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Biosorption of Congo red and Indigo carmine by nonviable biomass of a new *Dietzia* strain isolated from the effluent of a textile industry

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ABSTRACT

A new bacterial strain, PD1, was isolated from the effluent of a textile industry. Phylogenetic analysis based on 16S rRNA gene sequence showed that the strain belonged to the genus *Dietzia*. The efficacy of the dried bacterial biomass as biosorbent for removal of acid dyes, namely Congo red (CR) and Indigo carmine (IC), from aqueous solutions was studied by performing batch equilibrium tests under different operating parameters such as initial dye concentration, pH, and temperature. The amount of dye adsorbed onto *Dietzia* sp. PD1 decreased with increasing pH while it increased with increasing temperature. The equilibrium biosorption data showed excellent fit to Langmuir isotherm as compared to Freundlich isotherm. The maximum biosorption capacity, calculated using the Langmuir model, were 170.34 and 188.71 mg g^{-1} for CR and IC, respectively. Analysis of kinetic data showed that the biosorption processes followed pseudo-second-order kinetics. The numerical value of the thermodynamic parameters (ΔG^0, ΔH^0, and ΔS^0) indicated that biosorption of CR and IC was feasible, spontaneous, and endothermic under the examined conditions. The study shows that the isolated *Dietzia* strain can be used as an inexpensive and efficient biosorbent for removal of acid dyes from aqueous solution.

Keywords: Biosorption; *Dietzia* sp. PD1; Congo red; Indigo carmine; Equilibrium; Kinetics

1. Introduction

Over the past few decades, dyes have become one of the major sources of severe water pollution as a result of rapid industrialization. Dyes present in the wastewater streams of industries, such as textile, leather, paper, printing, food, cosmetics, paint, pigments, petroleum, solvent, rubber, plastic, pesticide, and wood preserving chemicals, have triggered a major concern on the human health and marine lives, emphasizing the necessity for their removal [1,2].

Traditional wastewater treatment technologies like coagulation, biodegradation, electroflocculation, membrane filtration, ion-exchange, precipitation, and ozonation are both ineffective as well as expensive for handling colored effluents [3,4]. As a viable alternative, biosorption defined as the accumulation and concentration of organic and inorganic pollutants from aqueous solutions by biological materials through a combination of active and passive transport mechanisms, has received global attention due its simplicity and flexibil-

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ity of design, high selectivity and efficiency, low operating cost, and high-quality treated effluent [5–7]. There are reports on the use of biological materials such as yeast, fungi, algae, industrial wastes, agricultural wastes, and other polysaccharide materials as biosorbent for decolorization of dye wastewater [6]. However, there are few reports on biosorption of dyes by dead bacterial biomass and most research has focused on studying the biodegradation potential of bacteria [5]. Bacterial biomass can be used as a cheap source of biosorbent due to the presence of amino, carboxyl, phosphonate, and hydroxyl groups in the bacterial cell wall which are capable of binding dye molecules [8,9]. As compared with biodegradation, biosorption is a metabolism-independent process; thus there is no requirement for the supplement of any nutrients for maintaining growth of the biomass.

Dietzia is a fairly new emerging bacterial genus [10]. *Dietzia* strains are widely distributed in the environment and are being increasingly isolated from natural habitats because of their clinical, industrial, and environmental importance [11]. Till date, *Dietzia* species have been isolated from diverse environments worldwide, such as soda lakes, oil fields, soil, deep sea sediments, hot and cold deserts, decomposing reed rhizomes, skin and the intestinal tracts of marine fish, and clinical samples [11]. Here, we report for the first time a novel *Dietzia* strain isolated from the effluent of a textile industry that was capable of adsorbing acid dyes. The study focused on the possible use of the dried biomass of the *Dietzia* isolate as biosorbent for removal of Congo red (CR) and Indigo carmine (IC) from aqueous solution. We chose these dyes as model pollutants due to their wide presence in dyestuff effluents and reported toxicological effects [12,13]. The biosorption studies were carried out under various parameters such as pH, initial dye concentration, and temperature. Equilibrium biosorption data were analyzed by Langmuir and Freundlich isotherm models while the biosorption kinetic data were tested by the pseudo-first-order and the pseudo-second-order kinetic models. The thermodynamics of the biosorption processes was also investigated.

