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Journal of Hazardous Materials 141 (2007) 45–52

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Utilization of fermentation waste (*Corynebacterium glutamicum*) for biosorption of Reactive Black 5 from aqueous solution

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Received 11 March 2006; received in revised form 20 June 2006; accepted 21 June 2006
Available online 27 June 2006

Abstract

A fermentation waste, *Corynebacterium glutamicum*, was successfully employed as a biosorbent for Reactive Black 5 (RB5) from aqueous solution. This paper initially studied the effect of pretreatment on the biosorption capacity of *C. glutamicum* toward RB5, using several chemical agents, such as HCl, H_2SO_4, HNO_3, NaOH, Na_2CO_3, $CaCl_2$ and NaCl. Among these reagents, 0.1 M HNO_3 gave the maximum enhancement of the RB5 uptake, exhibiting 195 mg/g at pH 1 with an initial RB5 concentration of 500 mg/l. The solution pH and temperature were found to affect the biosorption capacity, and the biosorption isotherms derived at different pHs and temperatures revealed that a low pH (pH 1) and high temperature (35 °C) favored biosorption. The biosorption isotherm was well represented using three-parameter models (Redlich–Peterson and Sips) compared to two-parameter models (Langmuir and Freundlich models). As a result, high correlation coefficients and low average percentage error values were observed for three-parameter models. A maximum RB5 uptake of 419 mg/g was obtained at pH 1 and a temperature of 35 °C, according to the Langmuir model. The kinetics of the biosorption process with different initial concentrations (500–2000 mg/l) was also monitored, and the data were analyzed using pseudo-first and pseudo-second order models, with the latter describing the data well. Various thermodynamic parameters, such as ΔG°, ΔH° and ΔS°, were calculated, indicating that the present system was a spontaneous and endothermic process. The use of a 0.1 M NaOH solution successfully desorbed almost all the dye molecules from dye-loaded *C. glutamicum* biomass at different solid-to-liquid ratios examined.

Keywords: Biosorption; *Corynebacterium glutamicum*; Isotherm; Kinetics; Reactive dyes; Wastewater treatment

1. Introduction

Dyes are used extensively in industries including textiles, paper and leather. The effluents emanating from these industries are often highly colored, and the disposal of their wastes into the environment can be extremely undesirable. Once in the environment, they may show toxic and genotoxic effects toward organisms [1]. Dyes are usually of synthetic origin, with complex aromatic molecular structures, making them very stable and difficult to biodegrade [2], particularly reactive dyes. They differ from all other dye classes in that they bind to textile fibres, such as cotton, through covalent bonds [3]. Reactive dyes are typically azo-based chromophores combined with different types of reactive groups. They are extensively used in many textile industries because of their favorable characteristics such as bright color, water-fast and simple application techniques [2]. However, nearly 50% of reactive dyes may be lost in the effluent after the dyeing of cellulose fibres, and are highly recalcitrant to conventional wastewater treatment processes [4]. In fact, as much as 90% of reactive dyes can potentially remain unaffected after activated sludge treatment [5]. Therefore, alternative methods need to be implemented for effective pollution abatement of dye-containing effluents.

Besides its public acceptance, bioremediation can also be technically attractive, as available conventional methods (e.g., adsorption, filtration and coagulation-flocculation) present some operational problems and have high costs [6]. In recent years, biosorption has gained momentum as it employs low-cost biological materials (biosorbents) in the process. Biosorbents, in many cases, have proved effective as they show good binding capacity towards different dye groups [7,8]. Biosorption can be defined as the uptake of contaminants by inactive/dead bio-

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doi:10.1016/j.jhazmat.2006.06.081

logical materials through various physicochemical mechanisms. These mechanisms include ion-exchange, adsorption, complexation, chelation, and so on. The mechanism of binding depends on the types of biosorbent, the chemical nature of the pollutant, and the environmental conditions (pH, temperature and ionic strength).

The cost effectiveness and good removal performance are the main attractions of biosorption. Fermentation industries generate huge amounts of waste biomass of microbial origins. However, such wastes are not often recycled as animal feed or as organic manure but are incinerated or dumped at sea [9]. The potential use of fermentation waste in the removal/recovery of dyes remains largely untapped. *Corynebacterium glutamicum*, a Gram-positive organism, belonging to the order of Actinomycetes, is widely used for the biotechnological production of amino acids. Currently, the production of amino acids in fermentation processes with *C. glutamicum* amounts to 1,500,000 t of L-glutamate and 550,000 t of L-lysine per year [10]. Hence, use of the *C. glutamicum* waste as a biosorbent is of great interest.

