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Biosorptive removal of Reactive Yellow 2 using waste biomass from lysine fermentation process

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Abstract

The protonated waste biomass of *Corynebacterium glutamicum* discharged from an industrial lysine fermentation plant was used for the removal of Reactive Yellow 2 (RY2). To evaluate the biosorption capacity and characteristics, the effects of solution pH, dye concentration, and salts were investigated in a batch mode. Also, the influence of biomass leachate during biosorption process was specially focused. The optimum pH ranges for RY2 uptake was from 1 to 4, with the maximum sorption capacity of the biomass being as high as 178.5 ± 17.0 and 154.3 ± 14.7 mg g^{-1} at pH 1 and 2, respectively. As the solution pH increased, the dye uptake rapidly decreased, but was negligible under neutral conditions. At pH 7 and above, the biomass leaching was found to be an important factor in evaluating the biosorption performance. The biomass could be easily regenerated and successfully reused even up to the fourth cycle of sorption/desorption.

Keywords: Biosorption; Reactive Yellow 2; Isotherm; Desorption; Biomass leaching; *Corynebacterium glutamicum*

1. Introduction

The textile industry consumes large volumes of water and chemicals for the wet processing of textiles. The textile finishing industry has a high specific water demand of well over 100–500 L kg^{-1} of finished product [1,2]. Dyeing industry effluents constitute one of the most problematic wastewaters requiring treatment, not only for their high chemical and biological oxygen demands, suspended solids and content in toxic compounds, but also due to their colour, which is the first contaminant recognized by the human eye [3]. The presence of very low dye concentrations in the effluent discharged from these industries is highly visible and undesirable [4]. Due to their chemical structures, dyes are resistant to fading when exposed to light, water and many chemicals [5,6]. Dyes are usually of synthetic origin with complex aromatic molecular structures, which make them very stable and difficult to biodegrade. Brightly colored, water-soluble reactive and acid dyes are the most problematic, as they tend to pass through conventional treatment systems unaffected [7]. Municipal aerobic treatment systems, which depend on biological activity, have been found to be inefficient in the removal of these dyes [8].

Various physical, chemical and biological methods have been used for the treatment of dye-containing wastewater. Some chemical oxidations, using Fenton reagent, ozone, UV plus H_2O_2 or NaOCl result in aromatic ring cleavage, but may generate chemical sludge or byproducts that are more toxic [9]. Aerobic biological treatment is known to be ineffective for dye removal, but anaerobic bioremediation enables the decolorization of water-soluble dyes [10]. Physical biosorption technology, i.e. with the use of activated carbons, has recently gained favor due to its high efficiency in the removal of highly stable dyes, as well as being economically feasible compared to other methods [11]. However, activated carbons are expensive and not easily regenerated [9]. Although ion exchange resins can be easily regenerated, the high cost hinders their

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wide application in the treatment of dye-bearing wastewater. Consequently, low-cost sorbents, capable of binding dye molecules and be easily regenerated, have been extensively searched and tested [9].

Many sorbents have been tested for their dye removal ability, but most of them are non-regenerable throwaway products, such as bagasse pith, eucalyptus bark and the biomass of the brown seaweed, *Ecklonia*. Good sorption capacities of reactive dyes (60–420 mg g^{-1}) have been found for quaternized organic materials, such as cellulose, sugarcane bagasse, rice husk and coconut husk, but no successful regeneration has been reported for these also [12].

In this study, the waste biomass of *Corynebacterium glutamicum* was evaluated as a biosorbent for the treatment of an anionic reactive dye Reactive Yellow 2 (RY2) as a model pollutant. A great quantity of this biomass is generated from the full-scale fermentation process for the production of amino acids. Amino acid fermentation industries have been troubled with the production of huge amount of biological solid waste; mainly composed of the biomass of *C. glutamicum*. Although this fermentation byproduct is potentially recyclable, until now most of them have been dumped in sea. Therefore, the feasibility for the reuse of this solid waste warrants assessment as a biosorbent.

