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Removal of Basic Blue 3 from aqueous solution by *Corynebacterium glutamicum* biomass: Biosorption and precipitation mechanisms

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Abstract−The waste biomass generated from mono sodium glutamate fermentation process, *Corynebacterium glutamicum*, was evaluated as a biosorbent for the removal of Basic Blue 3 (BB 3), as a model cationic dye, from aqueous solution. A series of batch experiments to study pH edge, precipitation of dye, isotherms and kinetics were undertaken. The solution pH was found to be an important factor in biosorption of BB 3. With increasing the pH, the uptake of BB 3 increased, except at a pH below 2. At pH values below 2, the precipitation of BB 3 occurred rather than biosorption, which resulted in overestimation of the sorption performance. The sorption process could reach quickly to equilibrium after 1 min. The Langmuir and Freundlich models were used to fit the experimental data at different pH conditions. Between them, the Langmuir model described the experimental data very well with high correlation coefficients. Furthermore, *C. glutamicum* was easily eluted by shifting the solution pH, making repeated sorption/desorption cycle (up to 4 times) possible without significant performance decrease.

Key words: Biosorption, Basic Blue 3, pH Effect, Precipitation

INTRODUCTION

More than 100,000 commercially available synthetic dyestuffs are used extensively in the paper printing, leather tanning and textile industries [1], most of which are difficult to be decolorized due to their complex structure and synthetic origin. They are specifically designed to resist fading upon exposure to sweat, light, water and oxidizing agents; and as such are very stable and difficult to be degraded [2,3]. Projected reports are alarming that 10-20% of dyes in the textile sector are lost in residual liquors through incomplete exhaustion and washing operations [4]. Conventional dye treatment processes are currently able to remove only half of the dyes present in residual liquors, while the remaining ~400 t/day find their way into the environment, either dissolved or suspended in water [4].

Basic dyes are the brightest class of soluble dyes used in the textile industry [5]. Their tinctorial value is very high, with less than 1 ppm of the dye producing an obvious coloration. Therefore, the color-bearing effluents require a treatment that will remove the color/dye in an economical fashion and is prescribed concentration levels prior to their discharge into water bodies. Considering both discharge volume and effluent combustion, the wastewater from the textile industry is rated the most polluting among all industrial sectors [6]. Therefore, there is a definite need for a suitable and cost effective dye/color-removal technology that works under the above circumstances.

In general, there are five main methods for the reduction of color in textile effluent streams: adsorption, oxidation-ozonation, biological treatment, coagulation-flocculation and membrane processes. Currently, the adsorption process is one of the most effective and attractive processes for the treatment of these dye-bearing wastewaters, with the most commonly used agent being activated carbon, which has also been extensively studied for the removal of dyes [7-11]. However, the relatively high operating costs and problems with regeneration of the spent carbon hamper its large-scale application [12]. Therefore, a number of other non-conventional sorbents have been tried for the treatment of wastewaters. Natural materials, biosorbents and waste materials from industry and agriculture represent potentially more economical alternative sorbents [13], with many having been tested for the removal of dyes, e.g., apple pomace and wheat straw [14], coir pith [15], straw, corncobs or barley husks [16]. In addition, peat, steel plant slag, bentonite clay and fly ash have been used for the removal of color from wastewaters, and their effectiveness for dye adsorption compared with that achieved by using activated carbon [17].

The biomass of *Corynebacterium glutamicum* is generated in great quantities from the full-scale fermentation process for the production of mono sodium glutamate (MSG). MSG fermentation industries have been plagued with huge amounts of biological solid waste, which is mainly composed of the biomass of *C. glutamicum*. Although this fermentation byproduct is potentially recyclable, until now, most of it has been dumped at sea. In this study, *C. glutamicum* was selected as a potential biosorbent for the removal of basic dye (Basic Blue 3).

MATERIALS AND METHODS

1. Materials

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

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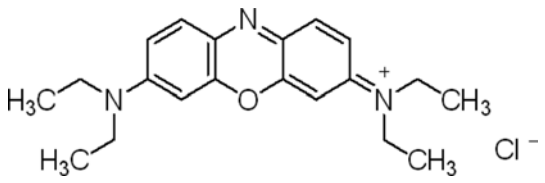

Fig. 1. The molecular structure of BB 3.

