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An Aminated Bacterial Biosorbent Capable of Effectively Binding Negatively Charged Pollutants in Aqueous Solution

Sung Wook Won¹, K. Vijayaraghavan¹, Juan Mao² and Yeoung-Sang Yun¹,²* (1) Division of Environmental and Chemical Engineering and Research Institute of Industrial Technology, Chonbuk National University, Jeonbuk 561-756, Korea. (2) Department of Bioprocess Engineering, Chonbuk National University, Jeonbuk 561-756, Korea.

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ABSTRACT: The main aim of this work was to enhance the biosorption capacity of Corynebacterium glutamicum for the remediation of wastewaters containing Reactive dyes. Amine groups were found to be responsible for accommodating negatively charged Reactive Red 4 (RR4) molecules via electrostatic interaction. Thus, increasing the number of amine groups on C. glutamicum, via amination, resulted in an enhanced RR4 biosorption capacity. The pH-edge experiments revealed that acidic conditions (pH = 2) favoured the biosorption of RR4 molecules. Isotherm experiments indicated that the aminated C. glutamicum exhibited the highest RR4 uptake, i.e. 133.8 mg/g at pH 2, compared to 96.8 mg/g for raw C. glutamicum. Of the two isotherm models considered, the Toth model provided a better description of the experimental isotherms, with high correlation coefficients and low percentage error values. Kinetic experiments revealed the importance of the initial dye concentration, with equilibrium being rapidly attained after ca. 1 h for all the concentrations examined. The non-linear form of the pseudo-second-order model described the biosorption kinetic data, with high correlation coefficients and low percentage error values compared to the pseudo-first-order model. Desorption was successful achieved at pH 10, with > 90.2% elution efficiencies for both the raw and aminated biomasses.

INTRODUCTION

The fermentation industry produces enormous quantities of microbial biomass as waste by-products, which present a considerable environmental menace. Several approaches are currently used for the disposal of biomass wastes, including land-fill and incineration (Puranik and Paknikar 1997). However, possible utilization of these wastes could possibly provide a more appropriate solution to this disposal problem, as this would enhance the value of the waste as well as benefiting local communities. The amino-acid fermentation industry, which faces severe problems in the handling of generated wastes, makes widespread use of Corynebacterium glutamicum — a gram-positive bacterium — in the biotechnological production of amino acids. Currently, the annual production of amino acids from fermentation processes using C. glutamicum amounts to 1 500 000 and 550 000 tonnes of L-glutamate and L-lysine, respectively (Hermann 2003). Hence, considerable amounts of C. glutamicum waste are generated after fermentation and, correspondingly, there is considerable interest in the potential utilization of this waste.

*Author to whom all correspondence should be addressed. E-mail: ysyun@chonbuk.ac.kr.
As fermentation wastes are capable of binding several heavy metal ions, dyes and other organic substances (Tsezos 2001; Vegliò and Beolchini 1997; Wang and Chen 2006), they offer potential as biosorbents. A number of these waste biomasses, such as Saccharomyces cerevisiae (Wang and Chen 2006), Aspergillus niger (Kapoor and Viraraghavan 1997) and Streptoverticillium (Puranik and Paknikar 1997), are known to act as biosorbents of heavy metal ions. However, little work has been conducted on employing these fermentation wastes for the removal of dyes from aqueous solutions.

Interest in increasing the sorption capacity of biomasses has escalated in recent years (Deng and Ting 2005; Yu et al. 2007). Several biomasses, regarded as wastes by the producing industries, possess low biosorption capacities. Since sorption mainly takes place on the biomass surface, increasing/activating the binding sites on the surface could provide an effective approach for enhancing the biosorption capacity. Carboxyl, amine, phosphonate, sulphonate and hydroxyl are well established as being the functional groups responsible for binding dyes (Fu and Viraraghavan 2001; Aksu 2005; Vijayaraghavan and Yun 2007a). In general, negatively charged functional groups can attract positively charged dye ions, and vice versa. However, a biosorbent will exhibit a low sorption capacity if the density of the binding groups is low. An ideal way of overcoming this problem is to convert the less important functional groups on the surface into active binding groups, a process which can be achieved via several chemical treatment methods. Thus, Li et al. (2007) employed citric acid to modify an alkali-saponified biomass, resulting in an increased total number of acidic sites but a decreased number of basic sites. In particular, biomass modified by treatment with 0.6 mol/ℓ citric acid at 80°C for 2 h was reported to exhibit a Cd²⁺ ion uptake capacity which was twice that of the raw biomass.