Considering these aspects, this study aimed to investigate the biosorption potential of *C. glutamicum* using Reactive Black 5 (RB5) as a model reactive dye. In an attempt to improve the biosorption capacity, the biomass was pretreated with different reagents. The influences of pH and temperature were also studied, and the isotherms described using several models. Attempts were also made at dye desorption using 0.1 M HNO_3 and NaOH solutions.

2. Materials and methods

2.1. Sorbate

RB5 ($C_{26}H_{21}N_5Na_4O_{19}S_6$) was purchased from Sigma–Aldrich Korea Ltd. (Yongin, Korea); it was 55% pure, and had a molecular weight of 991.82. The chemical structure of RB5 is given in Fig. 1.

2.2. Preparation of biosorbent

The fermentation wastes (*C. glutamicum* biomass) were obtained in a dried powder form from a lysine fermentation industry (BASF-Korea, Kunsan, Korea), and ground and sieved to obtain particle sizes within the range of 0.4–0.6 mm. The biomass was then pretreated using several chemical agents, which include contacting the biomass with several acids, alkalis and salts. For the pretreatment, 10 g/l of biomass was contacted with individual 0.1 M solutions of HCl, H_2SO_4, HNO_3, NaOH, Na_2CO_3, $CaCl_2$ and NaCl for 1 h at room temperature (25 °C). The biomass after each chemical pretreatment was washed with deionized water until the pH of the wash solution was approximately 7.0. The wet biomass was then dried in an oven at 60 °C for 12 h. The resultant dry biomasses were then sieved to obtain particle sizes within the range previously indicated, and thereafter used in the biosorption experiments.

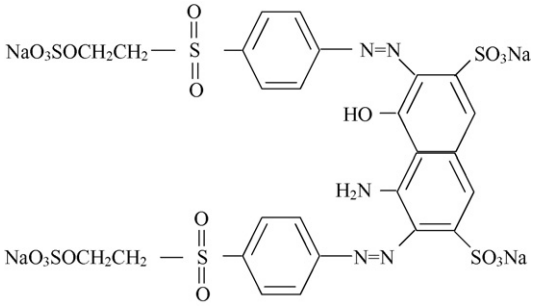

Fig. 1. Chemical structure of Reactive Black 5.

2.3. Biosorption studies

Biosorption isotherm experiments were conducted by bringing into contact 0.1 g of biomass with 40 ml dye solution, at the desired pH and temperature, in 50 ml plastic bottles (high-density polyethylene), which were maintained on a rotary shaker at 160 rpm. The pH of the solution was initially adjusted using either 0.1 M HCl or 0.1 M NaOH, which were subsequently used to control the pH during the experiments. After 12 h of contact with the dye solution, the bacterial biomass was separated by centrifugation at 3000 rpm for 5 min. The dye (RB5) concentration in the supernatant was determined using a spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan) at 597 nm, after appropriate dilution. Kinetic experiments were conducted same as the isotherm experiments except that the samples were collected at different time intervals to determine the time for attainment of biosorption equilibrium.

The amount of dye sorbed by the biomass was calculated from the differences between the concentrations of dye added to that in the supernatant using the following equation:

$$Q_e = \frac{V(C_0 - C_f)}{M} \quad (1)$$

where Q_e is the dye uptake (mg/g), C_0 and C_f the initial and equilibrium dye concentrations in the solution (mg/l), respectively, V the solution volume (l) and M is the mass of biosorbent (g).

2.4. Models to fit batch experimental data

Four equilibrium isotherm models were used to fit the experimental data. The isotherm models were as follows:

Langmuir model [11]:

$$Q_e = \frac{Q_m b C_f}{1 + bC_f} \quad (2)$$

Freundlich model [12]:

$$Q_e = K_F C_f^{1/n} \quad (3)$$

Redlich–Peterson model [13]:

$$Q_e = \frac{K_{RP} C_f}{1 + a_{RP} C_f^{\beta_{RP}}} \quad (4)$$

Sips model [14]:

