Surface modification of Corynebacterium glutamicum for enhanced Reactive Red 4 biosorption

Juan Mao a, Sung Wook Won b, K. Vijayaraghavan b, Yeoung-Sang Yun a,b,*

a Department of Bioprocess Engineering, Chonbuk National University, Jeonbuk 561-756, South Korea
b Division of Environmental and Chemical Engineering and Research Institute of Industrial Technology, Chonbuk National University, Jeonbuk 561-756, South Korea

Abstract

This study reports the possibility of enhancing the reactive dye biosorption capacity of Corynebacterium glutamicum via its cross-linking with polyethylenimine (PEI). The amine groups in the cell wall of C. glutamicum were found to electrostatically interact with reactive dye anions. Thus, cross-linking the biomass with PEI enhanced the primary and secondary amine groups, thereby increased the biosorption of reactive dye. The pH edge experiments revealed that acidic conditions, due to protonation of the amine groups, were found to favor Reactive Red 4 (RR 4) biosorption. According to the Langmuir model, the PEI-modified C. glutamicum recorded a maximum RR 4 uptake capacity of 485.1 mg/g compared to 171.9 mg/g of the raw C. glutamicum. The kinetic experiments revealed that chemical modification decreased the rate of biosorption. Desorption was successful at pH 9, with the biomass successfully regenerated and reused over four cycles.

1. Introduction

Biosorption is a technique that can be used for the removal of pollutants from waters, especially those that are not easily biodegradable. A variety of biomaterials are known to bind pollutants, including bacteria, fungi, algae, industrial wastes, agricultural wastes and other polysaccharide materials. The potential of bacteria to sorb and accumulate various organic/inorganic pollutants has been well established (Volesky and Holan, 1995; Aksu, 2005). Corynebacterium glutamicum, a gram positive bacterium, is widely used for the biotechnological production of amino acids. Currently, the productions of the amino acids, L-glutamate and L-lysine, from fermentative processes using C. glutamicum are 1,500,000 and 550,000 t per year, respectively (Hermann, 2003). Hence, potential utilization of this microbial waste is of interest.

In recent years, interest has focused on increasing the sorption capacity of biomass (Jeon and Höll, 2003; Yu et al., 2007; Vijayaraghavan and Yun, 2007). Several biomasses regarded as industrial wastes following certain processes possess low biosorption capacities. As sorption mainly takes place on the biomass surface, increasing/activating the binding sites on the surface would be an effective approach for enhancing the biosorption capacity. Therefore, this research focused on enhancing the biosorption of Reactive Red 4 (RR 4) via cross-linking the biomass with polyethyleneimine (PEI). PEI is well known for its metal chelation properties due to the presence of a large number of amine groups in a molecule (Ghoul et al., 2003; Deng and Ting, 2005); however, its performance in relation to dye adsorption is unknown well.

2. Methods

2.1. Dye, biosorbent and preparation

Reactive Red 4 (C32H24ClN8Na4O14S4), 50% pure, with a molecular weight of 931.3, was purchased from Sigma–Aldrich Korea Ltd. (Yongin, Korea). Analytical grade PEI ((H(NHCH2CH2)nNH2); molecular weight = 25,000 g/L), 4-bromobutyryl chloride (95%) and all other chemicals used in this study were also obtained from Sigma–Aldrich Korea Ltd. (Yongin, Korea).

The fermentation wastes, C. glutamicum, were obtained as a dried powder from a mono sodium glutamate fermentation industry (Deasang, Gunsan, Korea). The biomass, dried using a spray-drying process for 24 h, referred to as raw biomass, was subsequently used in the biosorption experiments. The particle size of raw biomass was less than 0.25 mm.