2. Materials and methods

2.1. Dyes and chemicals

CR (CI 22120, MF: $C_{32}H_{22}N_6Na_2O_6S_2$, FW: 696.7, λ_{max}: 570 nm) and IC (CI 73015, MF: $C_{16}H_8N_2Na_2O_8S_2$, FW: 466.36, λ_{max}: 610 nm) used in this study were of commercial grade and used as such without further purification. Dye stock solution (500 mg L^{-1}) was prepared by dissolving accurately weighed quantity of the dye in double distilled water. Experimental solutions of different concentration were prepared by diluting the stock solution with suitable volume of double distilled water. The initial solution pH was adjusted with 0.1 M HCl and 0.1 M NaOH solutions using a digital pH meter (LI 127, ELICO, India) calibrated with standard buffer solutions. All other chemicals used in this study were of analytical grade.

2.2. Isolation and identification of bacterial strain

Bacterial strain used in this study was isolated from dye effluent collected from a textile industry located in Rajarhat, Kolkata, India. The effluent sample was serially diluted 10-fold (1/10) seven times with 1% peptone water. The diluted effluent sample (0.1 mL) was spread on agar plates and incubated at 37 ± 1 °C for 5 days. Individual colonies appearing over this period were streaked onto mineral salt medium agar (MSMA) plates containing CR or IC (50 mg L^{-1}) as carbon source, and incubated at 37 ± 1 °C for 3 days. Among the five isolated bacterial strains, one strain was selected due to its high dye decolorization zone on the MSMA plates. The selected bacterial strain was designated PD1 and maintained on nutrient agar slants.

The isolate PD1 was identified by Merck Specialities Pvt. Ltd., Bangalore, India. The process involved isolation of the genomic DNA from pure culture pellet and amplification of the ~1.5 kB 16S rDNA fragment using *Taq* DNA polymerase, followed by bidirectional sequencing of the polymerase chain reaction product using forward, reverse, and an internal primer. The sequence data were then aligned and analyzed for finding the closest homologous microbes. The morphological, physiological, and biochemical characteristics of the strain were analyzed using standard procedures [14].

2.3. Biosorbent preparation

The strain PD1 was inoculated into 1 L Erlenmeyer flasks containing 500 mL nutrient broth (pH 5) and incubated for 24 h at 37 ± 1 °C, 100 rpm. At the exponential phase, it was autoclaved at 121 °C for 15 min. The cells were harvested by centrifugation at 10,000 × g for 20 min, rinsed several times with generous amounts of deionized water and then oven dried at 60 ± 1 °C for 24 h. The dried autoclaved cells were used as biosorbent.

2.4. Batch biosorption studies

Batch biosorption experiments were conducted in 250 mL glass-stoppered, Erlenmeyer flasks with 100 mL of dye solution (of desired concentration and pH). A

weighed amount (0.5 g) of biomass was added to the solution. The flasks were agitated at a constant speed of 150 rpm in an incubator shaker (Innova 42, New Brunswick Scientific, Canada) at a constant temperature of 40 ± 1°C (unless otherwise mentioned) until reaching equilibrium after which the biosorbent was separated from the solution by centrifugation at 8,000 rpm for 5 min. The residual dye concentration in the supernatant was then analyzed by monitoring the change in absorbance value at maximum wavelength (λ_{max}) using UV/VIS spectrophotometer (U-2800, Hitachi, Japan).

2.4.1. Effect of pH

The effect of pH on the biosorption capacity was studied in the range of 3–10 in batch experiments previously described. The amount of biomass was fixed at 0.5 g, and the initial dye concentration was 50 mg L^{-1}.

2.4.2. Effect of initial dye concentration

To investigate the effect of initial dye concentration on the biosorption efficiency, batch experiments were carried out by varying the initial dye concentration from 10 to 100 mg L^{-1}; the biomass dose fixed at 0.5 g.