$$Q_e = \frac{K_S C_f^{\beta_S}}{1 + a_S C_f^{\beta_S}} \quad (5)$$

where Q_m is the maximum dye uptake (mg/g), b is the Langmuir equilibrium coefficient (l/mg), K_F the Freundlich coefficient $(l/g)^{1/n}$, n the Freundlich coefficient, K_{RP} the Redlich–Peterson isotherm coefficient (l/g), a_{RP} the Redlich–Peterson isotherm coefficient $(l/mg)^{\beta_{RP}}$, β_{RP} the Redlich–Peterson model exponent, K_S the Sips model isotherm coefficient $(l/g)^{\beta_S}$, a_S the Sips model coefficient $(l/mg)^{\beta_S}$ and β_S is the Sips model exponent. All the model parameters were evaluated by non-linear regression using the Sigma Plot (Version 4.0, SPSS, USA) software. The average percentage error between the experimental and predicted values was calculated using:

$$\varepsilon(\%) = \frac{\sum_{i=1}^{N}(Q_{exp,i} - Q_{cal,i}/Q_{exp,i})}{N} \times 100 \quad (6)$$

where Q_{exp} and Q_{cal} represent the experimental and calculated dye uptake values, respectively, and N is the number of measurements.

2.5. Desorption studies

The dye-loaded biomass, which was exposed to 500 mg RB5/l at pH 1 and temperature 25 °C, was separated from the biomass-water slurry by centrifugation. The biomass was then brought into contact with two desorbents (0.1 M HNO_3 and 0.1 M NaOH), at different solid-to-liquid ratios, for 3 h on a rotary shaker at 160 rpm. The remaining procedure was the same as employed in the biosorption equilibrium experiments.

All experiments were performed in duplicates. The data were expressed as the mean values of two replicate experiments, with error bars indicated wherever necessary.

3. Results and discussion

3.1. Pretreatment

Initial experiments were conducted to study the effect of the pretreatment of *C. glutamicum* biomass on the biosorption of RB5. For this purpose, several chemical agents were used, including 0.1 M HCl, HNO_3, H_2SO_4, NaOH, Na_2CO_3, NaCl and $CaCl_2$ solutions. In order to determine the optimal pretreatment for the biomass, experiments were conducted under different pH conditions (pH 1–6) and the results are shown in Fig. 2. The raw biomass of *C. glutamicum* exhibited a high uptake of 146 mg/g at pH 1, which revealed the performance of the biomass, even in its native form. Here, it should be noted that the suitable range of pH for the removal of dye was too low to be used in actual process applications. If the biosorption is carried out at pH 1, the pH of effluent should be adjusted at around neutral pH prior to discharge. Even though the biosorption process is used in the middle of wastewater treatment, the pH adjustment makes the biosorption process costly. Therefore, it is needed to further study a proper way to modify the biomass able to bind dye molecules under moderate pH condition.

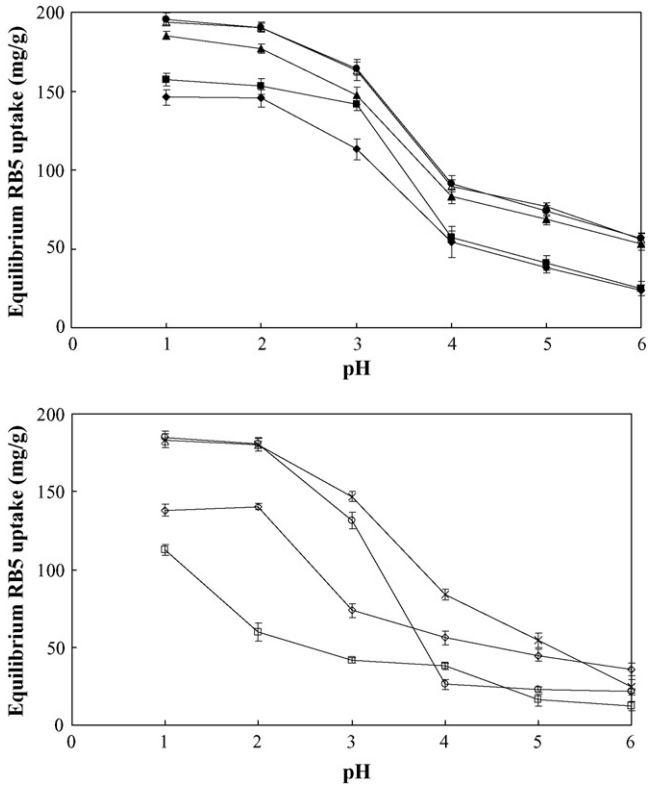

Fig. 2. The effect of pretreatment on RB5 biosorption by *C. glutamicum* biomass (initial RB5 concentration = 500 mg/l, temperature = 25 °C, agitation speed = 160 rpm). Biomass treated using: (♦) raw biomass; (■) deionized water; (▲) 0.1 M HCl; (●) 0.1 M HNO_3; (△) 0.1 M H_2SO_4; (□) 0.1 M NaOH; (◇) 0.1 M Na_2CO_3; (○) 0.1 M $CaCl_2$; (×) 0.1 M NaCl.