For PEI cross-linking, 10 g of dried raw biomass was brought into contact with 2.5 mL pyridine and 95 mL chloroform, followed by drop-wise addition of 5 mL of 4-bromobutyryl chloride. The reaction mixture was sealed and agitated in an incubated shaker at 25 °C for 12 h. The acylated biomass was rinsed with chloroform to remove any unreacted 4-bromobutyryl chloride before being...
immersed in a mixture of 10 g PEI and 0.1 g KOH in 90 mL tert-amy alcohol. After stirring the mixture at 75 °C for 24 h, the PEI-modified biomass was washed with methanol and deionized water. The washed biomass was finally freeze-dried, ground and sieved for uniform particle size (<0.25 mm), and subsequently used in the experiments.

2.2. FT-IR analysis

Infrared spectra of the biomass samples were obtained using a Fourier transform infrared spectrometer (FT-IR-8900, ABB Bomem, Quebec, Canada). The sample was prepared as a KBr disc and examined to identify the functional groups responsible for the biosorption over the range 400–4000 cm⁻¹.

2.3. Biosorption experimental procedure

For the biosorption experiments, the biomass (0.03 g) was brought into contact with 40 mL dye solution in a 50 mL falcon bottle (high-density polyethylene). The pH of the solution was initially adjusted and controlled using 1 M HNO₃ or NaOH. The tubes were then kept in an incubated rotary shaker at 160 rpm and 25 °C. After equilibrium had been attained, the biosorbent was separated by centrifugation at 3000 rpm for 5 min. The dye (RR 4) concentration in the supernatant was determined using a spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan) at 517 nm, after appropriate dilution. Kinetic experiments were conducted in the same manner as the isotherm experiments, with the exception that the samples were collected at fixed time intervals. Desorption experiments were performed by contacting the RR 4-loaded biomass with 40 mL of deionized water, with the pH of the suspension adjusted to pH 9, for 3 h at 160 rpm and 25 °C. The remaining procedure was the same as that employed in the biosorption equilibrium experiments. These cycles of biosorption followed by elution were repeated for four times to evaluate the sorbent capacity. All experiments mentioned above were carried out in duplicates, and the reported values were average values of two data sets.

3. Results and discussion

3.1. FT-IR analysis

For a more detailed investigation on the intensity and nature of the functional groups on the biomass surface, the FT-IR spectra of the raw and PEI-modified biomasses were analyzed. The FT-IR spectra (figure not shown) revealed the presence of broad, strong bands, ranging from 3800 to 2500 cm⁻¹, may have been due to the overlapping of OH and NH stretching vibrations, which was consistent with the peaks at 1080 and 1245 cm⁻¹ assigned to the out-of-plane bending absorption (Pavia et al., 2001).

Thus, the FT-IR results verified the presence of amine and hydroxyl groups on the raw C. glutamicum biomass surface. The acetylchlorine group in 4-bromobutyl chloride is very active, which easily reacts with hydroxyl and amine groups, while the hydrogen in the amine groups in PEI molecules can replace the bromine in 4-bromobutyl chloride (Deng and Ting, 2005). Thus, the amine and hydroxyl groups on the surface of C. glutamicum play an important role in the grafting of PEI onto the biomass surface.

3.2. pH edge

The solution pH will affect the availability of dye molecules and the activities of the functional groups on the biomass. In the present study, the pH edge experiments (Table 1) revealed that pH ≤ 3 favored RR 4 biosorption. Increasing the pH beyond 3 resulted in decreased RR 4 uptakes. The cell wall of a Gram-positive bacterium is mainly comprised of a peptidoglycan layer connected by amino acid bridges (Mera et al., 1992). Polyalcohols, known as teichoic acids, are imbedded in the Gram-positive cell wall, which give an overall negative charge to the bacterial cell wall due to the presence of phosphodiester bonds between the teichoic acid monomers (Beveridge et al., 1982). From the FT-IR results, the cell wall of C. glutamicum was found to be comprised of carboxyl and amine groups. Under acidic conditions, due to protonation of the functional groups, the biomass will have net positive charge. Conversely, reactive dyes release colored negatively charged dye ions into solution, which will exhibit electrostatic attraction towards the positively charged cell surfaces. In particular, the amine groups on C. glutamicum were found to be mainly responsible for the biosorption of reactive dyes, with the hydrogen ions acting as bridging ligands between the bacterial cell wall and the dye molecules (Vijayaraghavan and Yun, 2007).

