Reinforcement of carboxyl groups in the surface of Corynebacterium glutamicum biomass for effective removal of basic dyes

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ABSTRACT

The biomass of Corynebacterium glutamicum was treated with poly(amic acid) to improve the biosorption of Basic Blue 3 (BB3) from aqueous solution. The grafting of poly(amic acid) onto the biomass surface increased the density of the carboxyl groups. The UV-spectrum revealed that strong acidic (pH ≤ 2) and basic conditions (pH ≥ 11) resulted in the precipitation of BB3. Therefore, pH edge experiments were conducted only within the range 3–10; these results indicated that electrostatic attraction between carboxyl groups of C. glutamicum and BB3 dye cations was favored under alkaline conditions. From the Langmuir model, poly(amic acid)-modified biomass gave a maximum uptake of 173.6 mg/g at pH 9, compared to 52.8 mg/g by the raw biomass. The biosorption kinetics was found to be fast; with equilibrium attained within 10 min. The increase in the ionic strength strongly affected the uptake of BB3 for both forms of C. glutamicum.

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1. Introduction

Dyes are widely used in industry for the coloration of products, such as textiles, paper, plastics and leather. The effluents emanating from these industries often contain high concentrations of dye wastes. Two percent of the dyes produced are discharged directly in aqueous effluent, with a further 10% subsequently lost during the textile coloration process. Dyes are generally believed to be toxic and carcinogenic, or are prepared from other known carcinogens (Jin et al., 2007). Hence, the elimination of dyes from wastewaters is required prior to their discharge as effluents. This concern has led to the development of various methods for the decolorization of dye-bearing wastewaters, such as biological treatment, adsorption, precipitation and ozonation (Robinson et al., 2001).

Biosorption has been recognized as an alternative to conventional processes for the treatment of dye-bearing effluents (Fu and Viraraghavan, 2001; Aksu, 2005). It can be defined as the passive uptake of toxicants by dead/inactive biological materials or by materials derived from biological sources. Biosorption is due to a number of metabolism-independent processes, which essentially take place in the cell wall, where the mechanisms responsible for the pollutant uptake will differ according to the biomass type.

Microorganisms, including bacteria, fungi and algae, have been investigated in dye biosorption studies (Won et al., 2004; Fu and Viraraghavan, 2002a; Aksu and Tezer, 2005). Among the bacterial biomasses used for dye biosorption, Corynebacterium glutamicum is well known as a biosorbent, especially of reactive dyes (Won et al., 2004; Vijayaraghavan and Yun, 2007a), which is widely used for the biotechnological production of amino acids. Currently, the production of amino acids from fermentative processes using C. glutamicum account for 1,500,000 and 550,000 ton of L-glutamate and L-lysine, respectively, per year (Hermann, 2003). Hence, the waste C. glutamicum generated after fermentation is usually high, with great interest in its potential utilization.

Biosorbents can be pretreated to improve the biosorption performance via several techniques. 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. Chemical modification procedures include pretreatment, binding site enhancement, binding site modification and polymerization; whereas common chemical pretreatments include acid, alkaline, ethanol and acetone treatments of the biomass (Vijayaraghavan and Yun, 2007a; Selatnia et al., 2004; Gökşungur et al., 2005; Bai and Abraham, 2002). The success of a chemical pretreatment strongly depends on the cellular components of the biomass itself. In many instances, acidic pretreatment has proved successful, because some of the impurities and ions blocking the binding sites can be easily eliminated.

The modification of the specific binding sites on the biomass seems to enhance the biosorption capacity by many multiples.
Methods

2.1. Dye, biomass and chemical modification

Basic Blue 3 (C20H26ClN3O), 25% pure, was purchased from Sigma–Aldrich Korea Ltd. (Yongin, Korea).

The fermentation waste (C. glutamicum biomass) was obtained as a dried powder from a nucleic acid related fermentation industry (Deasang, Gunsan, Korea). The biomass was dried for 24 h using a spray-drying process, and is referred to as raw biomass, which was subsequently used in the biosorption experiments.

