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Valorization of *Escherichia coli* waste biomass as a biosorbent for removing reactive dyes from aqueous solutions

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ABSTRACT

Industrial waste biomass of *Escherichia coli* was developed as a biosorbent of Reactive Yellow 2 (RY2). The raw biomass was modified by esterification under acidic conditions to remove the carboxyl groups that could interfere with the binding of RY2 to the biomass. The maximum uptake of the esterified biomass was 335.16 ± 5.81 mg/g, which was 1.5 and 8.3 times higher than those of the raw biomass and ion-exchange resin (Amberjet 4200), respectively. This study can be a step forward to the feasible valorization of microbial fermentation wastes as an adsorbent.

Keywords: Valorization; Microbial waste; *Escherichia coli*; Biosorbent; Esterification; Reactive dyes

1. Introduction

Many different dyestuffs are discharged along with wastewater from various industries, including textiles, leather, paper, and plastics [1]. Due to their synthetic origin with aromatic molecular structures, effluent dyes are usually very stable and difficult to naturally degrade. Therefore, high chemical and biological oxygen are in demand for dyes-containing wastewater treatment. Dye contaminants can be recognized by humans at very low concentrations and can be toxic in aqueous environments. In addition, some dye compounds, particularly benzidine- and arylamine-based dyes, are reported for their carcinogenicities. Hence, the removal of dyestuffs from wastewater effluents has been regarded as an important environmental issue [2–4].

Various methods, including precipitation, adsorption and ion exchange, and membrane filtration, have been used for the treatment of dyestuffs in industrial wastewaters [5]. Especially, physical adsorption has recently attracted interest for dye removal due to its relatively high removal efficiency and economic benefits [6]. Although activated carbon has been widely used due to its high adsorption capacity, it is limited by relatively high operation costs, regeneration troubles, and difficulty in separating the treated activated carbon from the bulk solutions after use. Therefore, attentions have shifted to development of biological methods such as bioaccumulation and biosorption [5–7].

Biosorption is the technology which removes and/or recovers ionic solutes from wastewater and aqueous

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solutions using various biomaterials including bacteria [5], fungi [8], algae [9], and agricultural/industrial biowastes [10]. The biomaterials possess various functional groups including carboxyl, amine, hydroxyl, and sulfonate groups as binding sites for ionic materials [11,12]. The kind and amount of functional groups in biomaterials are important factors that affect biosorption capacities. Hence, according to functional group properties of biomass and interactions between sorbents and sorbates, the biomaterials are subjected to chemical modification for enhancing the sorption capacity [12,13]. Akar et al. reported the enhancement of the uptakes of Reactive Red 2 (RR2) and Basic Blue 9 (BB9) using quaternary ammonium salt-modified sugar pulp and using citric acid-modified *Thuja orientalis* cone powder, respectively [14,15]. In addition, polyethylenimine-modified biosorbents were proven to be high-performance sorbents for reactive dyes by Mao et al. [16], Low et al. [17] and Sun et al. [18].

A large amount of *Escherichia coli* waste biomass is generated from the full-scale fermentation process for L-phenylalanine production. Although potentially recyclable, most of this waste biomass is dumped at sea or burnt. The main purpose of this work is to evaluate the feasibility of the microbial waste valorization as a biosorbent for the removal of anionic dyes from aqueous solution. A simple esterification was applied in an attempt to enhance the adsorption capacity of the *E. coli* biomass. The biomasses, both raw and esterified, were applied to the removal of RY2 and their maximum sorption uptakes were compared with a commercial ion-exchange resin (Amberjet 4200).

2. Materials and methods

2.1. Materials

The spray-dried *E. coli* biomass was obtained from an L-phenylalanine fermentation industry (Daesang, Kunsan, Korea). The raw *E. coli* biomass (REB) was stored in desiccators prior to use as the biosorbent in the sorption experiments. Amberjet 4200 was purchased from Sigma–Aldrich Korea Ltd. The anionic dye, RY2 (Cibacron Brilliant Yellow 3G-P, $C_{25}H_{15}Cl_3N_9Na_3O_{10}S_3$, M.W.: 873.0, color index number (C.I.): 18972, dye content: 60–70%, λ_{max}: 404 nm), was purchased from Sigma–Aldrich Korea Ltd (Yongin, Korea). The structure of RY2 is shown in Fig. 1. Other chemicals used in this study were of analytical grades.