Table 1. Main characteristics of BB 3

Name	BB 3
Chemical formula	$C_{20}H_{26}ClN_3O$
Formula weight	359.89
Color index number	51104
λ_{max} (nm)	654

ments.

Basic Blue 3 (BB 3), a cationic dye, was purchased from Sigma-Aldrich Korea Ltd. and used as the adsorbate in the sorption experiments. As shown in Fig. 1, BB 3 is positively charged in aqueous solution. Table 1 summarizes the main characteristics of BB 3. All chemicals used in this study were of analytical grade.

2. Effect of pH

In the pH edge experiment, the final solution pH was adjusted to the desired value ranging from about 1-10 using 1 M HNO_3 or 1 M NaOH. The biomass, 0.4 g, was added to each 50 mL falcon tube, containing 40 mL BB 3 solution (250 mg/L), and agitated in a rotary shaker for 24 h at 160 rpm and 25±2 °C. After equilibrium, the final pH values of the working solutions were measured, with the samples then centrifuged, and the dye concentration remaining in the supernatant was analyzed by UV spectrophotometry (UVmini-1240, Shimadzu, Kyoto, Japan) at 654 nm, i.e., the maximum absorption peak.

3. Effect of Dye Precipitation on BB 3 Decolorization

In strong acidic condition, basic dyes have a tendency of precipitation. To evaluate the real sorption capacity of BB 3 by *C. glutamicum*, a series of control experiments were carried out with the pH ranging from 1-10 using 1 M HNO_3 and 1 M NaOH. 40 mL of a dye solution was added to a 50 mL falcon tube, without biomass, and was agitated in a rotary shaker for 24 h at 160 rpm and 25± 2 °C. The final pH of dye solutions was measured, and then centrifuged for solid-liquid separation. The supernatants were analyzed by using UV spectrophotometry, with the spectra measured in the ranging 800 to 400 nm.

4. Effect of Contact Time

The biosorption of BB 3 on *C. glutamicum* was conducted by agitating 0.4 g of biomass with dye solution (40 mL) in a 50 mL falcon tube at room temperature (25±2 °C) with a constant stirring speed of 160 rpm. Samples were then collected at different time intervals and analyzed for the dye concentration remaining.

5. Adsorption Isotherms

Isotherm studies were carried out by agitating a series of 50 mL falcon tubes containing 40 mL of BB 3 solution, with initial concentration ranging from 0-2,000 mg/L, with 0.4 g biomass at pH 6, 8 and 10, respectively, and at room temperature (25±2 °C) with a constant agitation speed of 160 rpm. After equilibrium was attained, the concentrations in the samples were analyzed as mentioned before.

6. Sorption/Desorption Experiments

To determine the reusability of the biomass, desorption experiments were conducted. The BB 3-loaded biomass was centrifuged at 3,000 rpm and then the supernatant removed. The settled biomass was subsequently resuspended with 30 mL of distilled water, and the pH of the suspensions adjusted to 3. The working solution was stirred at 160 rpm for 24 h at 25±2 °C to allow the dye molecules to be released from the biomass.

The desorption efficiency was calculated from the amount of dye adsorbed onto the biomass and the final dye concentration in the desorption medium. Therefore, the desorption efficiency was calculated from the following equation:

$$\text{Desorption efficiency } (\%) = \frac{\text{released BB 3}}{\text{initially sorbed BB 3}} \times 100\% \quad (1)$$

After desorption, the biomass was reused for subsequent sorption experiments. The sorption/desorption cycles were continued for four cycles to evaluate the feasibility of repeated reuse of the biosorbent.

The sorption efficiency of each cycle was calculated as a percentage of the uptake of the first sorption.