2.4.3. Effect of temperature

In order to study the effect of temperature on the biosorption performance, batch experiments as above described were carried out with temperatures of 20, 30, and 40°C, respectively. The amount of biomass was fixed at 0.5 g while the initial dye concentration was 50 mg L^{-1}.

2.4.4. Equilibrium and kinetic studies

Biosorption equilibrium studies were carried out by agitating 100 mL dye solution of different concentration with 0.5 g biomass in 250 mL glass-stoppered Erlenmeyer flasks at predetermined temperature of 40°C. The flasks were agitated for 12 h which was sufficient to reach equilibrium. After equilibrium, the biosorbent was separated from the solution by centrifugation at 8,000 rpm for 5 min. The residual dye concentration in the supernatant was then analyzed by using a UV/Vis spectrophotometer (U-2800, Hitachi, Japan).

Kinetic studies were performed by agitating 0.5 g of biomass with 100 mL (50 mg L^{-1}) of dye solution in a 250 mL glass-stoppered Erlenmeyer flask at 40°C. Samples were collected from the flask at fixed time intervals and the residual concentration of dye in each sample was analyzed as described before.

2.5. Calculations

The amount of dye adsorbed per unit biomass (mg dye per g biosorbent) was calculated according to a mass balance on the dye concentration using Eq. (1):

$$q_e = \frac{(C_0 - C_e)\,V}{m} \quad (1)$$

where C_0 is the initial dye concentration (mg L^{-1}), C_e is the equilibrium dye concentration in solution (mg L^{-1}), V is the volume of the solution (L), and m is the mass of the biosorbent in g.

The percent removal (%) of dyes was calculated using the following equation:

$$\text{Removal}\ (\%) = \frac{C_0 - C_e}{C_0} \times 100 \quad (2)$$

2.6. Statistical analyses

In order to ensure the accuracy, reliability, and reproducibility of the collected data, all biosorption experiments were performed in triplicate, and the mean values were used in data analysis. Relative standard deviations were found to be within ± 3%. Microsoft Excel 2007 program was employed for data processing. Linear regression analyses were used to determine slopes and intercepts of the linear plots and for statistical analyses of the data.

3. Results and discussion

3.1. Identification of the bacterial isolate

Phylogenetic analysis of the nearly complete 16S rRNA gene sequence (1,451 bp) of strain PD1 suggested that it belonged to actinobacteria and was a member of the genus *Dietzia*, showing the highest sequence similarity of 99% with *Dietzia maris*. A phylogenetic tree, constructed on the basis of multiple sequence alignment of 16S rRNA gene sequences, showing the phylogenetic position of PD1 is illustrated in Fig. 1. The isolated strain was named as *Dietzia* sp. PD1. The 16S rRNA gene sequence of *Dietzia* sp. PD1 isolate was deposited in the GenBank nucleotide sequence database under accession number JQ414030. The morphological, physiological, and biochemical characteristics of the isolate are presented in Table 1.

3.2. Effect of pH

The pH of the dye solution is an important monitoring parameter in biosorption studies. It influences not only the surface charge of the biosorbent but also

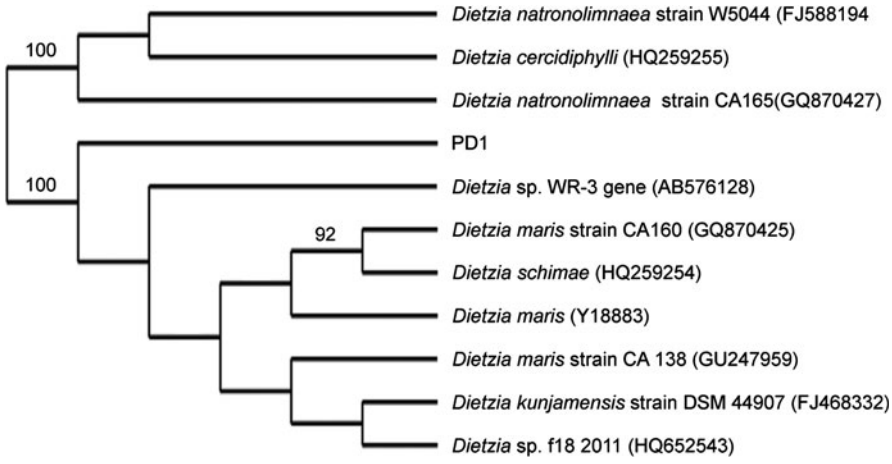

Fig. 1. Phylogenetic dendrogram for strain PD1 made using neighbor joining method showing the position of strain PD1 and species in the genus *Dietzia* based on the 16S rRNA gene sequence.