Washing the *C. glutamicum* with deionized water enhanced the RB5 uptake to 157 mg/g at pH 1, indicating the presence of impurities in the raw biomass and their influence on the uptake of dye. Treatment of the biomass with different mineral acids resulted in significant increases in the uptake of RB5, which is likely due to the opening up of new binding sites or the removal of ions blocking the sites. The pretreatment of *C. glutamicum* with Na_2CO_3 resulted in a decreased dye biosorption capacity to 138 mg/g, while 0.1 M NaOH pretreatment decreased the biosorption capacity to the greatest extent (113 mg/g). In contrary, the biomass pretreated with NaCl and $CaCl_2$, separately, performed well and exhibited RB5 uptakes close to that of the biomass treated with mineral acids.

The results of chemical pretreatment imply that HCl, HNO_3, H_2SO_4, NaCl and $CaCl_2$ enhanced the uptake capacity of RB5 by *C. glutamicum*. Apart from structural modification of biomass, the performance of mineral acids may also be attributed to the protonation of the functional groups responsible for biosorption. Our previous study identified the amino groups of *C. glutamicum* as being mainly responsible for reactive dye biosorption [15]. Most common amino acids have isoelectric points in the pH range 5–6 [16]. Thus, it is expected that amino groups in the biomass will be protonated under acidic conditions; and thus, the biomass will have a net positive charge. On the other hand, RB5 is negatively charged in aquatic solution, which will exhibit electrostatic attraction towards the positively

charged cell surface. This could explain the reason of maximum biosorption occurred in strong acidic pH ranges in all cases of pretreated biomass.

The reason why NaCl and $CaCl_2$ pretreatments increased biosorption capacity could be that Na^+ and Ca^{2+} are positive ions and thus could neutralize the negative charge (i.e. carboxyl group) on the surface of bacterial biomass. The carboxyl group has been known to inhibit the binding of anionic reactive dye molecule to amine [17]. Conversely, NaOH pretreatment reduced the RB5 biosorption capacity of the biomass likely because inhibitory carboxyl sites were increased during treatment with NaOH, which generally causes the swelled structure of microbial biomass [17].

Variation of the pH during the biosorption with each pretreated biomass was different in each case. The biomass treated with mineral acids had a tendency to decrease the solution pH, indicating the release of protons from the pretreated biomass. Conversely, the NaOH pretreated biomass had a tendency to raise the solution pH, indicated the release of OH^- from the pretreated biomass. For isotherm experiments, the solution pH was therefore maintained by the addition of either acid (0.1 M HCl) or base (0.1 M NaOH).

Of the chemical agents examined, 0.1 M HNO_3 was found to be most suitable for the pretreatment of *C. glutamicum* for the biosorption of RB5, giving the maximum uptake of 195 mg/g. Therefore, the pretreated biomass with 0.1 M HNO_3 was utilized in further experiments.

3.2. Effects of pH and temperature

The RB5 biosorption isotherms for *C. glutamicum* under different pH (1–3) and temperature (25–40 °C) conditions are presented in Fig. 3. The initial RB5 concentration was varied between 250 and 3000 mg/l to obtain the biosorption isotherms. In the examined range, the initial pH was found to play a significant role in the biosorption of RB5 with the highest uptake observed at pH 1. Under the examined pH conditions, the uptake of RB5 increased with increasing dye concentration, reaching saturation at a higher equilibrium concentration. The biosorption isotherm at pH 1 exhibited the steepest initial isotherm slope, which is the measure of the sorbent-solute affinity, with an eventual highest uptake value of 350 mg/g. The temperature also affected the equilibrium uptake of RB5 by *C. glutamicum*. Fig. 3 shows that the biosorption performance increased with increasing temperature, up to 35 °C, indicating the endothermic nature of biosorption process. This increase in binding could be due to increased surface activity and increased kinetic energy of the dye molecules [4].