2.2. Preparation of the esterified E. coli biomass

The chemically esterified *E. coli* biomass (EEB) was prepared by esterification of the carboxyl groups on the surface of REB. Twenty grams of REB was suspended in 2 L of methanol (99.8%), and 20 mL of concentrated hydrochloric acid (35%) was added to the suspension. The mixture was stirred for 6 h at 25°C. After completion of the esterification, EEB was separated by centrifugation, and then washed three times using methanol. The reaction proceeded as follows:

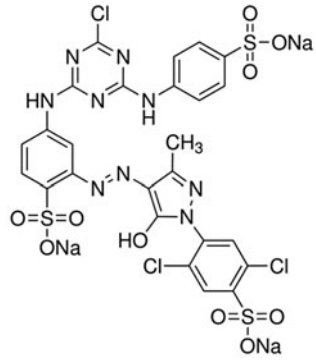

Fig. 1. The structure of Reactive Yellow 2 (RY2).

$$\text{Biomass–COOH} + \text{CH}_3\text{OH} \xrightarrow{\text{H}^+} \text{Biomass–COOCH}_3 + \text{H}_2\text{O} \quad (1)$$

2.3. Batch sorption experiments

Batch experiments (pH edge, isotherm, and kinetics) were conducted to test the RY2 biosorption performance of biosorbents. In the batch experiments, 0.1 g of dried REB and EEB and 0.2 g_{wet} of Amberjet 4200 (corresponding to 0.114 g_{dry}) were submerged in 40 mL of 500 mg/L RY2 solutions in conical tubes. The pH was adjusted using 1 M NaOH and 1 M HNO_3 solutions during the experiments. The mixtures were shaken for 24 h in an auto shaker at 160 rpm and 25°C. After reaching equilibrium, the final pH of each tube was measured. After the samples were centrifuged for liquid–solid separation, the supernatant portion was used to analyze the residual RY2 concentration.

Kinetics experiments were performed at pH 3 and 25°C until equilibrium states were achieved. The initial concentration of RY2 in REB and EEB was 300 mg/L. The mixtures were stirred until equilibrium state and the remaining amount of RY2 was analyzed at intervals of reaction time. The isotherm experiments were performed to evaluate the maximum sorption capacity (q_m) of the biosorbents and ion-exchange resin at pH 3 for 24 h. The sorbents were mixed with 40 mL RY2 solutions of different concentrations. The RY2 solution

was prepared by dilution with distilled water. The pH was adjusted to pH 3 using 1 M NaOH and 1 M HNO_3 solutions.

The RY2 concentration in aqueous solution was measured using a UV spectrophotometer (UV-2550, Shimadzu, Kyoto, Japan) at 404 nm, after the samples had been centrifuged at over 6,000 rpm for solid–liquid separation. The liquid phase was used for analysis of the dye concentration. The RY2 uptake was calculated using the following equation:

$$q = (V_i C_i - V_f C_f)/M \quad (2)$$

where q (mg/g) is the RY2 uptake, and V_i and V_f (L) are the initial and final (after the addition of acid or base solution to adjust the pH) working volumes, respectively. C_i and C_f (mg/L) are the initial and final concentrations of RY2, respectively. M (g) is the weight of the biomass.

2.4. Fourier transform infrared (FT-IR) analysis

The infrared spectrums of REB and EEB were obtained using a Fourier transform infrared spectrometer (FT/IR-4100, Jasco, Japan). The pellets for FT-IR analysis were prepared using KBr powder and were finely ground with the biomass. FT-IR analysis of biosorbents was performed in the peak range of 400–4,000 cm^{-1}.

3. Results and discussion

3.1. Variation of the functional groups on the biomass

The functional groups in the biomasses were characterized by FT-IR study. The FT-IR spectra of REB and EEB are presented in Fig. 2. As shown in Fig. 2(a), several functional groups on the *E. coli* biomass were revealed by the various adsorption peaks of the FT-IR spectrum. The broad and strong adsorption peaks in the range 3,500–3,000 cm^{-1} were derived from the overlapping of the O–H bond of the hydroxyl groups with the N–H bond of the amine groups on the *E. coli* biomass [19,20]. The strong peak at 2,925 cm^{-1} indicates the C–H symmetric stretching of the methylene groups ($–CH_2$) and deformation vibration of the methyl groups ($–CH_3$) [21]. A shoulder peak observed at 1,740 cm^{-1} indicates carbonyl stretching of unionized carboxylates [22,23]. The strong adsorption peak at 1,538 cm^{-1} was due to the amide I and/or C=O chelate stretching of the carboxyl group and/or amide II bands [24]. The peaks at 1,384 and 1,408 cm^{-1} were attributed to the symmetrical stretching of the carboxylate anion and