the degree of ionization of the dye present in the solution. Hence biosorption of the acid dyes by dried *Dietzia* sp. PD1 biomass was studied in the pH range of 3–10 at 50 mg L^{-1} initial dye concentration and the results are presented in Fig. 2(a). The biosorption of dyes by the bacterial biomass sharply decreases with increase in pH. The optimal pH value for dye removal is 3 and it was used for the subsequent experiments. This result is consistent with other works on biosorption of anionic dyes [15–17]. The medium pH affects the solubility of the dye and the ionization state of the functional groups like carboxyl, hydroxyl, phosphonate, and amino groups of the bacterial cell wall [8,9]. At low pH, the biosorbent is rich in positive dye-binding sites which attract the anionic dye molecule. On the other hand, at higher pH, the biosorbent carries a net negative charge resulting in electrostatic repulsion with the dye molecule. Lower biosorption at higher pH may also be due to the presence of excess OH^- ions competing with the negatively charged dye for the biosorption sites.

3.3. Effect of initial dye concentration

Batch biosorption experiments were also performed to study the effect of different initial dye concentration (10–100 mg L^{-1}) on the biosorption efficiency of dried biomass of *Dietzia* sp. PD1. Fig. 2(b) shows the plot of % dye removal against different initial dye concentration. The dye removal percentage decreases significantly with increase in initial dye concentration. This may be due to the saturation of the biosorption sites on the biosorbent with increase in dye concentration [18]. For a given biosorbent dose, the total number of available dye-binding sites is fixed, thereby adsorbing almost the same amount of dye, thus resulting in a decrease in the percentage removal of the dye with increase in initial dye concentration.

3.4. Effect of temperature

The effect of temperature on the biosorption of acid dyes by the bacterial biomass was investigated in the temperature range of 20–40°C and the results thus obtained are shown in Fig. 3(a). As seen from the figure, the dye uptake capacity increases with increase in temperature. The equilibrium biosorption capacity increases from 141.25 mg g^{-1} at 20°C to 165.28 mg g^{-1} at 40°C for CR while it increases from 147.28 mg g^{-1} at 20°C to 184.38 mg g^{-1} at 40°C for IC. Higher temperatures increase the rate of diffusion of the dye molecules across the external boundary layer and within the pores on the bacterial cell wall, due to decrease in the viscosity of the solution, thereby enhancing the dye-binding capacity of the biosorbent [1].

3.5. Biosorption isotherms

Equilibrium adsorption isotherms provide the most important piece of information in understanding a biosorption process. Therefore, in the present study, the experimental equilibrium data for biosorption of acid dyes by *Dietzia* sp. PD1 were fitted to the Freundlich and Langmuir adsorption isotherms.

The Freundlich model is described as [19]:

$$\log q_e = \log K_F + \left(\frac{1}{n}\right) \log C_e \quad (3)$$

where q_e (mg g^{-1}) is the equilibrium dye concentration on the biosorbent, C_e (mg L^{-1}) is the equilibrium dye

Table 1
Morphological, physiological, and biochemical characteristics of *Dietzia* sp. PD1

Morphological/physiological/ biochemical characteristics	*Dietzia* sp. PD1
Gram stain	+
Cell shape	Rod
Flagellum	Absent
Spore formation	Absent
Growth temperature (°C):	
Range	10–50
Optimum	37
pH for growth:	
Range	3–10
Optimum	5.0
LB agar culture characteristics	Smooth, circular, convex, wet, orange colonies
Indole test	–
Methyl red test	–
Voges–Proskauer test	–
Citrate utilization	–
Casein hydrolysis	–
Gelatin hydrolysis	–
Starch hydrolysis	–
Tween 80 hydrolysis	–
Catalase activity	+
Oxidase activity	–
Urease activity	–
Nitrate reduction	–
Growth on:	
Glucose	+
Fructose	–
Lactose	+
Maltose	–
Sucrose	+
Mannitol	+

concentration in solution, K_F (mg g^{-1}) (L g^{-1})$^{1/n}$ is the Freundlich constant related to sorption capacity, and n is the heterogeneity factor.