The modified biomass was prepared using a previously reported method (Yu et al., 2007). Firstly, in order to make a cross-linked biomass, 5.0 g of the raw biomass was added to 500 ml of glutaraldehyde solutions (0.5 wt% in water) and agitated in a shaker at 160 rpm and room temperature, 25 ± 1 °C, for 24 h. The cross-linked biomass was washed with distilled water and freeze-dried. Secondly, 150 ml of N,N-dimethylacetamide (DMAc) solution, containing 5.0 g of pyromellitic dianhydride (PMDA) and 1.0 g of thiourea, was shaken at room temperature for 2 h. After stirring, a clear yellow solution was obtained. The cross-linked biomass (4.0 g) was added to the yellow solution, with stirring continued for a further 4 h at 50 ± 1 °C. The biomass obtained was thoroughly rinsed with DMAc to remove residual monomer and polymer, and basified with the addition of 20 ml of 0.1 M NaOH solution. The chemically treated biomass, designated PAA-modified biomass, was washed three times with deionized water and freeze-dried before use. The resultant dried PAA-modified C. glutamicum biomass was stored in a desiccator, and subsequently used as the biosorbent in the sorption experiments.

2.2. Effect of pH in dye solution

In order to evaluate the stability of BB3 under different pH conditions, a BB3 solution with an initial concentration of 500 mg/l was prepared. 40 ml of this dye solution was placed in each bottle, without biomass, and adjusted to the desired pH during the experiment using either 0.1 M NaOH or 0.1 M HNO3. The dye solutions were agitated in a shaker at 160 rpm and room temperature for 24 h. After 24 h, the final pH was measured, with the samples taken and centrifuged at 3000 rpm for 5 min. Finally, the dye spectrum was measured within the range 800–400 nm, using a spectrophotometer (UV–2450, Shimadzu, Kyoto, Japan), after appropriate dilution.

2.3. Biosorption experiments

Stock solutions of BB3, without further purification, were prepared by dissolving accurately weighed samples of dye in deionized water to give a concentration of 550 mg/l, which was subsequently diluted when necessary.

pH edge experiments were conducted with 92 mg/l of the initial BB3 concentration and 2.5 g/l of the biomass. The pH was intentionally altered by the addition of 0.1 M NaOH or 0.1 M HNO3 to the working solution. The suspension was agitated at 25 ± 1 °C and 160 rpm in a shaker for 24 h. After the system had reached equilibrium, the final pH was measured and the biosorbent was separated by centrifugation at 3000 rpm for 5 min, with the dye (BB3) concentration in the supernatant determined, after appropriate dilution. Kinetic experiments were performed by fixing the BB3 concentration at 290 mg/l and the pH at 9.0. The samples were collected at different time intervals to determine the attainment of biosorption equilibrium. Biosorption isotherms of BB3 were obtained at different solution pHs. The isotherm experiments were conducted with 0.1 g of the biomass in a 40 ml of working solution volume. The initial dye concentration was altered from 0 to 550 mg/l, which resulted in different final dye concentrations after the sorption equilibrium had been achieved. Following the addition of the biomass into the dye-containing solutions, the solution pH was controlled to the desired value using 0.1 M NaOH or 0.1 M HNO3. All experiments mentioned above were carried out in duplicates, and the reported values were average values of two data sets.

The dissolved dye concentration of the samples was analyzed using a UV–Vis spectrophotometer at 654 nm, the maximum absorption peak of BB3. The amount of BB3 sorbed by the biosorbent was calculated from the difference between the concentrations of BB3 added to that in the supernatant, using the following equation:

\[ Q = \left( V_f C_f - V_i C_i \right) / M \]

where \( Q \) is the BB3 uptake (mg/g), \( C_o \) and \( C_f \) the initial and equilibrium BB3 concentrations in the solution (mg/l), respectively, \( V_o \) and \( V_f \) the initial and final solution volume (l), respectively, and \( M \) the mass of biosorbent (g).

2.4. Effect of ionic strength

To study the effect of ionic strength during the biosorption of BB3 by C. glutamicum biomass, experiments were conducted with the addition of different concentrations of NaCl (0.0–0.3 mol/l) to the solution, which contained 40 ml of BB3, with an initial concentration of 100 mg/l, and 0.1 g of biomass. The pH of the solution was maintained at pH 6.0 using either 0.1 M HNO3 or 0.1 M NaOH; the remaining procedure was the same as that for the biosorption isotherm experiments.

2.5. FTIR analysis

The infrared spectra of the biomass samples were obtained using a Fourier transform infrared spectrometer (Specter GX, Perkin Elmer, USA). The samples adjusted to pH 10 were prepared as KBr pellets and recorded within the range of 4000–600 cm⁻¹.