Attempts were also made to chemically enhance the amine groups via cross-linking of the biomass with PEI. As a result of enhancement, the biosorption capacity of C. glutamicum was increased 2.8 fold compared to that of the raw biomass, which confirmed the importance of amine groups in the binding of reactive dyes.

3.3. Biosorption isotherms and modeling

To evaluate the maximum biosorption potential of C. glutamicum, isotherm experiments were conducted at pH 2. The ratio between the RR 4 concentration remaining in solution and that sorbed onto the solid decreased with increasing RR 4 concentration, providing a concave curve, with a strict plateau (Fig. 1). Comparatively, PEI-modified biomass performed well compared to the raw biomass.

Attempts were made to model the RR 4 isotherm using the nonlinear forms of the Langmuir and Sips models, which can be represented as follows

Langmuir model: \[ Q = \frac{Q_{\text{max}}b_C C}{1 + b_C C} \] (1)

Sips model: \[ Q = \frac{K_C C_{S}^{b_C}}{1 + a_C C_{S}} \] (2)

where \(Q_{\text{max}} \) is the maximum dye uptake (mg/g), \( b_C \) the Langmuir equilibrium constant (L/mg), \( K_C \) the Sips model isotherm constant \((L/g)^{b_C}\), \( a_C \) the Sips model constant and \( b_C \) the Sips model exponent \((L/mg)^{b_C}\). The Langmuir constant “\(Q_{\text{max}}\)” is often used to compare the performance of biosorbents; while “\(b_C\)” characterizes the initial slope of the isotherm. Thus, for good biosorbents; in general, a high \(Q_{\text{max}}\) and steep initial isotherm slope (i.e., high \(b_C\)) are desirable.
(Kratochvil and Volesky, 1998). The parameters of the Langmuir model are shown in Table 1. The correlation coefficient \((R^2)\) values were greater than 0.968; whereas, the percentage error values were less than 6.2%. A three parameter model, viz the Sips model, was also used to improve the fit of the biosorption isotherm data. Very high \(R^2\) values (>0.979) and low percentage error values (<1.9%) were observed when the RR 4 biosorption isotherms were described using the Sips model (Table 1).

3.4. Biosorption kinetics and modeling

The experimental kinetic data revealed that more than 95% of raw biomass-RR 4 equilibrium was attained in the first 90 min; whereas, PEI-modified biomass took nearly 600 min to attain equilibrium (Table 1). This is because the capacity of PEI-modified biomass was found to be nearly 2.8 times higher than that of the raw biomass and also the binding of RR 4 anions to the cross-linked biomass was found to be 2.8 times higher than that of the raw biomass. The PEI-modified biomass was regenerated and reused over four successive sorption–desorption cycles. Thus, cross-linking the bacterial bio-

![Fig. 1. RR 4 biosorption isotherms for the raw and PEI-modified C. glutamicum biomasses (pH = 2; agitation rate = 160 rpm). Curves were predicted using the Sips model.](image)

### Table 1

Effect of biosorption process parameters (pH edge, kinetic and isotherm) on RR 4 removal by the raw biomass (RB) and PEI-modified biomass (PEIB)

