Corynebacterium glutamicum을 이용한 염료의 생체흡착: pH, 염도의 효과와 리고 결합자리의 특성

Biosorption of dyes using Corynebacterium glutamicum: effect of pH, salt concentration and characterization of binding sites

Juan Mao* · Sung Wook. Won* · K. Vijayaraghavan* · Yeoung-Sang Yun***
*Department of Bioprocess Engineering, Chonbuk National University, Jeonbuk, 561-756, Korea
**Department of Environmental and Chemical Engineering, Chonbuk National University, Jeonbuk, 561-756, Korea

Abstract

Corynebacterium glutamicum was prepared for the sorption of Reactive Red 4 (RR 4) and Methylene Blue (MB). The pH edge experiments showed that the pH of the solution was an important controlling parameter. An increased salt concentration strongly influenced the uptake of MB, but had no effect on RR 4. In order to identify the binding sites for the dye molecules, the biosorbent was potentiometrically titrated. The result showed that four types of major functional groups were present on the surface of biomass, which was confirmed by FTIR analysis. It was found that NH3+ were likely to be the binding sites for anionic RR 4, and –COO- played a role in electrostatic attraction with cationic MB.

1. Introduction

Biosorption can be defined as sequestering of organic and inorganic species including metals, dyes and odor causing substances using live or dead biomass or their derivatives. It is affected by the type of biomass (species, age), type of sorbates, presence of other competing ions and methods of biomass preparation (culture condition for live biomass), along with several physico-chemical factors (temperature, pH, ionic concentration) [1, 2].

The biomass of Corynebacterium glutamicum is generated in a great quantity from the full-scale fermentation process for mono sodium glutamate (MSG) production. In this study, C. glutamicum was used for the removal of reactive dye (reactive red 4) and basic dye (methylene blue) from the aqueous solution.

2. Materials and methods

2.1 Materials

In this study, C. glutamicum biomass was obtained in the form of powder from a lysine fermentation industry (Deasang, Gunsan, Korea). The powder biomass was dried at 60°C in an oven for 24 h, and then was stored in a desiccator and used as biosorbent in the sorption experiments.

Two kind of dyes, namely Reactive Red 4 (RR 4) and Methylene Blue (MB) were selected as the adsorbate. RR 4 is the representative of reactive anionic dye group, whereas MB is the representative of a wholly basic cationic dye group.

2.2 Biosorption isotherm

Adsorption experiments were conducted using the batch method. The equilibrium studies were carried out at 25 ± 2°C by varying the concentration of dye in aqueous solution from 0 to 3000 mg/L. In each experiment, 0.4 g biomass was added in a 50 mL falcon tubes containing 40 mL of dyes at pH 2, 4 and 9 with a constant agitation speed of 160 rpm. The desired pH values of working solution were adjusted using 1 M HNO3 or 1 M NaOH. After 24 h, which was sufficient to get equilibrium, the final pH values of working solutions were measured and the samples were then centrifuged and the left out concentrations in the supernatant solutions were analyzed using UV spectrophotometer (UV-2250, Shimadzu, Kyoto, Japan) at 517 nm and 661 nm, where the maximum absorption peak of RR 4 and MB exist.
respectively.

2.3 Effect of pH

In the pH edge experiment, the final solution pH was adjusted to the desired value ranging from about 2.11 using 1 M HNO₃ or 1 M NaOH. Biomass 0.4 g was added into each 50 mL falcon tube containing 40 mL RR 4 or MB solution (250 mg/L) and agitated in the shaker at 160 rpm for 24 h at 25 ± 2 °C. After equilibrium the concentrations in the samples were analyzed as mentioned before.

2.4 Effect of the salt concentration

Aqueous dye solutions of 500 mg/L dye (RR 4 or MB) concentration were prepared. 40 mL dye solutions having different concentration of NaCl and CaCl₂ (0°0.5 mmol/L) were added to 50mL falcon tubes with 0.4 g biomass at pH 2 and pH 9 for RR 4 and MB, respectively, and at room temperature (25 ± 2 °C) with a constant agitation speed of 160 rpm. Equilibrium dye concentrations were analyzed as mentioned before.

2.5 Potentiometric titration

The potentiometric data were regressed with following model [3]:

\[
[OH^-]_{\text{added}} = \frac{\sum b_i X_i}{\sum K_i [H^+]_i} - \frac{\sum b_i X_i}{\sum K_i [H^+]_i} + \frac{K_p}{[H^+]} \quad (1)
\]

Where \([OH^-]_{\text{added}}\) is the concentration of added hydroxide ions (mmol/L), \(K_i\) is the proton dissociation constant (M), \(b_i\) present the molar quantities of the functional groups in the biomass (mmol/g) and \(X_i\) is the concentration of biomass (g/L). Subscripts \(i\) and \(j\) indicate negatively and positively charged groups, respectively.

