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Diglycolic amic acid-modified *E. coli* as a biosorbent for the recovery of rare earth elements

Yukiho Hosomomi[a], Rie Wakabayashi[a], Fukiko Kubota[a], Noriho Kamiya[a,b], Masahiro Goto[a,b,*]

[a] Department of Applied Chemistry, Graduate School of Engineering, Kyushu University, 744 Motooka, Fukuoka 819-0395, Japan
[b] Center for Future Chemistry, Kyushu University, Fukuoka 819-0388, Japan

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ABSTRACT

Biosorption has recently attracted much attention as an alternative to conventional techniques for the recovery of rare earth elements (REEs). In this study, *Escherichia coli* (*E. coli*) was chemically modified to improve its performance as a biosorbent for REEs. The diglycolic amic acid group, which shows high affinity to REEs, was introduced by succinylation of the amine groups on the *E. coli*. Adsorption curves using the modified *E. coli* were characteristic of the diglycolic amic acid group. The adsorption performance for transition metal ions was not affected by the modification. These results suggest that modification of *E. coli* with a functional group with high affinity to REEs increases the effectiveness of adsorption. The maximum uptakes of REEs on the modified *E. coli* were doubled. Modification of *E. coli* is an effective method for enhancing the adsorption performance for REEs.

1. Introduction

Rare earth elements (REEs) are group of metals including scandium (Sc), yttrium (Y) and the 15 lanthanides. They have received considerable attention because of their increasing use in high-tech industries. Therefore, stable supply and production of REEs are very important for the development of cutting-edge technology. However, it is difficult to ensure their stable supply because their minable ores are sparsely located [1]. Currently, around 90% of REEs is produced in China. The international price of metal resources has changed remarkably depending on the global demand-and-supply balance or the economic plank of the main exporting countries. The prices of REEs rose steeply from 2009 to 2011 because of the reduction in China's export quota [2]. Consequently, the development of highly efficient recovery techniques from underutilized resources and electronic waste containing REEs [3] are necessary.

Various conventional methods have been used for the recovery of REEs, including precipitation [4], solvent extraction [5,6] and ion-exchange [7]. However, these methods are not environmentally friendly and have high energy requirements. Hence, ecofriendly and cost-effective recovery methods are desirable. In recent years, biosorption processes have attracted attention as novel methods for the recovery of REEs. In general, these methods have low operating costs and produce minimal volumes of chemical and/or biological sludge for waste treatment [8,9]. In biosorption, microorganisms, algae or biomaterials that adsorb metal ions are investigated for REE recovery [10–12]. In an earlier study, we showed that *Escherichia coli* could be used as an adsorbent for REE recovery [13]. However, the use of microbial biosorbents for industrial applications is limited because the adsorption efficiency for REEs is dependent on the microbial cell components. Because sorption primarily occurs on the biosorbents, the modification could greatly alter the sorption capacity and selectivity [14,15]. Therefore, modification of the *E. coli* could be used to improve its performance as a biosorbent for REE recovery.

Recently, *N,N*-dioctyldiglycol amic acid (DODGAA) was developed as an efficient extractant of REEs, and it was reported to show the higher affinity for the heavier REEs [16]. Furthermore, it was revealed that REEs from light to heavy groups are selectively extracted over other transition metals including Cu^{2+} and Al^{3+} [17]. In our previous paper, REEs such as Y^{3+}, Eu^{3+}, La^{3+} and Ce^{3+}

* Corresponding author at: Department of Applied Chemistry, Graduate School of Engineering, Kyushu University, 744 Motooka, Fukuoka 819-0395, Japan.
E-mail addresses: hosomomi@mail.cstm.kyushu-u.ac.jp (Y. Hosomomi), rie_wakaba@mail.cstm.kyushu-u.ac.jp (R. Wakabayashi), f_kubotcm@mail.cstm.kyushu-u.ac.jp (F. Kubota), nori_kamiya@mail.cstm.kyushu-u.ac.jp (N. Kamiya), m-goto@mail.cstm.kyushu-u.ac.jp (M. Goto).

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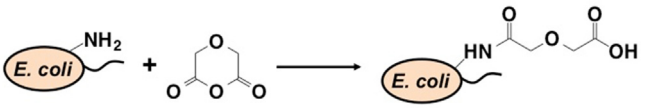

Fig. 1. Schematic illustration of the chemical modification of *E. coli*.

were successfully recovered from a leaching solution of a scrap containing a variety of metal ions using DODGAA [18]. The high performance of DODGAA has been attributed to electrostatic interaction, cation-oxygen-donor interaction, and the chelate effect, which are caused by the tridentate diglycol amic acid groups.

In this study, *E. coli* was chemically modified by succinylation of the amine groups with diglycolic anhydride to introduce diglycol amic acid groups (Fig. 1). *E. coli*, which has various functional groups, is easily available and readily cultured. The effect of this modification on the performance of *E. coli* as a biosorbent for recovery of REEs was investigated.

2. Materials and methods

2.1. Chemical modification of E. coli cells

Recombinant *E. coli* BL21 (DE3) harboring the plasmid vector pET22b (+) was cultivated in 10 mL of lysogeny broth supplemented with ampicillin (100 mg/L) in a shaking incubator at 37 °C. The overnight culture was used to inoculate 1 L of lysogeny broth supplemented with ampicillin (100 mg/L). The cells were grown at 37 °C to an optical density at 600 nm of 0.5–0.6. When this value was reached, the cells were harvested by centrifugation at 5800*g* and 4 °C for 10 min. The harvested cells were washed three times with 25 mmol/L Tris–HCl buffer (pH 8.0), and then lyophilized overnight and used as a negative control. These cells are referred to as unmodified *E. coli*.