<table>
<thead>
<tr>
<th>pH edge</th>
<th>RB, RR 4 uptake (mg/g)</th>
<th>PEIB, RR 4 uptake (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>90.9</td>
<td>20.5</td>
</tr>
<tr>
<td>3</td>
<td>82.6</td>
<td>49.3</td>
</tr>
<tr>
<td>4</td>
<td>70.6</td>
<td>82.1</td>
</tr>
<tr>
<td>5</td>
<td>47.6</td>
<td>94.4</td>
</tr>
<tr>
<td>6</td>
<td>25.4</td>
<td>135.4</td>
</tr>
<tr>
<td>7</td>
<td>7.5</td>
<td>160.1</td>
</tr>
<tr>
<td>8</td>
<td>7.2</td>
<td>151.9</td>
</tr>
<tr>
<td>10</td>
<td>6.0</td>
<td>156.0</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>RB, RR 4 uptake (mg/g)</th>
<th>PEIB, RR 4 uptake (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>171.9</td>
<td>485.1</td>
</tr>
<tr>
<td>20</td>
<td>0.589</td>
<td>0.172</td>
</tr>
<tr>
<td>45</td>
<td>0.971</td>
<td>0.968</td>
</tr>
<tr>
<td>60</td>
<td>102.3</td>
<td>149.9</td>
</tr>
<tr>
<td>90</td>
<td>0.595</td>
<td>0.300</td>
</tr>
<tr>
<td>120</td>
<td>0.983</td>
<td>0.711</td>
</tr>
<tr>
<td>300</td>
<td>0.985</td>
<td>0.979</td>
</tr>
<tr>
<td>600</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Kinetic

- **RB** 4.1 73.9 127.2 143.7 156.0 151.9 160.1 –
- **PEIB** 20.5 49.3 82.1 94.4 135.4 160.1 344.8 410.4

### Isotherm

- **Langmuir** $Q_{eq} = \frac{Q_{max} b L}{1 + C/L}$
- **Sips** $q_{eq} = q_0 (1 - \exp(-k_1 t))$

where $Q_{eq}$ is the amount of dye sorbed at equilibrium (mg/g), $q_t$ the amount of dye sorbed at time $t$ (mg/g), $k_1$ the pseudo-first order rate constant (L/min) and $k_2$ is the pseudo-second order rate constant (g/mg.min). In the case of the pseudo-first order model, the correlation coefficients were found to be above 0.921, with calculated $q_t$ close to the experimental $q_{eq}$ suggesting the applicability of the model for fitting the kinetic data from the present system.

### 3.5. Desorption and regeneration of biomass

Desorption experiments were aimed at evaluating the possibility of recycling the biomass over four cycles. Desorption of RR 4 from the dye-loaded PEI-modified C. glutamicum biomass was attempted at pH 9, where biosorption was found to be negligible, as under strongly basic (high pH) conditions, the amine groups became deprotonated and dye anions desorbed from the biosorbent. Desorption was successful, with an elution efficiency of 92.5% in the first cycle. However, slightly decreased elution efficiencies affected the RR 4 uptake in subsequent cycles. A decrease of 15.7% was observed when comparing the fourth cycle for the RR 4 uptake capacity of regenerated PEI-modified C. glutamicum to the first cycle.

### 4. Conclusions

Cross-linking the biomass via the reaction with polyethyleneimine introduced primary and secondary amine groups, which in turn enhanced the RR 4 biosorption capacity. Also the FT-IR spectra confirmed the presence of amine, hydroxyl and carboxyl groups. With the aid of pH edge data, acidic conditions were found to be mandatory for optimum RR 4 biosorption. According to the Langmuir model, the maximum uptake capacity of PEI-modified biomass was 2.8 times higher than that of raw biomass. The PEI-modified biomass was regenerated and reused over four successive sorption–desorption cycles. Thus, cross-linking the bacterial bio-
mass via polyethylenimine is an attractive option for enhancing the biosorption potential towards reactive dyes.

Acknowledgements

This work was supported by the Korea Science and Engineering Foundation (KOSEF) NRL Program Grant funded by the Korea government (MEST) (No. R0A-2008-000-20117-0) and, in part, by Ministry of Environment as “The Eco-technopia 21 project”.

References