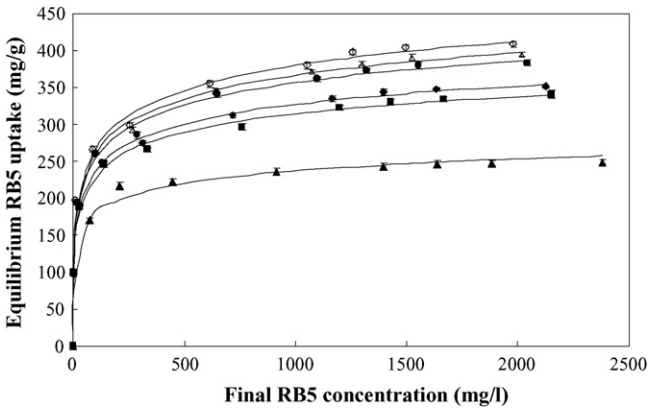

Fig. 3. RB5 biosorption isotherms for *C. glutamicum* under different conditions (agitation rate = 160 rpm). pH and temperature: (♦) 1 and 25 °C; (■) 2 and 25 °C; (▲) 3 and 25 °C; (●) 1 and 30 °C; (◊) 1 and 35 °C; (△) 1 and 40 °C. Curves predicted by the Sips model.

3.3. Equilibrium modeling

Langmuir, Freundlich, Redlich–Peterson, and Sips models were used to describe the non-linear equilibrium relationship between the dye sorbed onto the bacterium (Q) and that left in solution (C_f). The main reason for the extended use of these isotherm models was that they incorporate easily interpretable constants. The model coefficients along with correlation coefficients (R^2) and average percentage error (ε) values obtained from the four isotherm models are listed in Table 1.

The Langmuir sorption isotherm has traditionally been used to quantify and contrast the performance of different biosorbents. In its formulation, binding to the surface was primarily by physical forces and implicit in its deviation was the assumption that all sites possess equal affinity for the sorbate. Its use was extended to empirically describe the equilibrium relationships between the bulk liquid and solid phases [18]. In this study, the Langmuir model produced reasonably good agreement with the biosorption isotherm data (Table 1). The Langmuir model served to estimate the maximum uptake values where they could not be reached in the experiments. The coefficient b represents the affinity between the sorbent and sorbate. Both Q_m and b increase with decreasing pH from 3 to 1, and with increasing temperature from 25 to 35 °C. High b values indicate that the biomass can remove RB5 even at trace level and high Q_m values shows a desirable high capacity of dye binding [18]. The RB5 biosorption capacity observed in this study was superior when compared to the results published in the literature. Activated sludge exhibited 116 mg RB5/g [19], *Endothiella aggregata* biosorbed 44 mg RB5/g [20] and *Kluyveromyces marxianus* biosorbed 37 mg RB5/g [21] compared to 419 mg RB5/g by *C. glutamicum* in this study.

The Freundlich isotherm was originally empirical in nature, but later became interpreted as sorption to heterogeneous surfaces or surfaces supporting sites with varied affinities. The Freundlich coefficient K_F reached its corresponding maximum value at pH 1 and a temperature of 35 °C, implying the binding capacity had reached its highest value compared to the other conditions investigated (Table 1).

The Redlich–Peterson model, which incorporated features of both the Langmuir and Freundlich isotherms, described the RB5 biosorption data, with very high correlation coefficients and low percentage error values (Table 1). There are two limiting behaviors: Langmuir form for $\beta_{RP} = 1$ and Henry's law form for $\beta_{RP} = 0$ [22]. From Table 1, it is worth noting that the β_{RP} values were close to unity i.e., the data can preferably be fitted to the Langmuir model. Also, the Sips model better described the RB5

Table 1
Langmuir, Freundlich, Redlich–Peterson and Sips model parameters for RB5 biosorption by *C. glutamicum*

pH	T (°C)	Langmuir model				Freundlich model			
		Q_m (mg/g)	b (l/mg)	R^2	ε (%)	K_F (l/g)$^{1/n}$	$1/n$	R^2	ε (%)
1.0	25	350	0.032	0.92	6.1	109	0.16	0.99	1.5
2.0	25	341	0.031	0.91	4.4	107	0.15	0.99	1.4
3.0	25	250	0.027	0.84	10.8	98	0.12	0.96	6.1
1.0	30	383	0.040	0.88	7.3	137	0.13	0.97	5.7
1.0	35	419	0.042	0.88	11.0	142	0.14	0.97	8.6
1.0	40	397	0.043	0.92	9.1	139	0.14	0.97	8.5