For chemical modification, 300 mg of unmodified *E. coli* was suspended in 30 mL of sodium carbonate buffer (100 mmol/L, pH 9.0) solution at room temperature. Diglycolic anhydride (3.48 g), which was purchased from the Tokyo Chemical Industry Co. (Tokyo, Japan), was added dropwise to this suspension, and then the solution was stirred for 1 h at room temperature. The modified cells were harvested by centrifugation at 5800*g* and 4 °C for 10 min and washed three times with acetate buffer (100 mmol/L, pH 4.0). Finally, the modified cells were lyophilized overnight. In this study, the *E. coli* was used in the form of non-uniform small lumps.

2.2. Fourier-transform infrared spectroscopy (FT-IR)

To investigate the chemical characteristics of the biosorbents, the unmodified *E. coli*, *E. coli* soaked in sodium carbonate buffer (100 mmol/L, pH 9.0) for 1 h at room temperature, and the modified *E. coli* were analyzed by attenuated total reflectance FT-IR (Spectrum 65, PerkinElmer Inc., Waltham, MA). For FT-IR analysis, all samples were lyophilized overnight. All infrared spectra were recorded over the range 700–4000 cm^{-1} with a resolution of 4 cm^{-1}.

2.3. Adsorption experiments

Aqueous solutions containing six metal ions (Nd^{3+}, Dy^{3+}, Lu^{3+}, Fe^{3+}, Al^{3+} and Cu^{2+}) were prepared by diluting 1000 ppm standard solutions, which was supplied from Wako Pure Chemical Industries (Osaka, Japan), with a 0.1 mol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid solution. The pH of the solution was adjusted using 1 mol/L HNO_3 or 1 mol/L NaOH. The lyophilized cells were used as the adsorbent in the adsorption experiments. The adsorbent (5 mg) was added to 2.5 mL of the aqueous solution,

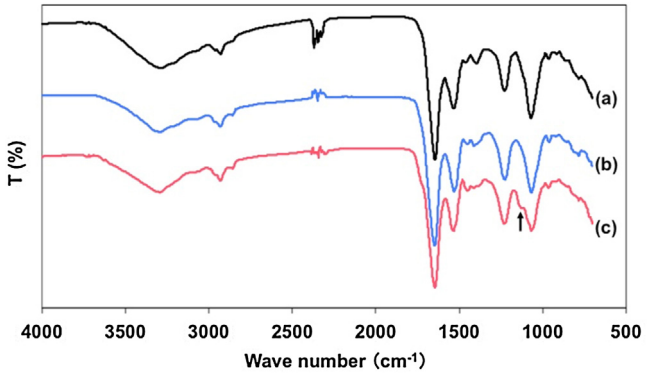

Fig. 2. FT-IR spectra of (a) unmodified *E. coli*, (b) *E. coli* soaked in sodium carbonate buffer (100 mmol/L, pH 9.0), and (c) modified *E. coli*. The arrow indicates the band corresponding to the C—O—C stretching vibration in the diglycol amic acid group.

and the mixture was shaken on a vortex mixer for 30 s, followed by stirring at 160 rpm for 60 min to reach equilibrium. After each adsorption experiment, the aqueous phase was separated from the adsorbent by centrifugation at 5800*g* for 10 min and filtration through a 0.20 μm membrane filter. All procedures were conducted at 30 °C. The initial and final concentrations of the metal ions were determined by inductively coupled plasma atomic emission spectrometry (Optima 5300 DV, PerkinElmer Inc.). The equilibrium pH (pH_{eq}) was measured using a pH meter (HM-60 G, DKK-TOA Co., Tokyo, Japan). The adsorption ratios of the metal ions and REE uptake values (Q_e, mg/g) were calculated using the following equations:

$$\text{Adsorptionratio} = (C_i - C_e)/C_i \quad (1)$$

$$Q_e = V(C_i - C_e)/M \quad (2)$$

where C_i and C_e are the initial and the final concentrations of the metal ions (mg/L), respectively; V is the volume of the solution (L); and M is the mass of the adsorbent (g). The experiment was repeated three times for the REEs and two times for other transition metals.

3. Results and discussion

3.1. FT-IR analysis

FT-IR spectra for the unmodified *E. coli*, *E. coli* soaked in sodium carbonate buffer, and the modified *E. coli* are given in Fig. 2. Broad transmittance bands were observed at 3000–3700 cm^{-1} for N—H stretching vibrations and at 2800–3000 cm^{-1} for O—H and C—H stretching vibrations in all three spectra. Differences among the spectra appeared at 700–2000 cm^{-1} (Fig. 2).

The large bands near 1653 and 1540 cm^{-1} in the three spectra were assigned to amide I and amide II bands. The band at 1460 cm^{-1} was attributed to O—H deformation in the carboxyl groups, while the bands around 1410 cm^{-1} were attributed to the carboxyl anion. Additionally, bands at 1240 and 1075 cm^{-1} were identified as phosphoric ester and free phosphate groups of phospholipids, and these were classed together as phosphate groups. There were no large changes between the spectra of the unmodified *E. coli* (Fig. 2(a)) and that of the *E. coli* soaked in sodium carbonates buffer (Fig. 2(b)). In the modified *E. coli* (Fig. 2(c)), a band corresponding to the C—O—C stretching vibration in the diglycol amic acid group appeared at 1140 cm^{-1}. This band was not observed in the spectra of the unmodified or sodium carbonate buffer-soaked *E. coli*. Thus, the FT-IR results suggested that the modification of *E. coli* was successful.