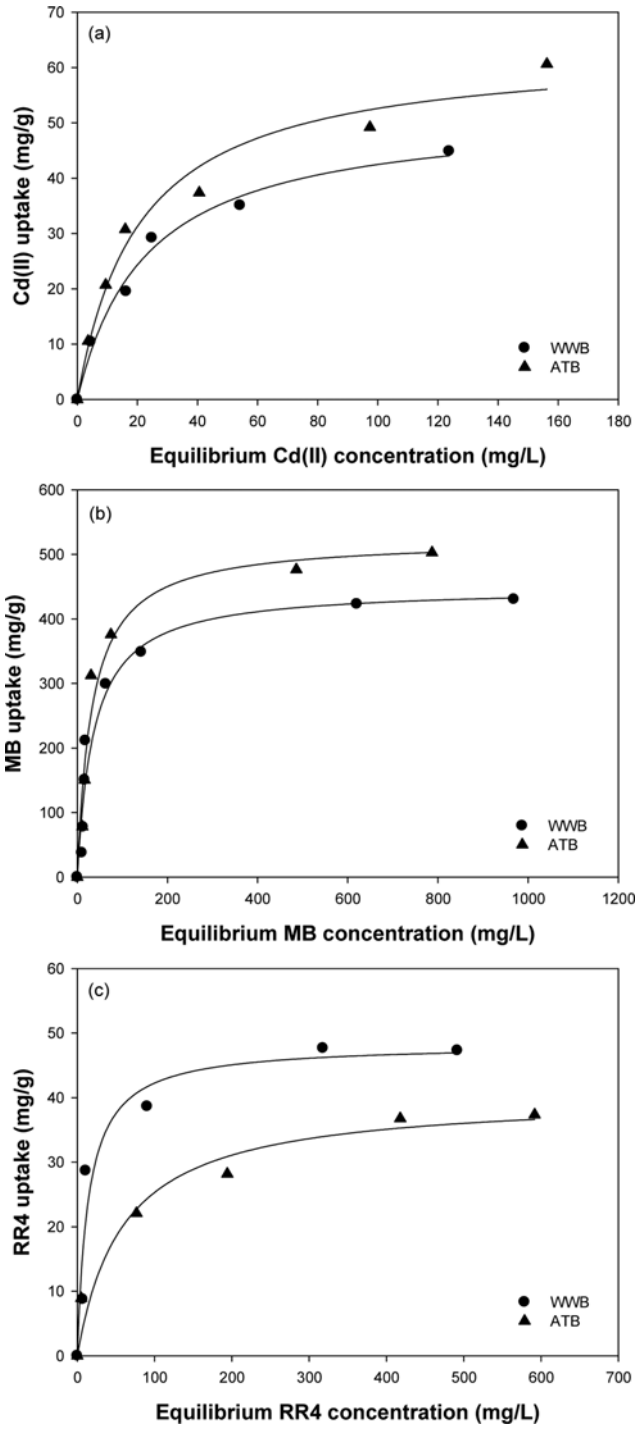

Fig. 1. Biosorption isotherms for Cd(II) (a), MB (b) and RR4 (c) by water-washed biomass and acid-treated biomass. The curves were obtained by fitting the Langmuir model.

tion and micrograph quality.

5. Potentiometric Titration

Potentiometric titration experiments of ATB and WWB were individually carried out with 10 g/L of biomass in 50-mL high-density polyethylene bottles. First, the biomass and 20 mL of water (CO_2-free) were put into each bottle. CO_2-free water was obtained by stripping water with nitrogen gas for 2 h with vigorous mixing. Different volumes of 1 M NaOH were added to each bottle containing the biomass suspensions. After the bottles were closed with caps, they were agitated at 200 rpm and 25 ºC for 24 h in a shaker. Preliminary tests showed that 24 h was sufficient to achieve proton sorption equilibrium. Thereafter, the equilibrium pH was measured using an electrode (Ingold), with nitrogen gas bubbling to maintain the CO_2-free condition to avoid the influence of inorganic carbon on the solution pH.