pH	T (°C)	Redlich–Peterson model					Sips model				
		K_{RP} (l/g)	a_{RP}(l/mg)$^{\beta_{RP}}$	β_{RP}	R^2	ε (%)	K_S(l/g)$^{\beta_S}$	a_S(l/mg)$^{\beta_S}$	β_S	R^2	ε (%)
1.0	25	177	1.37	0.87	1.00	0.1	103	0.199	0.31	1.00	0.1
2.0	25	136	1.04	0.87	1.00	0.1	101	0.208	0.31	1.00	0.2
3.0	25	120	0.86	0.92	0.93	6.0	95	0.248	0.27	0.97	4.3
1.0	30	190	1.37	0.86	0.94	5.8	105	0.181	0.31	0.97	4.6
1.0	35	202	1.54	0.85	0.98	2.9	108	0.171	0.32	0.98	2.5
1.0	40	198	1.58	0.85	0.99	2.7	107	0.178	0.31	0.99	3.0

biosorption data. At low sorbate concentrations, Sips isotherm effectively reduces to the Freundlich isotherm, and does not obey Henry's law. At high sorbate concentrations, the monolayer sorption capacity characteristic of the Langmuir isotherm is predicted [23]. The exponent β_S values found in this study were very low, implying the RB5 biosorption data obtained in this study were more of in the Freundlich form than that of the Langmuir, as confirmed in Table 1. In general, the three-parameter models represent biosorption isotherm data well, with high correlation coefficients and low percentage error values compared to the two-parameter models. A typical example of biosorption isotherm data (at pH 1 and temperature 35 °C), fitted using the four isotherm models, is shown in Fig. 4.

3.4. Kinetic studies

For any practical applications, the process design and operation control, the sorption kinetics are very important [24]. The sorption kinetics in wastewater treatment is significant, as it provides valuable insights into the reaction pathways and mechanism of sorption reactions [25]. Also, the kinetics describes the solute uptake which in turn controls the residence time of sorbate uptake at the solid–solution interface [26]. Taking this into account, kinetic experiments were performed by varying the initial RB5 concentration between 500 and 2000 mg/l (Fig. 5). As expected, the uptake of RB5 by *C. glutamicum* was strongly dependent on the initial dye concentration, with high RB5 uptakes observed at high initial RB5 concentrations. On changing the initial RB5 concentration from 500 to 2000 mg/l, the amount biosorbed increased from 188 to 355 mg/g. However, the removal efficiency of RB5 decreased from 94.1 to 44.4% as the RB5 concentration increases from 500 to 2000 mg/l. This was because at lower concentrations, the ratio of the initial moles of dye to the available surface area is low, with the subsequent fractional biosorption becoming independent of the initial concentration. However, at higher concentrations, the sites available for biosorption become fewer compared to the moles of dye present; hence, the percentage of RB5 removed is dependent on the initial dye concentration [27]. It was also observed that

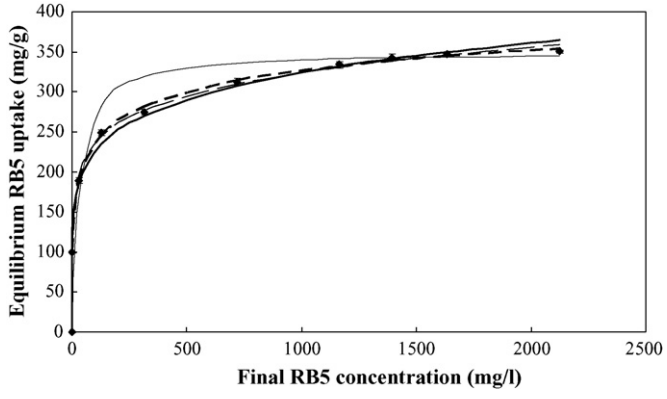

Fig. 4. Application of Langmuir (—), Freundlich (▬▬), Redlich–Peterson (- - -) and Sips (▬ ▬ ▬) models to experimental isotherm data (♦) at pH = 1, temperature = 25 °C and agitation rate = 160 rpm.

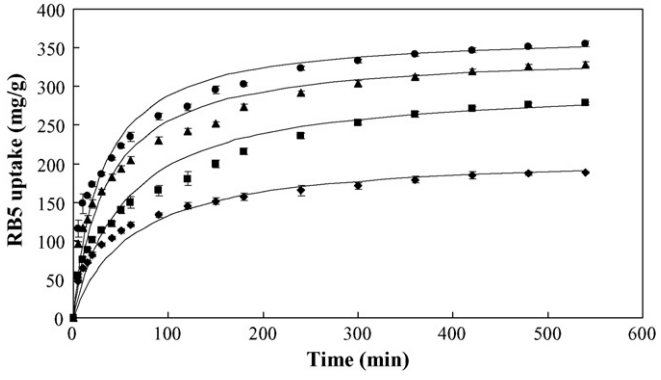

Fig. 5. Effect of the initial concentration on the biosorption of RB5 onto *C. glutamicum* (pH = 1; temperature = 25 °C; agitation rate = 160 rpm). Initial RB5 concentration: (♦) 500 mg/l; (■) 1000 mg/l; (▲) 1500 mg/l; (●) 2000 mg/l. Curves predicted by the pseudo-second order model.