Thus, the focus of the present work was on increasing the number of amine groups on C. glutamicum via its reaction with ethanolamine. Reactive Red 4 (RR4) was used as the model solute for comparing the performances of aminated and raw C. glutamicum biomasses.

MATERIALS AND METHODS

Biomass and chemical modification

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

The aminated biomass was prepared employing a previously reported method (Brady and Duncan 1994). To facilitate amination of the biomass, 5 g of the raw biomass was mixed with 100 ml of ethanolamine and 10.42 g of hydrochloric acid (HCl) as an acidic catalysis, with the reaction mixture being shaken on a rotary shaker for 6 h at 150 rpm. This treatment was expected to result in the amination of the carboxy groups via the general reaction:

\[
\text{Biomass–COOH} + \text{NH}_2\text{CH}_2\text{CH}_2\text{OH} \xrightarrow{H^+} \text{Biomass–COOCH}_2\text{CH}_2\text{NH}_2 + \text{H}_2\text{O}
\]

The chemically treated biomass, designated as the aminated biomass, was washed three times with de-ionized water and then dried in an oven at 60°C for 24 h. The resultant dried aminated C. glutamicum biomass was stored in a desiccator, and subsequently used as the biosorbent in the sorption experiments.
Biosorption experiments

Such experiments were conducted by allowing 0.1 g of biomass to come into contact with 40 ml of the dye solution, contained at the desired pH in 50 ml plastic bottles (high-density polyethylene). The resulting mixtures were then maintained on an incubated rotary shaker at 160 rpm and 25 ± 1°C. The pH of the solution was initially adjusted using either 0.1 M HNO₃ or 0.1 M NaOH; these solutions were subsequently used to control the pH during the experiments. After 24 h contact with the dye solution, the biosorbent was separated by centrifugation at 3000 rpm for 5 min. The concentration of the dye (RR4) in the supernatant was determined, after appropriate dilution, via a UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan) at 517 nm. Kinetic experiments were also conducted as above, except that the samples were collected at different time intervals to determine the attainment of biosorption equilibrium.

The amount of RR4 sorbed by the biosorbent was calculated from the difference between the concentration of RR4 initially added and that in the supernatant, using the following equation:

\[ Q = \frac{V(C_0 - C_f)}{M} \]  

where \( Q \) corresponds to the RR4 uptake (mg/g), \( C_0 \) and \( C_f \) are the initial and equilibrium concentrations of RR4 in the solution (mg/l), respectively, \( V \) is the solution volume (l) and \( M \) is the mass of biosorbent employed (g).

Desorption experiments

The RR4-loaded biomass, exposed to 300 mg/l RR4 at a pH value of 2 and a temperature of 25 ± 1°C, was centrifuged at 3000 rpm and the supernatant removed. The dye-loaded biosorbent was then brought into contact with 40 ml of de-ionized water and the pH of the resulting suspension adjusted to a value of 10. This suspension was agitated for 3 h on a rotary shaker at 160 rpm. The remaining procedure was the same as that employed in the biosorption equilibrium experiments mentioned above.

RESULTS AND DISCUSSION

pH-edge experiments

The data associated with the equilibrium sorption of RR4 as a function of pH (see Figure 1) clearly show that the pH value of the solution played an important role in the biosorption of RR4 by C. glutamicum biomass. Initial experiments conducted with raw C. glutamicum showed that the maximum uptake of RR4 occurred at a pH value of 2. Increasing the pH above this value led to a decrease in the dye uptake. The cell walls of Gram-positive bacteria are mainly comprised of a peptidoglycan layer connected by amino-acid bridges (Mera et al. 1992), with polyalcohols known as teichoic aids imbedded therein. Due to the presence of phosphodiester bonds between the teichoic acid monomers, an overall negative charge is imparted to the bacterial cell walls (Beveridge et al. 1982). The cell wall of C. glutamicum is known to be comprised of carboxy, phosphonate and amine groups (Won et al. 2004; Vijayaraghavan and Yun 2007b). Due to protonation of the functional groups, such as amino groups within the pKᵌ range between 8 and 11, the biomass will exhibit a net positive charge under acidic conditions. Conversely, Reactive dyes
release negatively charged coloured dye ions into solution and these are attracted electrostatically towards the positively charged cell-wall surfaces. In particular, the amine groups present on \(C.\) glutamicum have been shown 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 2007b). In the present work, the number of amine groups was increased via amination of the biomass in an attempt to enhance biosorption; the aminated \(C.\) glutamicum thus prepared performed well and exhibited an increased RR4 biosorption capacity.