3. Results and discussion

3.1. BB3 precipitation

The absorption spectra of BB3 (500 mg/l) solutions were measured under different pH conditions. The absorption peak, corresponding to the color of BB3 at \( \lambda_{max} = 654 \) nm, remained constant as the various pHs tested. Also, no new absorption bands appeared in the visible regions. The absorption spectra within the pH range 3–10 showed very similar patterns. However, the absorption spec-
trum at pH 2 was dramatically reduced. The absorbance was also decreased at pH 11 and 12. It appeared that the BB3 precipitated from solution under strong acidic (pH 2) and strong basic conditions (pH 11 and 12). As evidence of the precipitation of the dye at pH 2, 11 and 12, some solid mass was found at the bottom of dye solution after centrifugation. Therefore, sorption experiments with BB3 were only conducted within the pH range from 3 to 10.

3.2. FTIR analysis of raw and PAA-modified biomass

In order to confirm the enhancement of carboxyl groups in the surface of modified biomass, the FTIR spectra of the raw and PAA-modified biomass were analyzed and compared. The FTIR spectra of the two samples display a number of absorption peaks, indicating the complex nature of the examined biomass. According to our previous study (Won et al., 2004), the main functional groups of raw biomass are comprised of carboxyl (B—COO\(^-\)), phosphonate (B—HPO\(_4^2-\)) and amine (B—NH\(_2^+\)) sites. The broad and strong band, ranging from 3200 to 3600 cm\(^{-1}\), may be due to the overlapping of the N—H bond of the amino groups and the O—H bond of the hydroxyl groups (Pradhan et al., 2007; Li and Bai, 2005). The peaks at 1658, 1536 and 1233 cm\(^{-1}\) assigned to the bending of N—H bonds, stretching of H—N—C bonds and stretching of C—N bonds, respectively (Pavia et al., 2001). The phosphate groups showed characteristic absorption peaks around 1157 cm\(^{-1}\) (P=O stretching) and 1078 cm\(^{-1}\) (P—OH stretching) (Pagnanelli et al., 2000; Burnett et al., 2006). The spectrum showed obvious changes on the surface of the biomass after grafting between the biomass and poly(amic acid). The peak at 1242 cm\(^{-1}\) shifted from 1235 cm\(^{-1}\) associated with C—N stretching vibration and the relative intensity of this peak was observed to be higher than that of the raw biomass. Moreover, two characteristic peaks at 1583 and 1402 cm\(^{-1}\), which were attributed to C=O asymmetric and symmetric stretching in carboxylate ions were clearly shown and increased in FTIR spectrum of PAA-modified biomass. It may be due to the enhancement of carboxyl groups on the modified biomass. Therefore, these results indicated that poly(amic acid) was well grafted on the surface of the biomass.

3.3. Effect of pH

In the first series of experiments, the influence of the equilibrium pH on the biosorption of BB3 was examined (Fig. 1). The solution equilibrium pH was found to severely affect the BB3 biosorption capacity of the raw C. glutamicum, with maximum uptake obtained under alkaline pH conditions. The cell walls of Gram-positive bacterium are mainly comprised of a peptidoglycan layer connected by amino acid bridges. Imbedded in the Gram-positive cell wall are polyalcohols, known as teichoic acids, which give an overall negative charge to the bacterial cell wall, due to the presence of phosphodiester bonds between the teichoic acid monomers (Vijayaraghavan and Yun, 2008). The zero charge potential of the biomass was determined as 2.1 and; thus, the biomass will have a net negative charge above pH 2.1. Conversely, basic dyes will release colored positively charged dye ions when in solution, which will exhibit electrostatic attraction towards the negatively charged cell surface. In particular, the carboxyl groups present in C. glutamicum (Vijayaraghavan et al., 2008) were mainly responsible for the biosorption of BB3. The pK\(_a\) value of carboxylic groups usually lies within the range 3.8–5.0 (Schiewer and Voelsky, 2002). Therefore, the carboxyl groups have a negative charge at pHs approximately higher than five; therefore, will electrostatically bind BB3 to the bacterial biomass.

At low pH values, the carboxyl groups will be in their protonated form and; thus, the overall charge of the biomass will be positive. In this situation, the sorption of BB3 onto the carboxyl groups will be difficult and; hence, a low uptake was observed at acidic pH values (Fig. 1). Also, as the pH increases, the net biomass charge will become more negative and; hence, the biosorption of BB3 will increase.