7. Adsorption Isotherm Models

The Langmuir [18] and Freundlich [19] isotherm models, which are widely used to analyze data for water and wastewater treatment application, have been shown to describe the biosorption equilibrium. These equations can be written in the form given below to evaluate the adsorption capacities of the biosorbent.

$$\text{Langmuir: } q_e = \frac{q_m b C_e}{1 + b C_e} \quad (2)$$

$$\text{Freundlich: } q_e = K_f C_e^{1/n} \quad (3)$$

For the Langmuir model (Eq. (2)), C_e is the equilibrium concentration (mg/L), q_e the amount of dye adsorbed at equilibrium (mg/g), and q_m and b the Langmuir constant related to the maximum sorption capacity (mg/g) and energy of adsorption (L/mg), respectively. K_f is the Freundlich constant (L/g), and n the Freundlich exponent. K_f and n are indicative of the extent of the adsorption and the degree of non-linearity between solution concentration and adsorption, respectively.

8. Dye Uptake Calculation

To consider the change in the working volume (up to 5%) due to the addition of HNO_3 and NaOH solutions, the uptake of BB 3 was calculated from the mass balance equation:

$$q = \frac{V_0 C_0 - V_f C_f}{M} \quad (4)$$

where C_0 and C_f (mg/L) are the initial and the final dye concentrations, respectively, V_0 and V_f (L) the initial and final (initial plus added HNO_3 or NaOH solutions) volumes, respectively, and M (g) the mass of dried biomass of *C. glutamicum*.

RESULTS AND DISCUSSTIONS

1. Effect of pH

The pH value of the solution is an important controlling param-

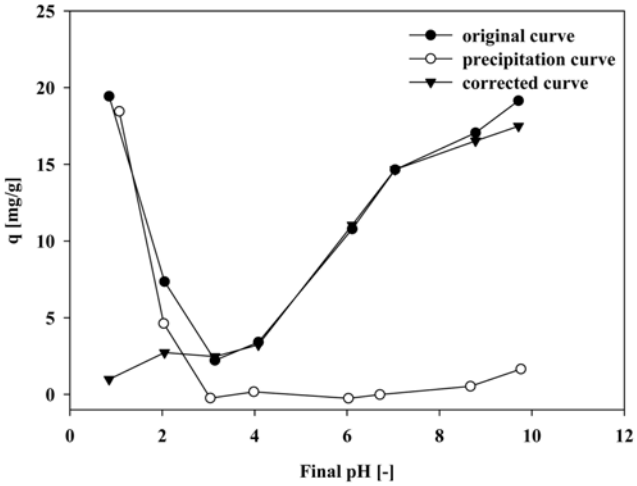

Fig. 2. The effect of pH on the biosorption of BB 3 at 25±2 °C. ● the original experimental curve. ○ the precipitation of BB 3. ▼ corrected experimental curve considering the effect of precipitation of BB 3.

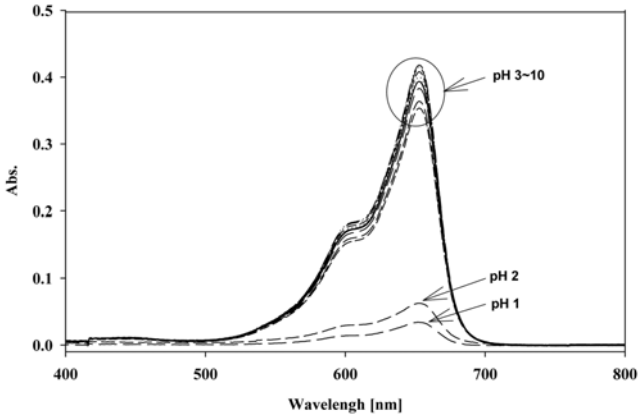

Fig. 3. UV-VIS adsorption spectra of BB 3 solution (250 mg/L) under different pH conditions and 25±2 °C.

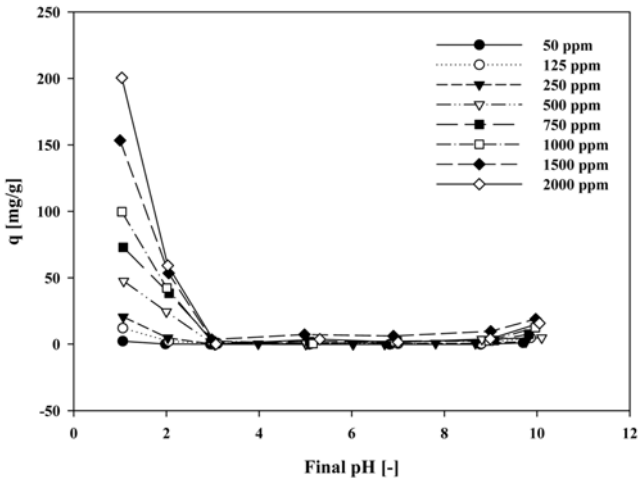

Fig. 4. The uptake by precipitation of BB 3 with different initial concentrations, ranging from 50-2,000 mg/L, at 25±2 °C.