Biosorption isotherms and modelling

The results outlined in the previous section indicate that the raw and aminated \(C.\) glutamicum biomasses were competent in the biosorption of RR4 under acidic conditions. Hence, isotherm experiments were conducted at pH values of 2 and 5 in order to examine the complete biosorption potential of the biomass. Typical H-shaped sorption isotherms (Limousin et al. 2007) were observed for both forms of \(C.\) glutamicum (Figure 2), i.e. the ratio between the RR4 concentration remaining in solution and that sorbed onto the solid decreased with increasing RR4 concentration, resulting in a convex curve with a well-defined plateau.

To evaluate the biosorption isotherms, two models were used in this study, viz. the Langmuir and Toth models, which can be represented as follows:

Langmuir model:

\[
Q = \frac{Q_{\text{max}} b C_f}{1 + b C_f} \tag{2}
\]

Toth model:

\[
Q = \frac{Q_{\text{max}} b_T C_f}{\left[ 1 + (b_T C_f)^{1/n_T} \right]^{n_T}} \tag{3}
\]

where \(Q_{\text{max}}\) is the maximum dye uptake (mg/g), \(b\) the Langmuir equilibrium constant (\(\ell/\text{mg}\)), \(b_T\) the Toth model affinity constant and \(n_T\) the Toth model exponent. The main reason for the extended use of these isotherm models is that they incorporate easily interpretable constants. These constants, along with the correlation coefficients (\(r\)) and percentage error values obtained from the two isotherm models, are listed in Table 1.
The Langmuir sorption model was used to estimate the maximum dye uptake values. In this model, the constant $b$ represents the affinity between the sorbent and sorbate. It was found that the Langmuir model parameters were largely dependent on the solution pH, with maximum values being observed at pH values of 2. For favourable biosorption, a high value of $Q_{\text{max}}$ and steep initial isotherm slope (i.e. a high value of $b$) are desirable. Of the two biomass forms examined, the aminated $C. \text{glutamicum}$ performed well, giving high $Q_{\text{max}}$ and $b$ values. It was found that the sorption capacity of the aminated biomass was ca. 1.4-times greater than that of the raw biomass.

To improve the fit of the biosorption isotherm data, a three-parameter isotherm model, viz. the Toth model, was also used. The Toth isotherm, derived from potential theory, has proved a useful model for describing sorption in heterogeneous systems, e.g. the sorption of phenolic compounds onto carbon. It assumes an asymmetrical quasi-Gaussian energy distribution with a widened

### TABLE 1. Isotherm Constants for the Biosorption of RR4 by Raw and Aminated $C. \text{glutamicum}$ Biomasses

<table>
<thead>
<tr>
<th>Isotherm models</th>
<th>Raw biomass</th>
<th>Aminated biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 2</td>
<td>pH 5</td>
</tr>
<tr>
<td>Langmuir</td>
<td>$Q_{\text{max}}$ (mg/g)</td>
<td>96.8</td>
</tr>
<tr>
<td></td>
<td>$b$ (l/mg)</td>
<td>1.99</td>
</tr>
<tr>
<td></td>
<td>$r$</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>$\varepsilon$ (%)$^a$</td>
<td>0.28</td>
</tr>
<tr>
<td>Toth</td>
<td>$Q_{\text{max}}$ (mg/g)</td>
<td>95.8</td>
</tr>
<tr>
<td></td>
<td>$b_T$ (l/mg)</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>$n_T$</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td>$r$</td>
<td>0.998</td>
</tr>
<tr>
<td></td>
<td>$\varepsilon$ (%)$^a$</td>
<td>0.02</td>
</tr>
</tbody>
</table>

$^a$Error value.
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 $r$ and low percentage error values (Table 1). The successful application of the Toth model to the present data supports the view that the biosorbent surfaces were heterogeneous and contained different functional groups.