RESULTS AND DISCUSSION

1. Effect of Acid Treatment on Sorption Capacity

Biosorption isotherms of WWB and ATB were performed with different initial concentration ranges of Cd(II), MB and RR4 because the sorption uptakes of biomasses were different according to the types of sorbates. Initial investigations were carried out with cadmium. Biosorption isotherms for Cd(II) onto the WWB and ATB are presented in Fig. 1(a). The solution pH was controlled at 6 because that is the optimal pH for Cd(II) biosorption. As mentioned in our previous report [16], pH 6 was selected as a suitable pH level at which only biosorption occurred without precipitation because cadmium ions exist in the form of Cd(II) below pH 7. The isotherm experimental data were fitted by the Langmuir model to evaluate the maximum Cd(II) uptakes by the WWB and ATB. The Langmuir parameters and coefficient of determination, R^2, are listed in Table 1. According to the results, the q_{max} and b of WWB were estimated to be 52.2 mg/g and 0.044 L/mg, respectively. ATB showed 63.4 mg/g of the maximum Cd(II) uptake and 0.049 L/mg of the affinity constant. Both the maximum Cd(II) uptake and affinity of ATB were increased to about 20 and 10%, respectively, compared to those of WWB.

In case of MB biosorption, *C. glutamicum* biomass showed that equilibrium was reached within 5 h and optimal pH was greater than or equal to 7 [17]. MB biosorption isotherms by the WWB and ATB were evaluated at pH 9 and their experimental data were described using the Langmuir model. The results were given in Fig. 1(b). The pattern of the MB biosorption isotherms was similar to that of Cd(II) biosorption. As shown in Table 1, the WWB and ATB exhibited the maximum capacities (q_{max}) of 448.9 and 524.0 mg/g, respectively, with affinity coefficients (b) of 0.027 and 0.030 L/mg, respectively. Therefore, ATB was superior to WWB in MB biosorption.

Biosorption isotherms for RR4 showed a different isotherm pattern compared to Cd(II) or MB isotherms (Fig. 1(c)). According to our previous report [18], the uptake of RR4 by *C. glutamicum* biomass was increased as the pH decreased up to 1. The solution pH was adjusted at 4 during isotherm experiments because this pH is proper to confirm the change between the WWB and ATB. The maximum uptake of RR4 by WWB and ATB was 48.3 and 40.3 mg/g, respectively. The affinity constants were estimated to be 0.070 and 0.017 L/mg for WWB and ATB, respectively. Consequently, both the maximum uptake and affinity of ATB were lower than those of WWB.

From the results of isotherm experiments of cationic and anionic pollutants, the acid-treatment led to positive effect on the sorption of cationic pollutants, but negative effect on the sorption of anionic solute. To understand the sorption phenomena, one needs to consider which functional groups on the surface of the biomass are responsible for ionic pollutant biosorption. The surface of the *C. glutamiucm* biomass contains carboxyl, phosphate and amine groups as major functional groups [19]. Among them, negatively charged functional groups such as carboxyl and phosphate groups can bind the cationic pollutants [20,21]. Positively charged amine groups can play a role of binding sites for anionic pollutants in aqueous media [22,23]. To check the variation of functional groups before and after acid treatment requires FTIR analysis of WWB and ATB.

2. FTIR Spectra of WWB and ATB

FTIR spectra of WWB and ATB are shown in Fig. 2. The infrared bands are composed of a combination of protein, lipid and

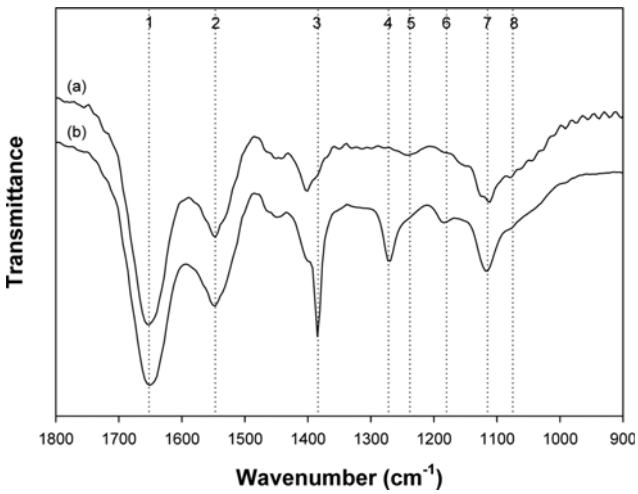

Fig. 2. FTIR spectra of water-washed biomass (a) and acid-treated biomass (b). Numbers 1-8 in figure represent assigned main peaks.