2. Materials and methods

2.1. Materials

The fermentation wastes (*C. glutamicum* biomass) were obtained, in dried powdered form, from a lysine fermentation industry (BASF-Korea, Kunsan, Korea). The protonated biomass was prepared by treating the raw biomass with a 1 M HNO_3 solution for 24 h; thereby, replacing the natural mix of ionic species with protons. The acid-treated biomass, designated as protonated biomass, was washed several times with deionized distilled water and then dried at 60 °C for 24 h in an oven. The resultant dried *C. glutamicum* biomass was stored in a desiccator prior to use as the biosorbent in the sorption experiments.

All chemicals used in this study were of analytical grade. The RY2 was purchased from Sigma–Aldrich Korea Ltd. (CI 18972, Yongin, Korea). As shown in Fig. 1, RY2 has three sulfonate groups, which have negative charges in aqueous solution. The general characteristics of RY2 are summarized in Table 1.

2.2. pH edge experiments

The pH edge experiments were carried out as follows: an equilibrium relationship was obtained between the dye uptake and final pH, which is helpful in understanding the pH dependence of biosorption [13]. The pH edge experiments were conducted with a 500 mg L^{-1} of initial RY2 concentration and 10 g L^{-1} of the biomass. The pH was intentionally altered by the addition of either 1 M NaOH or 1 M HNO_3 to the bottles. The suspension was agitated for over 24 h in a shaker at 160 rpm and at room temperature of 25 ± 2 °C. After reaching

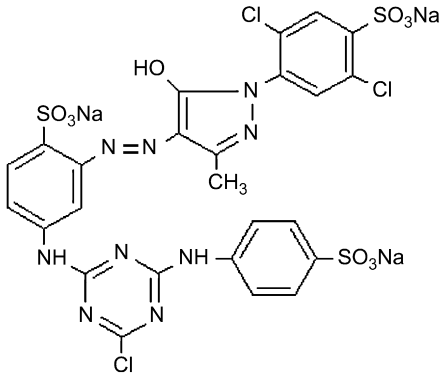

Fig. 1. Chemical structure of RY2.

equilibrium, the final pH of the system was measured, and then the samples were taken and centrifuged for liquid–solid separation. The supernatant portion, after an appropriate dilution, was used to analyze the residual RY2 concentration.

2.3. Biomass leaching experiments

To evaluate the extent of leaching and influence of leachate from the biomass, experiments were conducted with 10 g L^{-1} of the biomass within the pH range 2–12. The pH was intentionally altered by the stepwise addition of either 1 M NaOH or 1 M HNO_3 to the bottles. Other experimental conditions were the same as those used in the pH edge experiments. To characterize the leached component from the biomass, light absorption spectra of biomass leachate were measured within the range 700–380 nm.

2.4. Isotherm experiments

To evaluate the sorption capacity of the biomass, biosorption isotherms of RY2 were obtained at different solution pHs, with 0.4 g of the biomass in a volume of 40 mL working solution. The initial concentration was altered from 0 to 3000 mg L^{-1}, resulting in different final dye concentrations after the sorption equilibrium was achieved. Following the addition of the biomass into the dye-containing solutions, the solution pH was controlled at the desired value using 1 M HNO_3, as the pH tended to increase during binding of the RY2 to the biomass. All other conditions were the same as those used in the pH edge experiments.

2.5. Desorption and repeated reuse experiments

To evaluate the desorption efficiency, the RY2-loaded biomass was centrifuged at 3000 rpm and the supernatant was

Table 1
General characteristics of Reactive Yellow 2

Molecular formula	Molecular weight	Colour index number	Dye content (%)	λ_{max} (nm)
$C_{25}H_{15}Cl_3N_9Na_3O_{10}S_3$	873.0	18972	60–70	404

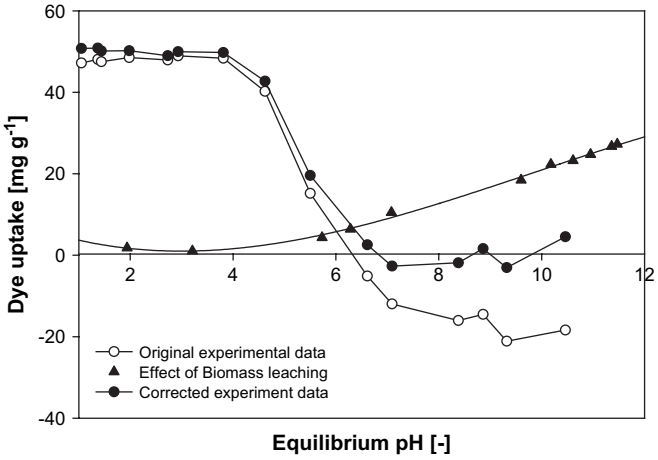

Fig. 2. The effect of pH on the biosorption of RY2. The open and closed circles represent the original data and corrected data with the biomass leaching effect, respectively. The triangles represent the mislead data caused by leachate from the biomass.