PAA, from the reaction of PMDA and thiourea, is comprised of a large number of carboxyl and amide groups. Thus, via the grafting of PAA onto C. glutamicum, the biosorption of BB3 was increased (Fig. 1). It is interesting to note that the effect of pH was less pronounced in the case of the PAA-modified C. glutamicum, which may be due to the presence of a greater number of carboxyl groups as a result of the chemical treatment, i.e., more binding sites available compared to the initial BB3 concentration (92 mg/l) used.

3.4. Biosorption isotherms and modeling

The results in the previous section revealed that both the raw and PAA-modified biomass of C. glutamicum can be competent for the biosorption of BB3 at near to alkaline conditions; therefore, isotherm experiments were conducted at pH 6 and 9 to elucidate the complete biosorption potential of the biomass. Typical biosorption isotherms were observed for both forms of C. glutamicum (Fig. 2). The isotherms obtained for the BB3-raw biomass were close to “C-isotherms”, which means that the ratio between the concentration of BB3 remaining in solution and that sorbed on the biosorbent was almost the same at any concentration (Limousin et al., 2007). Conversely, the isotherms originating from the PAA-modified biomass and BB3 were “H-isotherms” i.e., the ratio between the BB3 concentration remaining in solution and that sorbed on the solid decreases with increasing BB3 concentration, resulting a concave curve with a strict plateau. The isotherms were steep, indicating the high affinity of the sorbate towards the sorbent. Also, the PAA-modified C. glutamicum exhibited very high BB3 uptakes, approximately 3.3 times higher than the raw biomass at pH 9.

Modeling of the BB3 isotherm data was attempted using the Langmuir, Freundlich and Toth models, which can be represented as follows:

Langmuir model: \[ Q = \frac{Q_{\text{max}} b_i C_f}{1 + b_i C_f} \]  
(2)

Freundlich model: \[ Q = K_f C_f^{1/n} \]  
(3)
A high PAA-modified biomass exhibited high and sorbate. On comparing the two forms of sorbents generally have high isotherm, indicating the desirable high affinity. Thus, for good bio-

The Langmuir isotherm is one of the models most commonly used to describe the sorption of a solute onto solid sorbents, and was derived based on the assumptions of the presence of a finite number of binding sites homogeneously distributed over the sorbent surface; thus, presenting the same affinity for sorption of a single layer. Also, the Langmuir model serves to estimate the maximum dye uptake values, where these can not be found experimentally. The constant $b_L$ represents the affinity between the sorbent and sorbate. On comparing the two forms of *C. glutamicum*, the PAA-modified biomass exhibited high $Q_{\text{max}}$ and $b_L$ values (Table 1). A high $b_L$ value is reflected in the steep initial slope of a sorption isotherm, indicating the desirable high affinity. Thus, for good biosorbents generally have high $Q_{\text{max}}$ values and a steep initial isotherm slope (i.e., high $b_L$).

The Freundlich isotherm, originally empirical in nature, was later interpreted as the sorption to heterogeneous surfaces or to surfaces supporting sites of varying affinities. It is assumed that the stronger binding sites are initially occupied, and that the binding strength then decreases with increasing degree of site occupation. For the PAA-modified biomass, both the $K_F$ and $n$ values reached their corresponding maxima, which imply that the binding capacity reaches its highest value; the affinity between the biomass and BB3 was also higher than that of the raw biomass (Table 1).

The Toth isotherm, derived from potential theory, has proved a useful model for describing the sorption in heterogeneous systems, such as phenolic compounds onto carbon. It assumes an asymmetrical quasi-Gaussian energy distribution, with a widened left-hand side, i.e., most sites have sorption energy less than the mean value (Ho et al., 2002). As expected, the Toth model described the isotherm data well, with high $R^2$ and low % error values (Table 1). The successful application of the Toth model to the present data supports that the surfaces of the biosorbent were heterogeneous and contained different functional groups.