Table 1. Langmuir isotherm parameters for ionic pollutant biosorption by water-washed biomass and acid-treated biomass

Sorbate	Water-washed biomass			Acid-treated biomass		
	q_{max} (mg/g)	b (L/mg)	R^2	q_{max} (mg/g)	b (L/mg)	R^2
Cd	52.2 (3.2)	0.044 (0.008)	0.988	63.4 (4.2)	0.049 (0.011)	0.976
MB	448.9 (27.9)	0.027 (0.006)	0.955	524.0 (31.9)	0.030 (0.007)	0.967
RR4	48.3 (3.9)	0.070 (0.027)	0.941	40.3 (3.8)	0.017 (0.007)	0.963

Table 2. Assignment of major FTIR absorption peaks for water-washed biomass and acid-treated biomass

No.	Wavenumber (cm^{-1})	Assignment	Functional group	Reference
1	1652	N-H bending	Amine	[21]
		C=O stretching	Secondary amide I	[23,24]
2	1547	H-N-C stretching	Secondary amide II	[23,24]
3	1384	COO^- stretching	Carboxyl	[24]
4	1272	C-O stretching	Carboxyl	[21]
5	1238	C-N stretching	Amine	[21]
6	1180	P=O stretching	Phosphonate	[22]
7	1115	P-O-C stretching	Phosphonate	[22]
8	1075	P-OH stretching	Phosphonate	[22]

carbohydrate functional group peaks. The main functional groups and corresponding infrared frequencies are summarized in Table 2. Because carboxyl groups are known to be major binding sites of cationic metals, considerable attention was paid to the bands corresponding to the carboxyl groups. The biomass was prepared at pH 8 in order to change the carboxylic acid (COOH) to the carboxylate anion (COO^-). A distinct change was observed in the carboxylate anion band between the WWB and ATB (No. 3). Similar results have been described for the cyanobacteria, confirming the change of intensity in the carboxylate anion band [24]. Moreover, the C-O stretching band of the carboxyl group was observed in the ATB, while it was very weak or not observed in case of WWB (No. 4).

Before and after acid treatment, there was no significant change in amine (No. 1, 5) [25] and phosphonate (No. 6, 7, 8) [26] groups, which can also behave as binding sites for heavy metals and dye molecules. The peaks at 1,652 (No. 1) and 1,547 (No. 2) cm^{-1} can be assigned to the C=O stretching of the secondary amide I and H-N-C stretching of the secondary amide II, respectively [27,28].

3. Potentiometric Titration

Potentiometric titrations can determine the acid dissociation constants (pK_H) and contents of the major functional groups on bacterial cell surface. The biomass titration curves display distinct characteristics depending upon the type and amount of functional groups present. The titration results of WWB and ATB are shown in Fig. 3. An inflection point was obvious between pH 2 and 4, indicating the presence of a functional group with a pK_H value in the same range. The buffering effect was found in other pH range, implying that other functional groups were present.

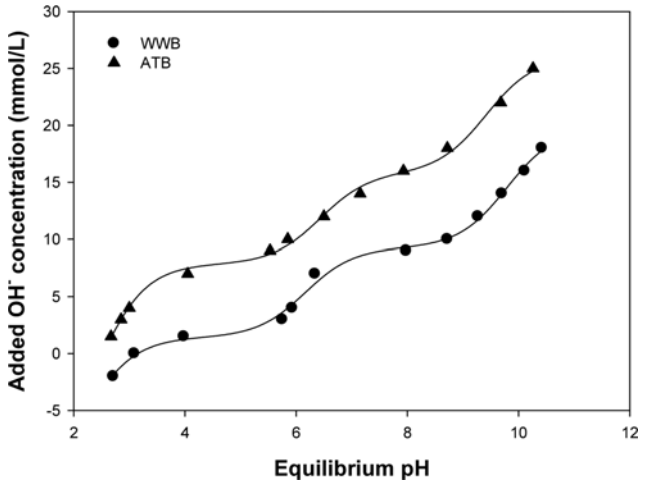

Fig. 3. Potentiometric titration of water-washed biomass and acid-treated biomass. The solid lines represent the theoretical curves predicted from the three-site proton-binding model.

To quantitatively evaluate the properties of the functional groups, we tried proton binding modeling. With respect to a certain group (B_jH), its reaction with a proton and its related equilibrium constant (K_j) may be defined as follows:

$$B_jH \leftrightarrow B_j^- + H^+ \quad K_j = \frac{[B_j^-][H^+]}{[B_jH]} \tag{3}$$

Using the mass balance of functional groups and electro neutrality condition, Eq. (3) can be derived. A more detailed derivation has been reported by [12].

$$[Na]_{added} = \sum_{j=1}^{N} \frac{a_j X}{1 + [H^+]/K_j} + \frac{K_w}{[H^+]} - [H^+] \tag{4}$$

where $[Na]_{added}$ is identical to the concentration of hydroxide ions added. a_j and X represent the quantity of specific functional groups per unit mass of biomass (mmol/g) and the biomass concentration (g/L), respectively. The change in the working volume due to the addition of NaOH was compensated during data processing.