The Langmuir isotherm can be written as [19]:

$$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{K_L q_m C_e} \quad (4)$$

where q_m (mg g^{-1}) is the maximum biosorption capacity and K_L (L mg^{-1}) is the biosorption equilibrium constant.

The Freundlich model constants K_F and n were determined from the intercept and slope of the plots between log q_e and log C_e (plots not shown) while the Langmuir model parameters q_m and K_L were calculated from the intercept and slope of the plots between $1/q_e$ and $1/C_e$ (Fig. 3(b)). The model

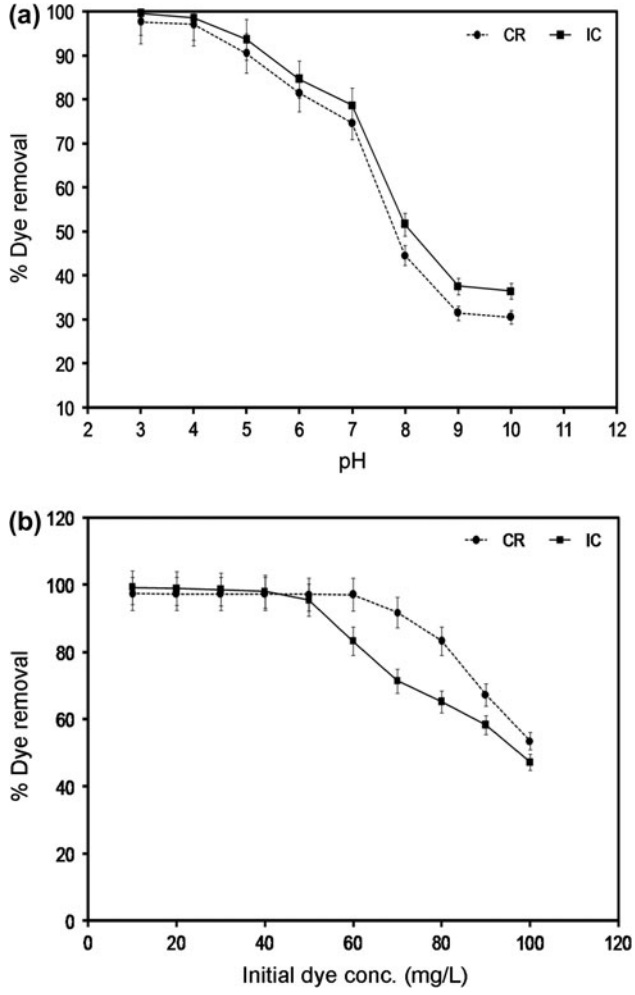

Fig. 2. (a) Effect of pH on the dye biosorption efficiency of *Dietzia* sp. PD1 and (b) Effect of initial dye concentration on biosorption of acid dyes by *Dietzia* sp. PD1.

parameters and constants thus obtained along with the correlation coefficients (R^2) are listed in Table 2. The low R^2 values for the Freundlich model suggest that this model was not suitable for describing the equilibrium biosorption data of CR and IC. The Freundlich constant n gives a measure of favorability of biosorption with the value of n between 1 and 10 representing a favorable biosorption [20]. In the current investigation, the values of n obtained for biosorption of CR and IC are $1 < n < 10$, suggesting that biosorption of CR and IC onto *Dietzia* sp. PD1 biomass can be considered as favorable. Unlike the Freundlich model, the comparatively high R^2 values for the Langmuir model establish the fact that biosorption took place at specific homogeneous sites within the biosorbent and that once the dye molecule occupied a site, no further adsorption could take place at that site, thereby forming a monolayer [20].