**Biosorption kinetics and modelling**

Figure 3 shows a typical set of results from kinetic experiments for the biosorption of RR4 onto C. glutamicum biomass at different initial concentrations (100–500 mg/ℓ). The initial dye concentration provides an important driving force for overcoming all mass-transfer resistances of the dye between the aqueous and solid phases; hence, the uptake of a dye increases with increasing initial dye concentration. In the present studies, for instance, when the initial RR4 concentration was increased from 100 to 500 mg/ℓ, the amount of dye biosorbed by the aminated C. glutamicum increased from 40 mg/g to 133.8 mg/g; however, the RR4 removal efficiency decreased from 100% to 67%. This arose because, at lower concentrations, the ratio of the initial number of moles of dye to the available surface area was low, with the subsequent fractional sorption becoming independent of the initial concentration. However, at higher concentrations, the available sorption sites become fewer in number compared to the number of moles of dye present and, hence, the percentage removal of RR4 becomes dependent on the initial dye concentration.

Indeed, it was observed that the attainment of equilibrium depended strongly on the initial concentration of dye. At low concentrations (100–200 mg/ℓ), ca. 10–20 min was sufficient to remove more than 90% of the dye molecules. Thereafter, the uptake rate decreased, with equilibrium finally being attained in ca. 15–40 min. The higher sorption rate during the initial period may have been due to the increased number of vacant sites initially available on the surface of the biosorbent, resulting in an increased concentration gradient between the sorbate in solution and that on the biosorbent surface. With increasing time, this concentration gradient was reduced due to the sorption of RR4 molecules onto the vacant sites, leading to a decrease in the sorption

![Figure 3](image-url). Biosorption kinetics for raw and aminated C. glutamicum biomasses. Data points correspond to the following samples: ◆, raw biomass (100 mg/ℓ); □, raw biomass (200 mg/ℓ); △, raw biomass (500 mg/ℓ); +, aminated biomass (100 mg/ℓ); ×, aminated biomass (200 mg/ℓ); ○, aminated biomass (500 mg/ℓ). Experimental conditions employed: temperature = 25 ± 1°C, agitation speed = 160 rpm. Curves predicted by the non-linear pseudo-second-order model.
rate during the later stages. Also, it is noteworthy that at low initial dye concentrations (100–200 mg/l), the performances of the raw and aminated biomasses were the same; however, at an initial RR4 concentration of 500 mg/l, the aminated biomass clearly outperformed the raw biomass. This was because the number of amine groups on the raw biomass made the latter more capable of adsorbing all the RR4 anions at low concentrations. Also, the attainment of equilibrium on the aminated biomass was delayed at low RR4 concentrations relative to the situation with the raw biomass. This may possibly have been due to the difficulty experienced by the RR4 anions in accessing the chemically introduced amine groups on the aminated biomass surface.

The experimental biosorption kinetic data were modelled using both the pseudo-first- and pseudo-second-order kinetic equations, which can be represented in their non-linear forms as follows:

\[ q_t = q_e [1 - \exp(-k_1 t)] \]  

\[ q_t = \frac{q_e^2 k_2 t}{1 + q_e k_2 t} \]

where \( q_e \) is the amount of dye sorbed at equilibrium (mg/g), \( q_t \) is the amount of dye sorbed at time \( t \) (mg/g), \( k_1 \) is the pseudo-first-order rate constant (l/min) and \( k_2 \) is the pseudo-second-order rate constant [g/(mg min)]. The rate constants, predicted equilibrium uptakes, corresponding correlation coefficients and percentage error values for all the tested concentrations were calculated and are summarized in Table 2.