Eq. (4) contains two parameters per functional group: the equilibrium constant (K_j) and the amount of that particular functional group per unit weight (a_j). To compare the number of functional groups of WWB and ATP, the titration model, Eq. (4) was fitted to individual titration data with the same biomass concentration (10 g/L). As a result, the three-site model (two negative sites and one positive site) could successfully describe the titration results (Fig. 3). Meanwhile, two- or one-site functional group models were insufficient to fit the titration data (data was not shown). It means that both WWB and ATB have at least three different functional groups. The estimated parameters are summarized in Table 3.

The first group, of which the pK_H values were 2.29±0.14 and 2.42±0.06 in the cases of WWB and ATB, respectively, was believed to be carboxyl groups. Although the pK_H value of carboxyl groups is lower than reported values, ranging from 3.5 to 5.0 in biological polymers [29]. It was considered to be the carboxyl group due to the peak changes of carboxyl groups observed in the FTIR spectra. The pK_H values of the second binding site of WWB and ATB were estimated to be 6.16±0.12 and 6.44±0.14, respectively. The pK_H

Table 3. Dissociation constants (K) and contents (a) of three functional groups in water-washed biomass and acid-treated biomass[a]

Biosorbent	Functional groups	First group	Second group	Third group
	Charge	–	–	+
Water-washed biomass	pK_H	2.29 (0.14)	6.16 (0.12)	9.79 (0.15)
	a (mmol/g)	1.19 (0.11)	0.78 (0.07)	1.05 (0.10)
Acid-treated biomass	pK_H	2.42 (0.06)	6.44 (0.14)	9.42 (0.17)
	a (mmol/g)	1.81 (0.09)	0.79 (0.06)	1.02 (0.08)

[a]Standard errors are given in parentheses, and the coefficient of determination (R^2) of WWB and ATB were 0.996 and 0.998, respectively.

values were similar to that of the phosphonate group. In general, the phosphonate groups of phospholipids present in the plasma membrane of the biomass have a similar range of pK_H values [30]. The last one was considered to be of amine groups, compared with pK_H values for various biomaterials ranging between 8 and 10 [29]. The results of potentiometric titration and FTIR analysis indicated the existence of carboxyl, phosphonate and amine groups on the surface of WWB and ATB.

Comparing the amount of functional groups before and after acid treatment, *a* notable change in the carboxyl groups was observed. The a value of the carboxyl groups in WWB was 1.19±0.11 mmol/g. Conversely, the *a* value of carboxyl groups in ATB was 1.81±0.09 mmol/g, which was 1.52 times higher than that of WWB. However, the second and third types of functional groups corresponding to phosphonate and amine groups showed no remarkable changes. The amount of the second functional group in WWB and ATB was 0.78±0.07 and 0.79±0.06 mmol/g, respectively. Similarly the amount of the third functional group in WWB and ATB was 1.05±0.1 and 1.02±0.08 mmol/g, respectively. It indicates that the acid treatment of the biomass leads to the increase of the carboxyl groups, but does not affect two other functional groups such as phosphate and amine groups.

As mentioned in section 1, the effect of acid treatment on sorption capacity, the maximum uptake of ATB was higher than that of WWB in the cases of cationic solutes (Cd(II) and MB). Meanwhile, the uptake of anionic solute RR4 decreased after acid treatment. According to the results of FTIR and potentiometric titration, the difference in the maximum capacity between WWB and ATB can be explained by the effect of the increase in carboxyl groups. Since a carboxylic acid changed to a carboxylate anion above its pK_H value, the groups have a negative charge in the solution. Thus, the negatively charged sites can easily bind the positively charged Cd(II) and MB through electrostatic attraction. On the other hand, the carboxyl group may act as an interfering site in binding of anionic RR4 due to electrostatic repulsion. Therefore, it can be noted that acid treatment is effective only for preparation of cation biosorbents.

4. Discussion on the Reason of the Increase of Carboxyl Groups

The enhancement of the carboxyl sites on the surface of ATB was confirmed from the results of FTIR and potentiometric titration. However, the reason for 'why the amount of carboxyl groups on the ATB was increased' needs to be understood. In this section, two possible reasons are suggested.