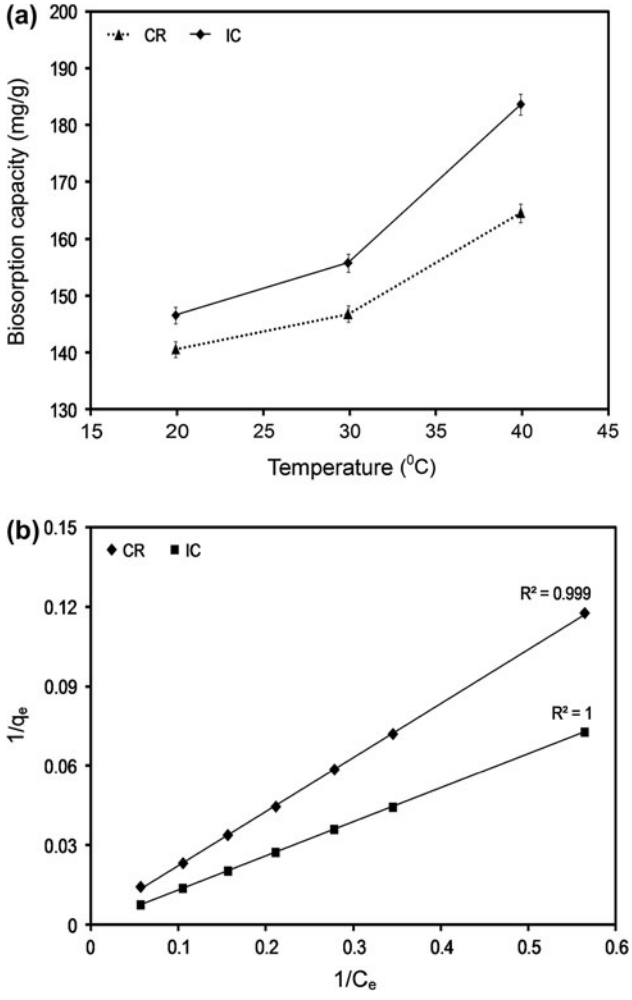

Fig. 3. (a) Effect of temperature on the equilibrium dye biosorption capacity of *Dietzia* sp. PD1 and (b) Linearized Langmuir isotherm plots for biosorption of acid dyes by *Dietzia* sp. PD1.

Table 3 outlines the comparison of q_m value of various biosorbents including *Dietzia* sp. PD1 for biosorption of CR and IC. *Dietzia* sp. PD1 biomass has higher dye biosorption capacity in comparison with many of the other reported biosorbents. Thus, it seems that this new isolate could be considered as an efficient and low-cost alternative biosorbent for treatment of dye-contaminated wastewaters.

Table 3
Comparison of maximum biosorption capacity of *Dietzia* sp. PD1 for CR and IC with other sorbents

Dye	Biosorbent	Maximum sorption capacity ($mg\,g^{-1}$)	Reference
CR	Orange peel	14.0	[21]
	Aspergillus niger	14.7	[22]
	Banana peel	18.2	[21]
	Cattail root	38.79	[23]
	Neem leaf powder	41.20	[24]
	Egg shells	69.45	[12]
	Pleurotus pulmonaris	93	[5]
	Dietzia sp. PD1	170.34	This study
IC	Bottom ash	0.00017	[25]
	De-oiled soya	0.00038	[25]
	Chitin	0.0058	[26]
	Chitosan	0.072	[26]
	Brazil nut shell	1.09	[27]
	Rice husk ash	29.28	[28]
	C. versicolor	98	[5]
	Dietzia sp. PD1	188.71	This study

3.6. Biosorption kinetics

The pseudo-first-order and pseudo-second-order kinetic models were applied in this study to investigate the reaction pathways and potential rate limiting steps of biosorption of CR and IC onto nonviable biomass of *Dietzia* sp. PD1.