Table 2
Pseudo-first and pseudo-second order model kinetic parameters at different initial RB5 concentrations

Initial concentration (mg/l)	Experimental, Q_e (mg/g)	K_1 (min^{-1})	Predicted, Q_e (mg/g)	R^2	K_2 (10^5 g/mg min)	Predicted, Q_e (mg/g)	R^2
500	188	0.009	139	0.95	6.0	196	1.00
1000	278	0.009	244	0.96	6.1	303	0.99
1500	329	0.008	234	0.97	8.2	345	0.99
2000	355	0.008	227	0.99	9.4	370	1.00

the uptake of RB5 was rapid for the initial 2 h, but thereafter proceeded at a slower rate, until saturation was finally attained. This two-stage behavior may be due to the heterogeneity of the biomass. The higher biosorption rate over the initial period (2 h) may be due to an increased number of vacant sites available during the initial stage, resulting in an increased concentration gradient between the sorbate in solution and that at the biosorbent surface. With increasing time, this concentration gradient became reduced due to the biosorption of RB5 molecules onto vacant sites, leading to a decrease in the biosorption rate during the latter stages.

The experimental biosorption kinetic data were modeled using pseudo-first and pseudo-second order kinetics. The linearized form of the pseudo-first and pseudo-second order models [28] are shown below as Eqs. (7) and (8), respectively:

$$\log(Q_e - Q_t) = \log(Q_e) - \frac{K_1}{2.303}t \quad (7)$$

$$\frac{t}{Q_t} = \frac{1}{K_2 Q_e^2} + \frac{1}{Q_e}t \quad (8)$$

where Q_e is the amount of dye sorbed at equilibrium (mg/g), Q_t the amount of dye sorbed at time t (mg/g), K_1 the pseudo-first order rate constant (min^{-1}) and K_2 is the pseudo-second order rate constant (g/mg min). The rate constants, predicted equilibrium uptakes and corresponding correlation coefficients for all concentrations tested have been calculated, and are summarized in Table 2.

In the case of the pseudo-first order model, the correlation coefficients were found to be above 0.95, but the calculated Q_e was not equal to the experimental Q_e, suggesting the insufficiency of the model to fit the kinetic data for the initial concentrations examined [28]. The reason for these differences in the Q_e values was due to a time lag, possibly as a result of a boundary layer or an external resistance controlling the beginning of the sorption process [29]. In most cases in the literature, the pseudo-first order model does not fit the kinetic data well over the entire contact time range; therefore, generally underestimate the Q_e values [28,30]. Thus, good linearity of the Lagergren plots is no guarantee that the interactions will follow first order kinetics [31].

The pseudo-second order model is based on the sorption capacity of the solid phase. Contrary to the pseudo-first order model, it predicts the sorption behavior over the entire study range [29]. This was consistent with the better results obtained with the pseudo-second order model (Table 2). The correlation coefficients were always greater than 0.99. The predicted equilibrium biosorption capacity values showed reasonably good agreement with the experimental equilibrium uptake values.

3.5. Thermodynamic parameters of biosorption

Biosorption isotherm data, obtained at different temperatures, were used to calculate the important thermodynamic properties, such as the standard Gibbs free energy change (ΔG°), standard enthalpy change (ΔH°) and standard entropy change (ΔS°). The Langmuir coefficient b (l/mg) was used to calculate the standard Gibbs free energy change (ΔG°) according to the following equation [32]:

$$\Delta G^\circ = -RT \ln b \quad (9)$$

where R is the gas constant (8.314 J/mol K) and T is the absolute temperature (K). Standard enthalpy and entropy changes were obtained from a plot of $\ln b$ versus $1/T$; the equations are as follows:

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \quad (10)$$

From Eqs. (9) and (10), we get,

$$\ln b = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} \quad (11)$$

The ΔG° values obtained were −26.1, −26.7, −27.2 and −27.8 kJ/mol at 25, 30, 35 and 40 °C, respectively. A negative value of free energy change indicates the feasibility of the RB5 biosorption process and confirms affinity of the biosorbent towards the sorbate. The values of ΔH° and ΔS° obtained from the plot of $\ln b$ versus $1/T$ ($R^2 = 0.98$) were 7.4 kJ/mol and 0.11 kJ/mol K, respectively. The positive enthalpy value indicates that the RB5 biosorption process is endothermic, with the positive entropy value indicating the increasing randomness at the solid–liquid interface during the biosorption process [32,33]. The increase in temperature affects not only the solubility of the dye but also the chemical potential of the sorbate, the latter being a controlling factor in biosorption (this was confirmed by the thermodynamic parameters).