removed. Thereafter, the settled biomass was resuspended with 40 mL of deionized distilled water, and the pH of the suspension was adjusted to >7, where the uptake of RY2 was found to be negligible in the pH edge experiments. The suspension was shaken at 160 rpm for 24 h to allow the dye to be released from the biomass. Thereafter, the desorbed dye was analyzed and the desorption efficiency was calculated as follows:

$$\text{Desorption efficiency}(\%) = \frac{\text{released RY2(mg)}}{\text{initially sorbed RY2(mg)}} \times 100 \quad (1)$$

After desorption, the biomass was again reused for subsequent sorption experiments. The sorption/desorption cycle was performed up to eight times to evaluate the feasibility of repeated biosorbent reuse. The sorption efficiency of each cycle was calculated as a percentage of the uptake of the first sorption.

2.6. Measurements of dye uptake

The concentration of the dissolved dye samples was analyzed at 404 nm, using a spectrophotometer (UVmini-1240, Shimadzu, Kyoto, Japan), where the wavelength of the maximum absorption peak exists. Before analysis of the RY2 concentration, samples were centrifuged at 3000 rpm for solid–liquid separation. To compensate for the change of working volume (up to 5%) due to the addition of HNO_3 solution, the dye uptake (q) was calculated from the mass balance, as follows:

$$q = \frac{V_0 C_0 - V_f C_f}{M} \quad (2)$$

where V_0 and V_f are the initial and final (initial plus added acid solution) volumes, respectively. C_0 and C_f are the initial and final concentrations of RY2, respectively, and M is the weight of the biomass used.

2.7. Estimation of model parameters

The parameters of the Langmuir were obtained by fitting the model to experimental data using the Marquardt–Levenberg nonlinear regression algorithm [14]. The Sigma Plot (version 4.0, SPSS, USA) computer software was used for the nonlinear regression.

3. Results and discussion

3.1. pH edge

The pH of a dye solution plays an important role in the whole biosorption process, particularly in the biosorption capacity. The variation in the biosorption of RY2 was studied within the pH range 1–11, the results of which are shown in Fig. 2. The original experimental data are represented using an open circle. From this plot, it is clear that the optimum pH for the removal of RY2 is present within 1–4. As the pH increased, the uptakes of RY2 decreased. At elevated pH, the uptake showed minus values, which were a strange but reproducible result. To understand this extraordinary phenomenon, the following study on the effect of biomass leaching was carried out.

3.2. The extent and effect of leachate from the biomass

The extent and characteristics of leachate were evaluated to find a reason for the results shown in the pH edge experiments. As shown in Fig. 3, when the biomass was contacted with deionized water not containing any dyestuff, the absorption by the supernatant solution increased as the wavelength decreased. This indicated that certain constituents were released

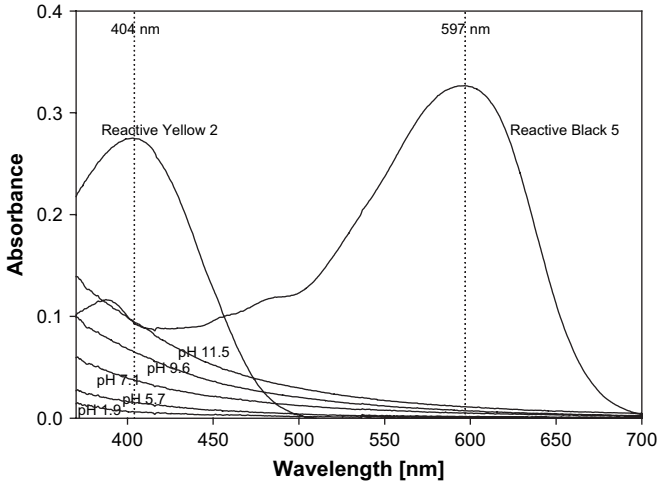

Fig. 3. Spectra of the biomass leachate at different pHs and of pure dye solutions.

from the biomass and the leachate can absorb well a low range of light. As the solution pH increased, the extent of biomass leaching became more significant.