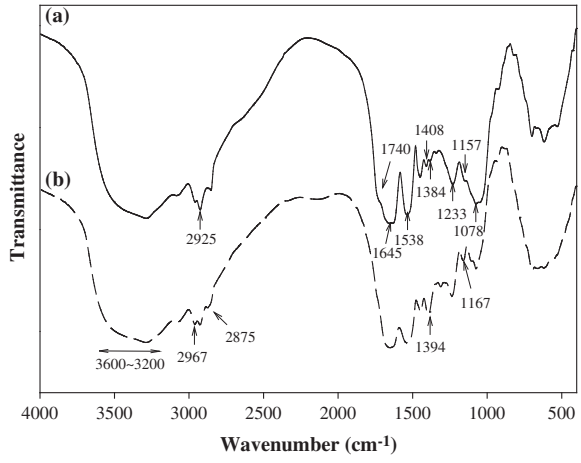

Fig. 2. FT-IR spectrum of (a) the raw *E. coli* biomass (REB) and (b) the esterified *E. coli* biomass (EEB).

carboxylic acid, respectively [25]. The spectrum of the adsorption peaks at 1,233 and 1,157 cm^{-1} indicate the C–O stretching vibration of ketones, aldehydes, and lactones or carboxyl groups [26]. The adsorption peaks around 1,157 cm^{-1} (P–O stretching) and 1,078 cm^{-1} (P–OH stretching) were indicators of the presence of phosphonate groups [27]. The FT-IR analysis revealed amine, carboxyl, and phosphonate groups as the main functional groups for adsorption on REB. Some of these adsorption peaks were changed for EEB (Fig. 2(b)). The peak at 2,967 cm^{-1} (aliphatic C–H) [28] was enhanced and the peak at 2,857 (aromatic C–H) [28] was shifted to 2,875 cm^{-1}. During esterification, the peak at 1,408 cm^{-1} was enhanced and shifted to 1,394 cm^{-1}. The REB shoulder peak at 1,740 cm^{-1} was removed. In addition, the peak at 1,157 cm^{-1} was enhanced and shifted to 1,167 cm^{-1} after chemical modification.

3.2. The effect of pH

The effect of pH on RY2 adsorption is displayed in Fig. 3. The RY2 sorption was significantly affected by the pH of solutions. The RY2 adsorption capacities of the biomasses decreased with increasing pH. The RY2 uptake of REB rapidly decreased with increasing pH and significantly decreased from around pH 5. Furthermore, the RY2 uptake was almost zero from pH 8. The pK_a values of the amine group for biomaterials are between 8 and 10 [23]. Therefore, the amine groups of biomass should be positively charged at pH < 8 by protonation of amine groups. On the other hand, carboxyl groups of the biomass have pK_a values ranging from 3.5 to 5.0 [29]. Therefore, it is possible that the number of negatively charged carboxyl

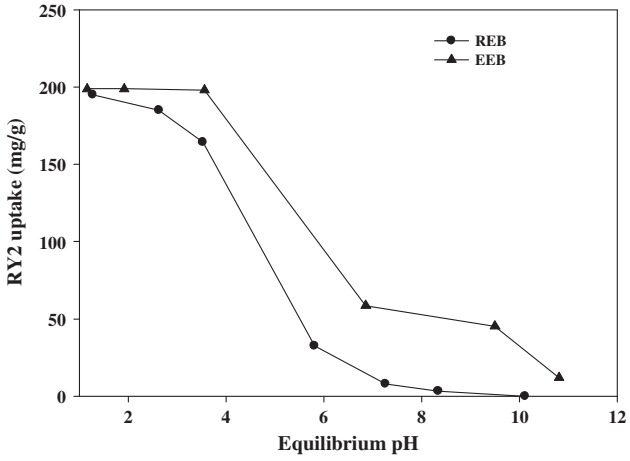

Fig. 3. Effect of pH on the biosorption capacity of RY2 (●) REB and (▲) EEB.