eter in the adsorption process [20]. The effect of pH on BB 3 uptake was investigated over the pH range of 1 to 10. As shown in Fig. 2, the uptake of BB 3 decreased from pH 1 to 2, as well as increased with increases in the pH from 3 to 10. In our previous studies, the surface of the biomass of *C. glutamicum* was found to be three kinds of functional groups: carboxyl, phosphonate and amino group [21,22]. According to the result of potentiometric titration (not shown here), the pK_H values of carboxyl and phosphate groups were 4.9 and 6.8, respectively. When the pH value was higher than 4.9, the carboxyl group (-COOH) would exist in the form of carboxylate anion ($-COO^-$), which would be involved in electrostatic binding of the cationic dye molecules. Accordingly, the uptake of BB 3 was increased with increasing pH. When pH was more than 6.8, the phosphate group also became negatively charged ($-PO_4^-$), which resulted in the increases of BB 3 uptake. At pH 10, the uptake of BB 3 was maximal, with a value of 19.15 mg/g. While pH<4.9, the non-ionic form of carboxyl groups would exist. Indeed, according to the biosorption mechanism for basic dyes under these conditions, the sorption capacities of BB 3 were expected to be negligible. However, as shown in Fig. 2, at pH 1 and pH 2, the uptake of BB 3 reached 19.44 mg/g and 7.35 mg/g, respectively, both of which were higher than the uptake of BB 3 at pH 3. It was confirmed by replicate experiments that this phenomenon was not attributed to experimental error. Consulting the previous literature reported by [23], it was found that they just studied the pH effect from pH 2 to 5 for avoiding precipitation of Basic Red 5 and color change, without any correlative evidence shown in that literature. So further testing and discussion might be needed and carried out in the next section.

2. Effect of Precipitation on BB 3 Decolorization

To provide a reasonable explanation for the exceptional sorption points below pH 2, a series of UV-VIS absorption spectra of BB 3 solutions were obtained. All the spectra were measured in the range of 800 to 400 nm. Fig. 3 shows the spectra of BB 3 (250 mg/L) solutions without biomass under different pH conditions. The absorption peak, corresponding to the color of BB 3 at λ_{max}=654 nm, did not vary over the pH range, without new absorption bands appearing in the visible regions, which meant no ionic form of the dye changed and no new chemical form appeared. However, as indicated by the absorbance at 654 nm, more decolorization of BB 3 solution was accomplished by low pH (pH 1 and pH 2). Furthermore, some solids were observed visually at the bottom of microcentrifuge tubes after centrifugation of dye solutions controlled at pH 1 and 2. Hence, the formation of precipitation can be ascribed to the removal of BB 3 at pH 1 and 2, but not biosorption.

The removals of BB 3 by precipitation at different initial BB 3 concentrations are shown (Fig. 4). As shown in Fig. 4, the higher the concentration of BB 3, the more precipitation that occurred. And the precipitation only occurred at pH 1 and pH 2, while no precipitation was observed in dye solutions under other pH conditions.

Thus, a new pH edge curve was constructed considering the precipitation (Fig. 2). The pH edge by pure biosorption coincided with precipitation from the underlying mechanism (electrostatic interaction) which was discussed in the previous section Effect of pH.