3. Result and discussion

3.1 Biosorption isotherm

![Figure 1. Isotherm of RR4 on the biomass of C. glutamicum at pH 2, 4 and 9. The lines were simulated according to the Langmuir model.](image1)

![Figure 2. Isotherm of MB on the biomass of C. glutamicum at pH 2, 4 and 9. The lines were simulated according to the Langmuir model.](image2)

Biosorption isotherms of RR 4 and MB onto C. glutamicum are shown in Figure 1 and Figure 2, respectively. The isotherm were evaluated by varying initial dyes concentrations in the range of 0°3000 mg/L with a constant biomass dosage of 10 g/L. The uptakes of dyes increased with increasing equilibrium concentration and eventually reached a certain saturated value depending on the different pH values.

3.2 Effect of pH

As shown in Figure 3, the pH values of dye solution significantly affected the uptake of RR 4 and MB. In the case of RR 4, as pH increased from 2 to 10, the uptake decreased from 52 mg/g to 1 mg/g. In contrary, as pH increased, the uptake of dye also increased and maximum MB uptake was obtained at pH ≥ 9.
Figure 3. Effect of pH on biosorption of RR 4 and MB at 25 ± 2 °C. • represents RR 4 pH edge curve. ▽ symbolize pH edge curve of MB.

3.3 Effect of the salt concentration

The effect of the salt concentration on the uptake of RR 4 and MB were investigated. From Figure 4, the effect of NaCl and CaCl₂ concentration on the uptake of RR 4 could be negligible. It indicated that Cl⁻ ions do not compete with sulphonate groups of RR 4 for amine sites on the surface of biomass. From Figure 5, NaCl and CaCl₂ exist in solution affected the MB adsorption onto the biomass. It was seen that as the salt concentration increased from 0 to 0.5 mol/L, the uptake of MB decreased from 51.8 mg/g to 33.9 mg/g and 52.4 mg/g to 28.9 mg/g for NaCl and CaCl₂ respectively. It could be attributed to the competitive effect between MB ions and cations (Na⁺, Ca²⁺) from the salt for the sites available for the sorption process. And another reason was that ionic strength might affect the activity of dye and active sites on the surface of biomass. Ca²⁺ has more contribution to ionic strength and more positive charge than Na⁺, the effect of Ca²⁺ on adsorption was more commandable than Na⁺.

Figure 4. Effect of the salt concentration on the uptake of RR 4. Initial RR 4 concentration: 500 mg/L. Adsorbent dose: 10 g/L, pH 2.

Fig. 5: Effect of the salt concentration the uptake of MB. Initial MB concentration: 500 mg/L. Adsorbent dose: 10 g/L, pH 9

3.4 Characterization of binding sites

In order to quantitatively evaluate the functional group properties, Equation (1) was applied to the potentiometric data. As shown in figure, the proton-binding model was fitted to titration experimental data very well for the high correlation coefficient $r^2$ (0.9997). As a result, the four-site model (three types of negative group and one positive) was able to
completely describe the potentiometric titration data (Figure 6), whereas three-, two- or one-site functional group models lacked representation of the data, especially at a high pH (data not shown). The estimated parameters of the proton-binding model are summarized in Table 1.

![Graph showing potentiometric titration data](image)

Figure 6. Potentiometric titration of *C. glutamicum*. The solid line represents the theoretical curves predicted from proton-binding model.

Table 1. Estimated parameters of the proton–binding model.

<table>
<thead>
<tr>
<th>Functional group&lt;sup&gt;b&lt;/sup&gt;</th>
<th>First group</th>
<th>Second group</th>
<th>Third group</th>
<th>Fourth group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>pK&lt;sub&gt;H&lt;/sub&gt; (--)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.47 (0.09)</td>
<td>4.90 (0.09)</td>
<td>6.82 (0.05)</td>
<td>10.35 (0.08)</td>
</tr>
<tr>
<td>b (mmol/g)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.57 (0.34)</td>
<td>0.41 (0.02)</td>
<td>0.63 (0.02)</td>
<td>3.85 (0.35)</td>
</tr>
</tbody>
</table>

<sup>a</sup>The coefficient of determination was 0.9997. Standard errors of the estimated parameters are given parenthetical.

<sup>b</sup>The first functional group was considered as a sulphonate site; the second functional group indicates a carboxylic site; the third group is possibly a phosphonate site; and the fourth group a primary amine site.

<sup>c</sup>The pK<sub>H</sub> values represent the dissociation constants of the functional groups.

<sup>d</sup>The b value are the numbers of the functional groups.

Conclusion

*C. glutamicum* biosorbents were applied successfully for the sorption of RR 4 and MB. Equilibrium sorption data were modeled using Langmuir equation and were fitted very well. The pH edge experiments showed that pH was an important controlling parameter in the sorption process. And salt concentration had adverse effects on MB sorption but no effect on RR 4. Potentiometric titration experiments identified four binding sites on the biomass surface, which was confirmed by FTIR analysis. The positively charge amine groups were likely the binding sites for RR 4 and negatively charge carboxylic group played a role in electrostatic attraction with MB. So *C. glutamicum* biosorbents might have potential to removal dye contamination.

Reference