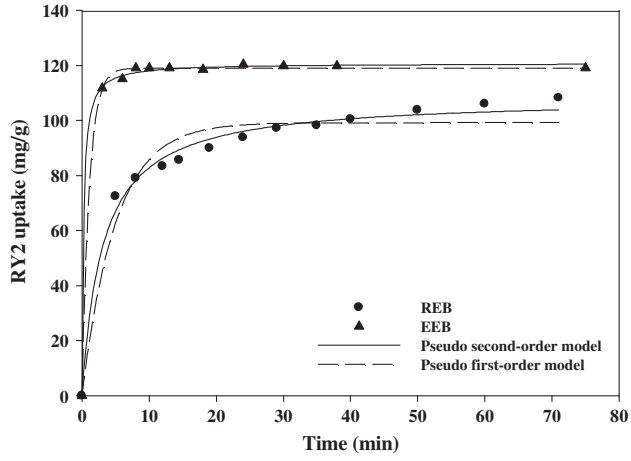

Fig. 4. Biosorption kinetics of RY2 onto REB (●) and EEB (▲) at pH 3.

groups increased by deprotonation at pH higher than their pK_a and that these groups interfere in the binding of the reactive dye to the amine group.

The uptake of RY2 by EEB remained constant up to around pH 3.5, due to the removal of interfering carboxyl groups by esterification, as intended. Enhancement of uptakes of anionic solutes through esterification have been successful in different kinds of biomass [6,30]. In addition, after modification of the biomass, the pH edge data were shifted to the right. This implies that EEB requires smaller amount of acid and base to alter sorption/desorption by pH control than REB.

3.3. Sorption kinetics

Fig. 4 shows the plots of RY2 uptake versus contact time at pH 3. Almost complete adsorption equilibrium was attained within 60 min for REB, but after only 10 min for EEB. Furthermore, the equilibrium RY2 uptake by EEB (119.75 mg/g) was 13% higher than that by REB (105.97 mg/g), due to removal of the interfering carboxyl group. To estimate the equilibrium state, the pseudo-first- and pseudo-second-order kinetic models were applied to the kinetic data. These can be represented as follows:

$$\text{Pseudo-first-order model} \quad q_t = q_e(1 - \exp(-k_1 t)) \tag{3}$$

$$\text{Pseudo-second-order model} \quad q_t = q_e^2 k_2 t/(1 + q_e k_2 t) \tag{4}$$

where q_e is the amount of dye adsorbed at equilibrium (mg/g), q_t is the amount of dye adsorbed at time t (mg/g), k_1 is the pseudo first-order rate constant (1/min), and k_2 is the pseudo-second-order rate constant (g/mg min). The estimated kinetic parameters from each model are summarized in Table 1.

In the case of the pseudo-first-order model, the calculated correlation coefficients (R^2) were 0.954 (R_1^2) and 0.999 (R_1^2), and the q_e values were 99.18 ± 2.26 and 118.97 ± 0.45 mg/g for REB and EEB, respectively. The first-order rate constants (k_1) were 0.199 ± 0.026 and

Table 1
Estimated parameters of pseudo first- and second-order kinetic models

	Parameters					
	Pseudo-first-order model			Pseudo-second-order model		
Adsorbent	k_1 (1/min)	q_e (mg/g)	R^2	k_2 (g/mg min)	q_e (mg/g)	R^2
REB	0.199 (0.026)	99.18 (2.26)	0.954	0.003 (0.4×10^{-3})	108.35 (1.75)	0.989
EEB	0.911 (0.060)	118.97 (0.45)	0.999	0.036 (0.005)	120.76 (0.48)	0.999

Note: Standard errors are shown in parentheses.

0.911 ± 0.06 1/min for REB and EEB, compared to second-order rate constants (k_2) of 0.003 ± 0.0004 and 0.036 ± 0.005 g/mg min, respectively. These results revealed faster sorption kinetics for EEB than for REB, suggesting that the affinity for RY2 was enhanced by esterification, as further demonstrated by the isotherm data presented below.

3.4. Isotherms

Adsorption isotherm experiments were performed to evaluate the maximum RY2 uptakes of REB and EEB at pH 3 (Fig. 5). The RY2 uptake increased with increasing initial RY2 concentration and reached equilibrium state. The Langmuir model was applied to isotherm data to evaluate the maximum adsorption capacity. The Langmuir equation can be written in the form below:

$$q_e = q_m b C_e / (1 + b C_e) \quad (5)$$

where q_e is the adsorbed amount of dye, C_e is the equilibrium dye concentration, q_m is the monolayer biosorption capacity, and b is a Langmuir constant related to the free energy of biosorption [4]. The estimated results of the parameters are summarized in Table 2.