As also can be seen in Fig. 3, the absorption by RY2 increased as the wavelength decreased and at 404 nm, the maximum peak was present. To analyze the concentration of RY2, the absorbance was measured at 404 nm. Accordingly, it was noted that the leachate from the biomass resulted in overestimation of RY2 concentration in samples especially under alkaline condition.

In order to take the leachate interference into account, the effect of leachate (dark triangle) is plotted in Fig. 2 together with pH edge data. Thereafter, the original pH edge data were corrected (dark circle). As shown in Fig. 2, the corrected pH edge indicates that the uptake of RY2 was negligible over pH 7.

To our knowledge, the biomass leaching effect has not been studied in previous literatures. It should here be noted that the leachate interference should be considered in the (bio)sorption research, especially for dyestuffs having the absorption peak at a low wavelength (λ_{max}). In the case of dyes having high λ_{max} like Reactive Black 5 (RB5), the effect of biomass leaching is not significant as shown in Fig. 3.

Because the uptake was preferential under acidic condition (Fig. 2), the sorption step should be operated at a low pH, where the biomass leaching effect is negligible. However, the desorption step can be highly affected by leachate and the biomass leaching may cause the loss of biosorbent. Therefore, to minimize the biomass leaching, the desorption should be carried out at around pH 7, where the biomass leaching is not significant. Therefore, it should be noted that the information on the biomass leaching can be useful for accurate evaluation of biosorption performance and also for establishment of operation strategies of biosorption/desorption process.

3.3. Biosorption isotherms

The uptake of RY2 increased with increasing equilibrium concentrations and eventually reached a certain saturated value (Fig. 4). Although the Langmuir equation cannot provide a mechanistic understanding of the sorption phenomena, it may be conveniently used to estimate the maximum uptake of dye from experimental data. The Langmuir biosorption isotherm [15] assumes that biosorption takes place at specific homogeneous sites within the adsorbent and has found successful application in many sorption processes of monolayer biosorption. The Langmuir isotherm can be written in the form:

$$q_e = \frac{q_m b C_e}{1 + b C_e} \tag{3}$$

where q_e is the adsorbed amount of the dye, C_e is the equilibrium concentration of the dye in solution, q_m is the monolayer biosorption capacity and b is a Langmuir constant related to the free energy of biosorption.

The Langmuir parameters were estimated using the nonlinear regression method [16], which are summarized in Table 2.

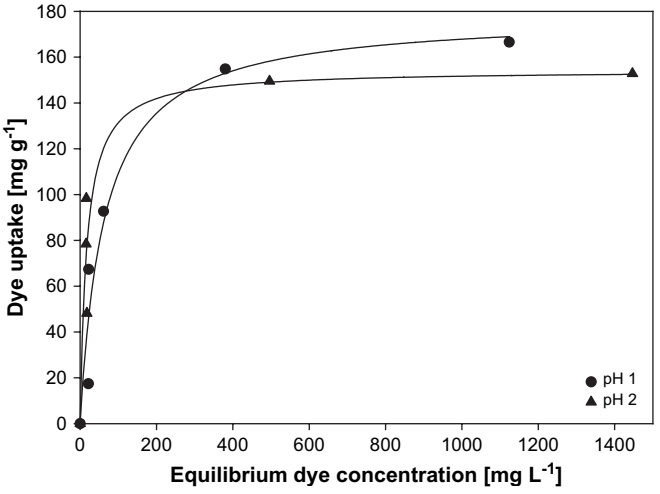

Fig. 4. Isotherms of RY2 for the protonated *C. glutamicum* biomass at pHs 1 and 2. The lines were produced using the Langmuir model. Experimental data: pH 1(●) and pH 2 (▲).