Although the precipitation of BB 3 was significant at pH 1 and 2, from a practical perspective, the utilization of *C. glutamicum* biomass as a biosorbent for the removal of BB 3 does not cause any

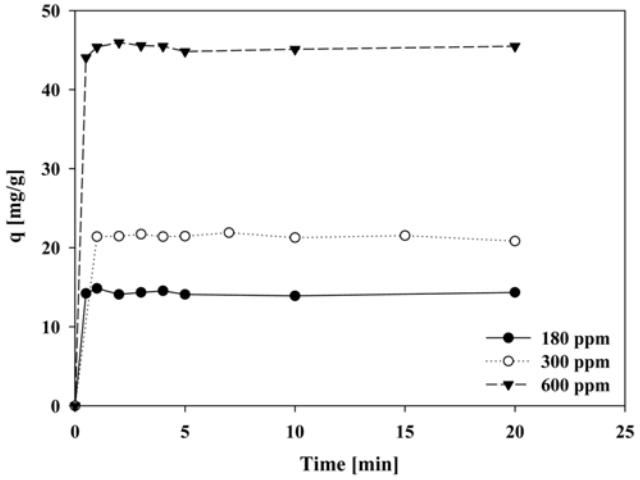

Fig. 5. The uptake of BB 3 onto *C. glutamicum* as a function of time for various initial dye concentrations at pH 7 and 25±2 °C.

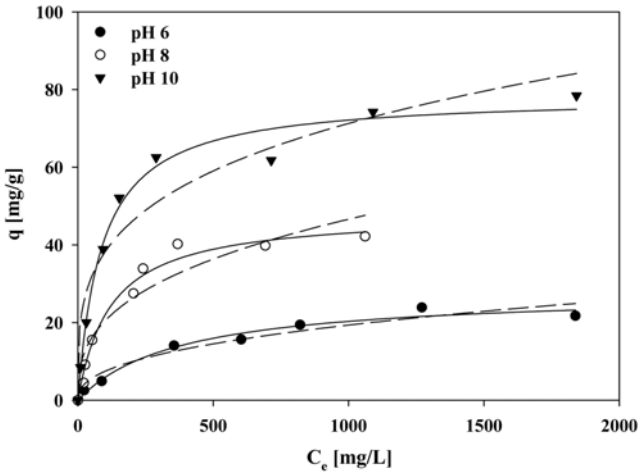

Fig. 6. The isotherms for the sorption of BB 3 onto the biomass of *C. glutamicum* at pH 6, 8 and 10, respectively. Solid represents Langmuir model, dashed symbolizes Freundlich model.

problems, because the sorption process is conducted under basic condition and the desorption at a mild acidic pH, i.e., pH 3.

3. Effect of Contact Time

Time needed for the treatment of wastewater dye is an important factor from the economical point of view. Fig. 5 shows that the BB 3 uptake by *C. glutamicum* as a function of time for different initial dye concentrations. As a result, the sorption process was found to be very rapid, and the equilibrium of sorption process could be reached within approximately 1 min. The uptake of BB 3 increased with the initial dye concentration because of increase in driving force [24,25]. Hence, it can be noted that a higher initial concentration of dye makes the adsorption process faster [26].

4. Adsorption Isotherms

An adsorption isotherm is a basic requirement for designing any sorption system, which expresses the relation between the mass of dye adsorbed at constant temperature per unit mass of the adsorbent and the liquid phase dye concentration. In order to optimize the use of an adsorption system to remove dye from solutions, it is important to establish the most appropriate correlation for the equilibrium curve.

Experimental BB 3 biosorption isotherms at different pH conditions are presented in Fig. 6. The uptake of BB 3 increased with increasing equilibrium concentration, and eventually reached to a certain saturated value depending on various pH values. To investigate the biosorption isotherms, Langmuir and Freundlich models were used in this study. And all of the model parameters were calculated by non-linear regression using the Sigma Plot (Version 8.02, SPSS, USA) software. The model parameters along with correlation coefficients (R^2) obtained from the two isotherm models are shown in Table 2.

Although the Langmuir model cannot provide any mechanistic understanding of the sorption phenomena, the Langmuir model may be conveniently used to estimate the maximum dye uptake from experimental data. As the pH increased from 6 to 10, both of q_m and b increased. The b value represents affinity between the sorbent and sorbate. High b value indicates that the biomass can adsorb BB 3 even at trace level, and high q_m value shows a desirable high capacity of dye binding [27]. Freundlich coefficient K_f reached its corresponding maximum value at pH 10 (Table 2), which indicated that the binding capacity had reached its highest value compared to other pH conditions investigated. From Table 2, it was noticed that the non-linear correlation coefficient R^2 values of Langmuir model were higher than that of Freundlich model, which means the Langmuir model can describe the data very well at all conditions examined.