### 3.5. Biosorption kinetics

For practical applications, the process design and operation control, the sorption kinetics is very important. Sorption kinetics in wastewater treatment is significant, as it provides valuable insights into the reaction pathways and the mechanism of the sorption reactions. Also, the kinetics describes the solute uptake, which in turn controls the residence time of sorbate uptake at the solid-solution interface (Ho et al., 2000). Fig. 3 shows the BB3 uptake by the raw and PAA-modified biomass as a function of time. For both raw and PAA-modified biomass, the complete biosorption equilibrium was attained within 10 min. The rapid kinetics observed with different forms of *C. glutamicum* biomass represents a significant advantage for its application in wastewater treatment systems and implies that the material would also be suitable for continuous flow systems.

The experimental biosorption kinetic data was modeled by pseudo-first-order kinetic, which is represented in their non-linear forms by the following equation:

$$q_t = q_1 (1 - \exp(-k_1 t))$$

where $q_t$ is the amount of solute sorbed at equilibrium (mg/g), $q_1$ is the amount of solute sorbed at any time, $t$ (min), $k_1$ is the pseudo-first-order rate constant (l/min). The pseudo-first-order ki-

### Table 1

<table>
<thead>
<tr>
<th>Isotherm models</th>
<th>Raw biomass</th>
<th>PAA-modified biomass</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>pH 6</td>
<td>pH 9</td>
</tr>
<tr>
<td>Langmuir</td>
<td>$Q_{\text{max}}$ (mg/g)</td>
<td>29.2</td>
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<tr>
<td></td>
<td>$b_L$ (l/mg)</td>
<td>0.012</td>
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<tr>
<td></td>
<td>$R^2$</td>
<td>0.917</td>
</tr>
<tr>
<td>Freundlich</td>
<td>$Q_{\text{max}}$ (mg/g)</td>
<td>36.2</td>
</tr>
<tr>
<td></td>
<td>$b_L$ (l/mg)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td>0.636</td>
</tr>
<tr>
<td>Toth</td>
<td>$Q_{\text{max}}$ (mg/g)</td>
<td>63.04</td>
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</table>
Table 2

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<th>Sorbent</th>
<th>(q_0) (mg/g)</th>
<th>(q_t) (mg/g)</th>
<th>(k_1) (l/min)</th>
<th>(R^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw biomass</td>
<td>53.6</td>
<td>53.8 (0.3)</td>
<td>3.63 (0.72)</td>
<td>0.996</td>
</tr>
<tr>
<td>PAA-modified biomass</td>
<td>105.5</td>
<td>106.0 (0.5)</td>
<td>0.88 (0.03)</td>
<td>0.998</td>
</tr>
</tbody>
</table>

Fig. 4. Effect of the salt concentration on the uptake of BB3 by the raw and PAA-modified C. glutamicum biomasses.

Table 3

<table>
<thead>
<tr>
<th>Salt concentration (mol/l)</th>
<th>BB3 uptake (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>Raw biomass</td>
</tr>
<tr>
<td>0.05</td>
<td>0.10</td>
</tr>
</tbody>
</table>

3.6. Effect of ionic strength

The increased ionic strength of the medium strongly affected the uptakes of BB3 for both forms of C. glutamicum (Fig. 4). This effect may be ascribed to the competition between ions, changes in the dye activity, or in the properties of the electrical double layer. When two phases, e.g. biomass surface and solute in aqueous solution, are in contact, they will inevitably be surrounded by an electrical double layer due to electrostatic interactions. Since the biosorption mechanism for BB3 is significantly via electrostatic attraction, the adsorption decreases with increasing ionic strength (Dönmez and Aksu, 2002).

4. Conclusions

The chemical modification of C. glutamicum by poly(amic acid) leads to significant enhancement in the uptake of BB3. The mechanism responsible for the biosorption of basic dye onto bacterial surfaces involves the electrostatic attraction of the dye cations towards the negatively charged carboxyl groups; while the grafting of poly(amic acid) enhanced carboxyl groups, which in turn increased the biosorption of BB3. The solution pH plays an important role, with maximum biosorption obtained under alkaline conditions. Under optimal pH conditions, i.e., pH 9, according to the Langmuir model, the maximum uptake of BB3 by the PAA-modified C. glutamicum was 173.6 mg/g; while that of the raw biomass was 52.8 mg/g. For both the raw and chemically modified biomasses, the biosorption kinetics was found to be fast, with equilibrium attained within 10 min. With a 3.3-fold enhancement compared to that of the raw biomass, this study has illustrated that poly(amic acid) is a good chemical agent for the enhanced biosorption of a basic dye by bacterial biosorbents.

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