The maximum uptakes at pHs 1 and 2 were estimated to be 178.5 ± 17.0 and 154.3 ± 14.7 mg g^{-1}, respectively. Some kinds of bacteria showed their maximum biosorption capacities in the range of 52.4–124.3 mg g^{-1} for RY2 [17], and the dried activated sludge showed biosorption of 119.4 mg g^{-1} at pH 5 [18]. Activated carbons show large variations in dye uptake performance (50–560 mg g^{-1}) depending on the types of activated carbons and dyes [19]. Therefore, it can be noted that the biomass of *C. glutamicum* used in this experiment is superior to other biosorbents and is comparable to that of commercially available sorbents. In addition, the affinity (b) of the biomass toward RY2 molecules was as high as 0.016 and 0.057 L mg^{-1} at pHs 1 and 2, respectively, indicating that the sorbents can effectively bind with RY2, even at low concentration levels.

The shape of an isotherm can be used to predict whether a sorption system is 'favourable' or 'unfavourable', within fixed-bed systems as well as batch processes. The essential characteristics of the Langmuir isotherm can be expressed in terms of either a dimensionless constant separation factor or equilibrium parameter, R_L [20].

$$R_L = \frac{1}{1 + b C_0} \tag{4}$$

where R_L is a dimensionless separation factor, C_0 is the initial concentration (mg L^{-1}) and b is the Langmuir constant (L mg^{-1}). The parameter R_L indicates the shape of isotherm, as follows:

Value of R_L	Type of isotherm
$R_L > 1$	Unfavorable
$R_L = 1$	Linear
$0 < R_L < 1$	Favorable
$R_L = 0$	Irreversible

Table 2
Estimated parameters of the Langmuir model

Parameters	pH 1	pH 2
q_{max} (mg g^{-1})	178.5 (17.0)	154.3 (14.7)
b (L mg^{-1})	0.016 (0.005)	0.057 (0.020)
R^2	0.95	0.91

All values of R_L, calculated using Eq. (4) were found to be less than unity. Positive values of R_L less than 1 confirm the favorability of the sorption isotherm.

3.4. Effect of salt concentration

In general, reactive dyes are applied to fabric in high salt concentrations in order to lower the dye solubility [21]. NaCl is the main salt used to enhance the bath dye exhaustion. Therefore, unfixed dye in wastewater is accompanied by a large amount of salt, which likely interferes with dye biosorption. The effect of the salt concentration in synthetic wastewater on the uptake of RY2 was investigated (Fig. 5). At an initial RY2 concentration of 500 mg L^{-1}, the effect of the salt concentration on the dye uptake was negligible, which is similar to the result of a previously reported study on Reactive Orange 16 biosorption [22], reflecting that Cl^- ions do not compete with the sulfonate groups of the RY2 molecules for the amine sites of the biomass. In addition, it was noted that an elevated ionic strength due to NaCl does not significantly interfere with the binding of RY2 to the biomass. From a practical point of view, these results imply that the waste biomass of *C. glutamicum* can be used for the removal of RY2 from salt-containing wastewaters.

3.5. Repeated reuse

To be a good sorbent for dye removal, a dye-loaded sorbent should be able to be regenerated; otherwise disposal of the waste and fresh sorbents is required. In this study, after the protonated biomass of *C. gluatmium* had been used for RY2 sorption, it was eluted from the dye-loaded biomass by adjusting the pH of the solution to >7, where the dye uptake was minimal and the biomass leaching is not significant (Fig. 2). Fig. 6(A) shows the results of the repeated reuse experiments. Although the sorption/desorption cycle continued for up to four times, the sorption and desorption efficiencies remained almost constant. However, the desorption efficiency was much higher than for the sorption. Because the reuse experiments were conducted at pHs > 7, the main reason for abnormal increase in the desorption efficiency is because of the effect of biomass leaching described previously.