With the pseudo-first-order model, the correlation coefficients were found to be greater than 0.961, with the calculated value of \( q_e \) being reasonably close to that found experimentally, thereby suggesting that the model was applicable for fitting the kinetic data obtained for the initial concentrations examined. The slight differences in the \( q_e \) values (Table 2) was due to a time lag, possibly as a result of a boundary layer or external resistance controlling the initial sorption process (McKay et al. 1999). In most cases described in the literature, the pseudo-first-order model does not provide a good fit to the kinetic data over the entire contact time range and, as

<table>
<thead>
<tr>
<th>TABLE 2. Kinetic Constants for the Biosorption of RR4 by Raw and Aminated C. glutamicum Biomasses</th>
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<tbody>
<tr>
<td>Kinetic models</td>
</tr>
<tr>
<td></td>
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<tr>
<td>Experimental ( q_e ) (mg/g)</td>
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<tr>
<td>Pseudo-first-order ( q_e ) (mg/g)</td>
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<tr>
<td>( k_1 ) (l/min)</td>
</tr>
<tr>
<td>( r )</td>
</tr>
<tr>
<td>( \varepsilon ) (%)a</td>
</tr>
<tr>
<td>Pseudo-second-order ( q_e ) (mg/g)</td>
</tr>
<tr>
<td>( k_2 ) [g/(mg min)]</td>
</tr>
<tr>
<td>( r )</td>
</tr>
<tr>
<td>( \varepsilon ) (%)a</td>
</tr>
</tbody>
</table>

*aError value.*
a result, the \( q_e \) values are generally under-estimated (Ho and McKay 1998; Reddad et al. 2002). Thus, good linearity of the Lagergren plot is no guarantee that the interactions follow first-order kinetics (Gupta and Bhattacharyya 2006). In contrast to the equilibrium uptake values, the values of the pseudo-first-order rate constant (\( k_1 \)) decreased with increasing initial dye concentration.

The pseudo-second-order model is based on the sorption capacity of the solid phase. In contrast to the pseudo-first-order model, it is capable of predicting the sorption behaviour over the entire range studied (McKay et al. 1999), giving consistently better results than those obtained with the pseudo-first-order model (cf. the corresponding \( r \) values listed in Table 2). In this case, the correlation coefficients were always greater than 0.992, and the predicted curves showed excellent agreement with the experimental data (see Figure 3). As expected, the pseudo-second-order rate constant (\( k_2 \)) decreased with increasing initial dye concentration.

**Desorption**

If a biosorption process is to be used as an alternative in wastewater treatment schemes, regeneration of the biosorbent is both crucial for maintaining low process costs and providing the possibility of recovering the dye molecules extracted from the liquid phase (Volesky 2001). The optimal eluent must be effective, non-damaging to the biomass, non-polluting and cheap (Kuyucak and Volesky 1989). In the present study, attempts have been made to effect the desorption of RR4 from the dye-loaded biomass of *C. glutamicum* at a pH value of 10, where the dye uptake was minimal (Figure 1). This is because under strongly basic (high pH) conditions, the number of negatively charged sites on the sorbent surface increases. These negatively charged sites favour desorption of dye anions due to electrostatic repulsion. Such desorption was effected successfully under the conditions described, with elution efficiencies greater than 90.2% for both the raw and aminated biomasses (Figure 4).

![Figure 4. Desorption of RR4 from the dye-loaded raw and aminated *C. glutamicum* biomasses. Experimental conditions employed: \( C_0 = 300 \text{ mg/l} \), pH = 10, temperature = 25 ± 1°C, agitation speed = 160 rpm.](image-url)
CONCLUSIONS

The following conclusions arise from the data obtained in the present study:

- The biomass of *C. glutamicum*, when aminated, was found to exhibit an enhanced biosorption capacity towards RR4.
- With the aid of pH-edge data, positively charged amine groups on the surface of the biosorbent were confirmed as being responsible for the binding of RR4 anions, with maximum biosorption being obtained under acidic conditions.
- The biosorption isotherms were modelled using the Langmuir and Toth models. According to the Langmuir model, at a pH value of 2, the aminated biomass exhibited an uptake of 133.8 mg RR4/g biomass compared to a value of 96.8 mg RR4/g biomass for the raw biomass. Based on the correlation coefficients and percentage error values, the Toth model provided a better representation of the RR4 biosorption isotherms under all conditions examined.
- Biosorption kinetic experiments showed that the rate of RR4 uptake was very fast, with equilibrium being attained within 1 h. The pseudo-second-order kinetic model was found to predict the experimental data with a high precision and good correlation for all concentrations examined.
- Desorption was achieved successfully at a pH value of 10, with elution efficiencies greater than 90.2%.
- Thus, amination of bacterial biomass provides an attractive option for enhancing its biosorption potential towards Reactive dyes.

ACKNOWLEDGEMENTS

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