On the basis of the Langmuir analysis, the maximum adsorption capacities were determined to be 27.95, 48.21 and 78.46 mg of BB 3 per gram of biomass at pH 6, 8 and 10, respectively, which was relatively high compared to other types of biosorbents, such as rice

Table 2. Isotherm constants of Langmuir and Freundlich models for BB 3 biosorption onto *C. glutamicum* at different pH conditions

Models	Parameters	pH		
		6	8	10
Langmuir	q_m (mg/g)	27.95 (2.21)	48.21 (2.48)	78.46 (2.74)
	b (L/mg)	0.0027 (0.0007)	0.0083 (0.0015)	0.0114 (0.0017)
	R^2	0.981	0.982	0.986
Freundlich	K_f (L/g)	1.105 (0.515)	3.911 (1.575)	10.909 (3.147)
	n	2.41 (0.39)	2.79 (0.51)	3.68 (0.60)
	R^2	0.952	0.913	0.930

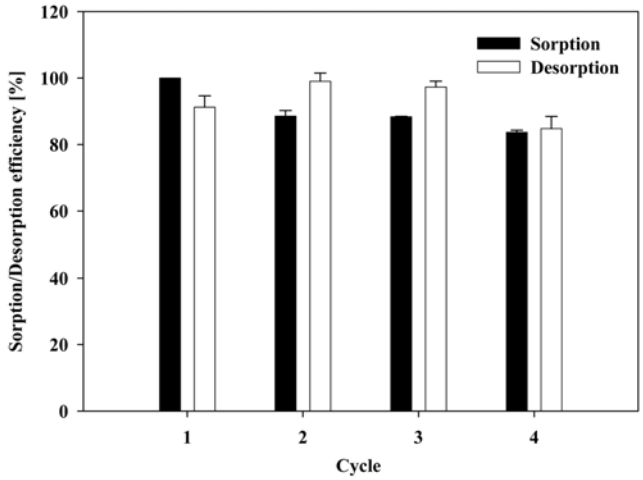

Fig. 7. Repeated reuse sorption/desorption cycles.

hull (14.68 mg/g) [28], maize starch/acrylonitrile (7.20 mg/g) [29] and kudzu (69.00 mg/g) [30].

5. Sorption and Desorption

The regeneration of the biosorbent is likely to be a key factor in assessing its potential for commercial application [30]. The dye was eluted from the dye-loaded biomass by adjusting the solution pH to 3, where the BB 3 uptake was minimal (Fig. 2).

Fig. 7 shows the results of repeated reuse experiments using the *C. glutamicum* biomass. The sorption/desorption experiments cycle were performed up to 4 times, with high sorption/desorption efficiencies (>84.67%). Besides, it is noted that the biomass can be regenerated easily in a simple method which is by only adjusting the solution pH. Considering the commercial sorbents such as activated carbons which are difficult to be regenerated [28], the *C. glutamicum* biomass has a great potential as a reusable dye biosorbent.

CONCLUSIONS

The present investigation showed that the pH value of the solution is an important controlling parameter in the adsorption process. The removal of BB 3 increased with increasing pH. However, the experimental data showed that the uptakes of BB 3 were higher at pH 1 and 2. It was ascribed to the precipitation of BB 3 under strongly acidic conditions, but not the biosorption. Control experiments without the biomass, under different pH conditions, confirmed the precipitation of BB 3 at pH below 2. Hence, the pH edge curve was corrected for BB 3 precipitation under acidic conditions. Langmuir and Freundlich models were employed to fit the experimental data, which were found to be well represented by the Langmuir isotherm model according to the high correlation coefficient. The maximum dye uptake values, q_m, were 27.95 mg/g, 48.21 mg/g and 78.46 mg/g at pH 6, 8 and 10, respectively, which was relatively high compared to other types of biosorbents. Furthermore, a swift biosorption process and facile regeneration make *C. glutamicum* show a great potential as a biosorbent in the future.

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