With respect to the biomass leaching, the desorption efficiency was recalculated and the corrected efficiencies are shown in Fig. 6(B). As a result, the sorption/desorption efficiencies of RY2 were at satisfactory level up to four repeated cycles. This indicates that the biomass leaching did not cause the significant loss of biomass for four cycles. However, for

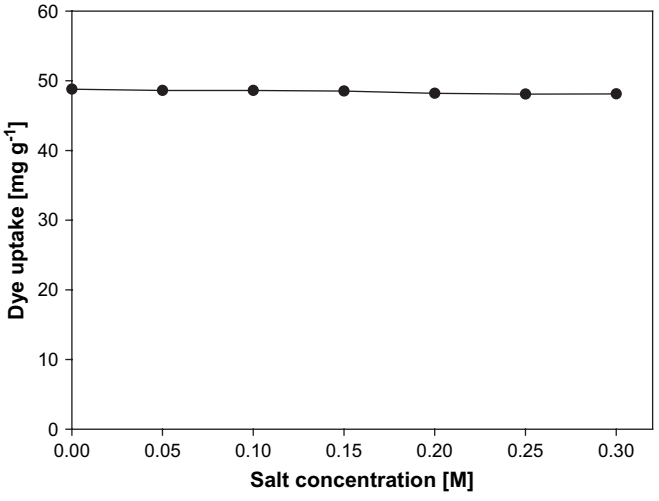

Fig. 5. Effect of the salt concentration on the uptake of RY2. The solution pH was controlled at pH 2 and the initial RY2 concentration was 500 mg L^{-1}.

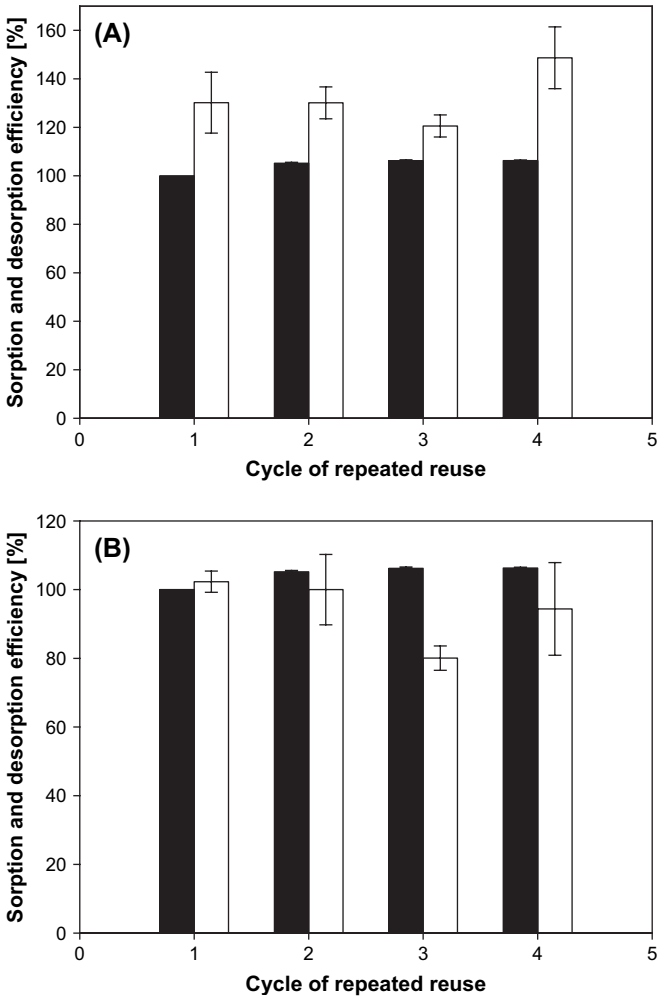

Fig. 6. Efficiencies of sorption and desorption during the repeated reuse experiments. The sorption was carried out at pH 2 and desorption at pH > 7. The black and white bars represent the sorption and desorption efficiencies, respectively. (A) The sorption/desorption efficiencies neglecting the biomass leaching effect. (B) The sorption/desorption efficiencies compensating for the biomass leaching effect.

more stable operation of biosorption process, the desorption should be carried out at around pH 7, where the biomass loss is negligible.

Considering that commercial sorbents, such as activated carbons, are hardly regenerated, this waste biomass has great potential as a regenerable dye sorbent. Moreover, it should be noted that the biomass can be easily regenerated by adjusting the solution pH to neutral conditions. With other types of biomass, desorption efficiencies range from 30 to 80%, although relatively expensive chemicals, such as methanol, ethanol and organic surfactants (i.e., Tween) are used for their regeneration.

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