3.6. Desorption studies

The RB5-loaded *C. glutamicum* biomass was subjected to desorption under acidic (0.1 M HNO_3) and basic (0.1 M NaOH) conditions at different solid-to-liquid ratios. The solid-to-liquid ratio can be defined as the mass of the dye-loaded biomass to the volume of desorbent. Thus, upon desorption of dye molecules

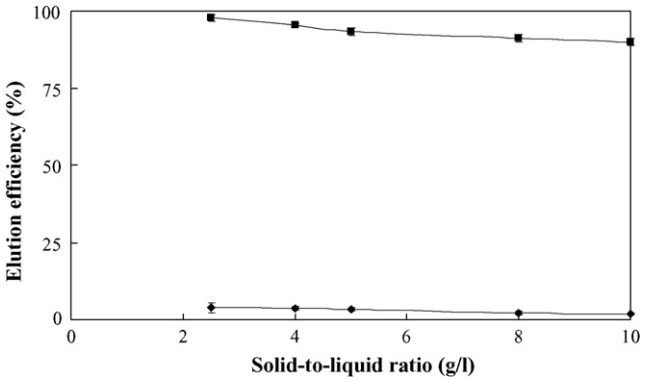

Fig. 6. Effect of the solid-to-liquid ratio on the desorption efficiency of RB5 using 0.1 M HNO_3 (♦) and 0.1 M NaOH (■).

from a biosorbent, it is desirable to use the smallest possible volume of desorbent so as to obtain the highest dye concentration. Fig. 6 illustrates the effect of the solid-to-liquid ratio on the RB5 desorption efficiency of the desorbing agents examined. The desorption efficiency was determined from the ratio of the dye mass in solution after desorption to the dye mass initially bound to the biosorbent [34,35]. The 0.1 M HNO_3 solution exhibited very low desorption efficiencies for all the solid-to-liquid ratios examined. In contrary, 0.1 M NaOH performed very well, and led to release of all the dye molecules from the biomass. The reason for this behavior can be explained, as follows: Under strong basic (high pH) conditions, the number of negatively charged sites increases. These negatively charged sites on the sorbent surface favor desorption of dye anions due to electrostatic repulsion [36]. Thus, 0.1 M NaOH performed well in RB5 desorption, as a significantly high electrostatic repulsion exists between the negatively charged surface of biomass and dye anions. It should also be noted that the desorption efficiency of 0.1 M NaOH appeared to be nearly independent of the solid-to-liquid ratio, for up to 10 g/l.

4. Conclusions

Fermentation industry wastes, which are generated in huge quantities over a short period of time, represent an important environmental problem in many parts of world. Hence, their effective management has drawn attention for many years, and the potential use of this waste may be a possible solution. On the other hand, successful biosorption processes require continuous supply of cheap biomass for the production of biosorbents. Taking this into consideration, it appears logical that fermentation industry wastes can serve as low-cost biosorbents. However, the effectiveness of waste to biosorb contaminants needs to be explored. Taking these aspects into account, this paper investigated the potential of fermentation waste (*C. glutamicum* biomass) for the biosorption of RB5. Acidic pretreatment enhanced the biosorption capacity of the biomass due to enhancement of positively charged cell surfaces, which exhibit electrostatic attraction toward negatively charged dye anions in solution. The solution pH and temperature affected the RB5 biosorption capacity of *C. glutamicum* biomass. Kinetic studies revealed that the uptake of RB5 was rapid during the initial 2 h, but thereafter proceeded at a slower rate, and finally attained saturation. Desorption of dye molecules from the dye-loaded biomass was successfully achieved using 0.1 M NaOH as the desorbing agent. Thus, this research has identified a low-cost, and highly efficient biomass for the biosorption of Reactive Black 5 from wastewaters.

Acknowledgements

This work was supported by the grant of Post-Doc. Program, Chonbuk National University (2005) and in part by KOSEF through AEBRC at POSTECH.

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