The maximum adsorption capacities were estimated as 196.89 ± 12.82 and 335.17 ± 5.81 mg/g for REB and EEB, respectively, i.e. 1.5 times higher for EEB than for REB. In addition, the affinity of EEB (0.125 ± 0.017 L/mg) was nearly double that of REB (0.063 ± 0.027 L/mg).

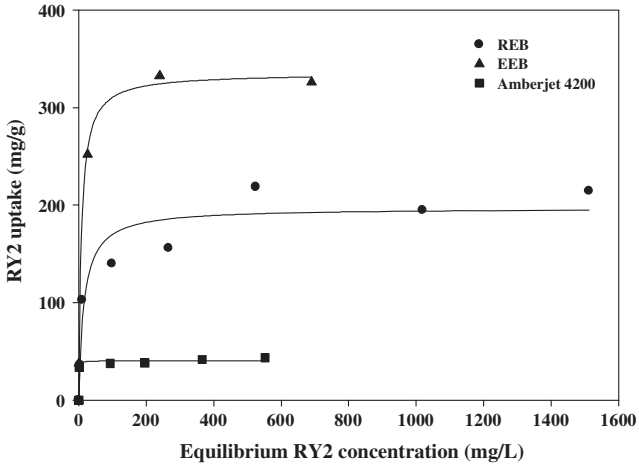

Fig. 5. Biosorption isotherms of RY2 by REB (●), EEB (▲), and Amberjet 4200 (■) at pH 3.

Table 2
Estimated parameters of the Langmuir model

Adsorbent	Parameter		
	q_m (mg/g)	b (L/mg)	R^2
REB	196.89 (12.82)	0.063 (0.027)	0.922
EEB	335.17 (5.81)	0.125 (0.017)	0.998
Amberjet 4200	40.36 (1.19)	2.415 (1.146)	0.983

Note: Standard errors are shown in parentheses.

To compare with the maximum sorption capacity of commercial ion-exchange resin, Amberjet 4200 was used as a model ion-exchange resin. The maximum sorption capacity of EEB (335.17 ± 5.81 mg/g) was 8.3 times higher than that of Amberjet 4200 (40.36 ± 1.19 mg/g) at pH 3, which was attributed to steric inhibition between functional groups of the resin and RY2 ions. The main functional group of Amberjet 4200 is trimethyl ammonium, whereas that of biomass is reported to be the primary amine group ($-NH_3^+$), which can bind with anionic pollutants [4,25,31]. The trimethyl ammonium group has a larger structure than that of the primary amine. The RY2 sorption occurred at the presented functional groups in the tiny pores and on the surface of Amberjet 4200. However, in case of biomasses, the RY2 molecules might be bound to functional groups on the surface of the biomasses. Therefore, RY2, as a large structural dye, was bound more easily on the biomass surface than on Amberjet 4200.

4. Conclusions

Microbial waste of *E. coli* biomass was successfully converted to biosorbent to remove reactive dyes by esterification of the interfering carboxyl groups of REB. The maximum sorption capacity of EEB was 1.5 and 8.3 times higher than that of REB and Amberjet 4200, respectively. The solution pH was a crucial factor and the uptake of RY2 was able to be controlled by changing the solution pH. Therefore, it can be mentioned that the result suggested a feasible option for valorization of microbial fermentation wastes as a powerful adsorbent.

Acknowledgments

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List of symbols

RY2 — Reactive Yellow 2
REB — raw *E. coli* biomass
M.W — molecular weight
λ_{max} — wavelength of maximum absorbance
C.I. — color index number
EEB — esterified *E. coli* biomass
q_m — maximum sorption capacity
q_t — amount of dye adsorbed at time t
q_e — amount of dye adsorbed at equilibrium state
V_i — initial volume of samples
V_f — final volume of samples after adding acid or base solution to adjust the solution pH
C_i — initial concentration of reactive dye
C_f — the final concentration of reactive dye
C_e — equilibrium dye concentration
M — weight of biomass
t — duration of adsorption
k_1 — pseudo first-order rate constant
k_2 — pseudo second-order rate constant
R^2 — correlation coefficient
b — Langmuir constant

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