One reason could be the hydrolysis of esters present on the biomass surface under acidic condition. Hydrolysis of esters results in formation of carboxyl groups. A carboxylic acid is produced from an ester when water is used as the solvent under acidic conditions, while the reaction of a carboxylic acid to yield an ester, called the Fischer esterification reaction, takes place by heating the carboxylic acid with an alcohol in the presence of an acid catalyst. Formation of carboxyl groups can also occur in the cell membrane. The central architectural feature of biological membranes is a double layer of lipids, which acts as a barrier to the passage of polar molecules and ions. The *C. glutamicum* biomass also has membrane lipids, in which two fatty acids are attached via an ester linkage to the first and second carbons of glycerol. Under the acidic condition, carboxylic acid can be produced by cleavage of an ester linkage. The head groups containing glycerol and other substituents can be removed from the fatty acid of the membrane lipid. Pratibha et al. [31] has reported that the MB uptake of acid-treated yeast was higher than that of untreated autoclaved yeast. They explained that the reason for increase in adsorption capacity of yeast was probably due to hydrolysis of certain biomolecules.

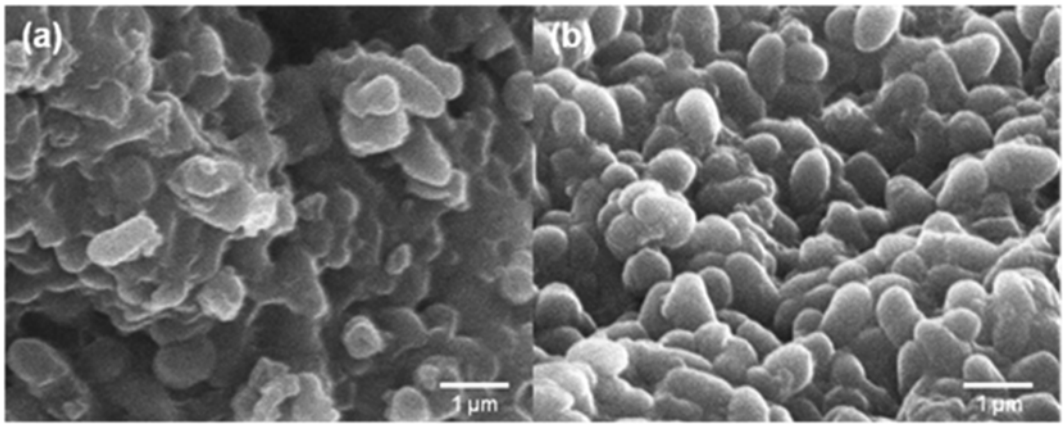

Fig. 4. SEM micrographs of water-washed biomass (a) and acid-treated biomass (b).

Another reason could be the effect of the removal of impurities on the biomass surface. To confirm the above claim, SEM analyses of WWB and ATB were performed (Fig. 4). In the case of WWB, the surface of the biomass was covered with various impurities which seem to be residual culture nutrients and salts. After acid treatment, impurities were removed from the surface of the biomass. The surface of the ATB exhibited cleaner than that of the WWB. Therefore, it is possible that the acid treatment of the biomass can lead to a large available surface area and to exposure of hidden functional groups. If acid treatment makes the hidden sites exposed, phosphonate and amine groups should be increased along with carboxyl group. However, according to the potentiometric titration and proton-binding modeling, only carboxyl groups increased after acid treatment. Although it is obvious that acid treatment results in the removal of impurities, the removal of impurities cannot guarantee the increase in all kind of functional groups.

CONCLUSIONS

This study evaluated the effect of acid treatment of *C. glutamicum* biomass and interpreted the reason why acid treatment affects the sorption capacity. The maximum uptakes of ATB were increased as 63.4 and 524.0 mg/g for cationic Cd(II) and MB, respectively, compared to those of WWB. However, in the case of anionic RR4, the maximum uptake of ATB was lower than that of WWB. According to the results of potentiometric titration and FTIR, the *C. glutamicum* biomass was largely composed of carboxyl, phosphonate and amine functional groups. After acid treatment of the biomass, the amount of carboxyl groups was increased due to the hydrolysis of esters in the biomass under the acidic condition, while the content of phosphonate and amine groups was not changed. Therefore, the increase of the carboxyl groups by acid treatment can enhance the sorption capacity for cationic pollutants such as heavy metals and cationic dyes.

ACKNOWLEDGEMENTS

This work was supported by the Korean Government through NRF (NRL 2008-0060070), KEITI (The Eco-Innovation project), and KETEP (20115020100060) grants. S.W. Won was supported by research funds of Chonbuk National University in 2013.

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