The pseudo-first-order kinetic model has the following formulation [29]:

$$\ln(q_e - q_t) = \ln q_e - k_1 t \quad (5)$$

where q_e ($mg\,g^{-1}$) is the equilibrium dye concentration on the biosorbent, q_t ($mg\,g^{-1}$) is the amount of dye

Table 2
Langmuir and Freundlich adsorption isotherm constants for biosorption of CR and IC by *Dietzia* sp. PD1

Dye	Langmuir			Freundlich		
	q_m ($mg\,g^{-1}$)	K_L ($L\,mg^{-1}$)	R^2	K_F ($mg\,g^{-1}$) ($L\,mg^{-1}$)$^{1/n}$	n	R^2
CR	170.346	2.589	0.999	1.728	2.051	0.882
IC	188.171	2.933	1.000	2.492	2.867	0.895

Table 4
Kinetic parameters for biosorption of CR and IC by *Dietzia* sp. PD1

Dye	$q_{e,exp}$ (mg g^{-1})	Pseudo-first-order			Pseudo-second-order		
		$q_{e,cal}$ (mg g^{-1})	k_1 (h^{-1})	R^2	$q_{e,cal}$ (mg g^{-1})	k_2 (g mg^{-1} h^{-1})	R^2
CR	165.286	144.573	1.542	0.925	166.032	0.312	0.997
IC	184.664	161.167	2.484	0.913	185.498	0.525	0.993

Table 5
Thermodynamic parameters for biosorption of CR and IC by *Dietzia* sp. PD1

Dye	ΔG^0 (kJ mol^{-1})			ΔH^0 (kJ mol^{-1})	ΔS^0 (J mol^{-1} K^{-1})
	20 °C	30 °C	40 °C		
CR	−4.25	−5.74	−7.38	41.63	156.92
IC	−6.41	−7.53	−8.96	29.58	122.76

adsorbed at time t, and k_1 (h^{-1}) is the pseudo-first-order rate constant. A plot of $\ln(q_e - q_t)$ vs. t gives a straight line, with the slope and intercept giving the values of k_1 and q_e, respectively.

The pseudo-second-order kinetic model is expressed by the following equation [29]:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \tag{6}$$

where k_2 (g mg^{-1} h^{-1}) is the pseudo-second-order rate constant. The constants k_2 and q_e can be calculated from the intercept and slope of the linear plot of t/q_t vs. t.

Table 4 presents the value of the pseudo-first-order and pseudo-second-order model constants together with the correlation coefficients (R^2) for biosorption of CR and IC dyes. The kinetic data show an excellent correlation with the pseudo-second-order kinetic model. The R^2 values from the pseudo-second-order kinetic equation are significantly higher than those from the pseudo-first-order kinetic model. The calculated equilibrium uptake ($q_{e,cal}$) values from the pseudo-second-order model show a good agreement with the experimental values ($q_{e,exp}$). These findings suggest that the biosorption processes were in accordance with pseudo-second-order kinetics and that the overall rate of dye biosorption onto *Dietzia* sp. PD1 biomass was controlled by a chemical process.

3.7. Biosorption thermodynamics

The thermodynamic parameters such as Gibbs free energy change (ΔG^0), enthalpy (ΔH^0), and entropy (ΔS^0) were calculated using the following equations [29]:

$$\Delta G^0 = -RT \ln K_C \tag{7}$$

$$K_C = \frac{C_a}{C_e} \tag{8}$$

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \tag{9}$$

where R is the universal gas constant (8.314 kJ mol^{-1}), T is the temperature in K, K_C is the distribution coefficient for adsorption, C_a is the equilibrium dye concentration on the biosorbent (mg L^{-1}), and C_e is the equilibrium dye concentration in solution (mg L^{-1}). The values of ΔG^0 for biosorption of CR and IC are listed in Table 5. The values of ΔH^0 and ΔS^0 obtained from the slope and intercept of the plots of ΔG^0 as a function of T are also summarized in Table 5. The ΔG^0 values are negative, suggesting that the biosorption processes were feasible and spontaneous in nature. The positive value of ΔH^0 confirms the endothermic nature of biosorption of CR and IC. The positive value of ΔS^0 suggests the affinity of the biomass for the dyes.

4. Conclusion

In this study, a new *Dietzia* strain was isolated from the effluent of a textile industry. The dried biomass of the isolated strain was tested as an alternative biosorbent for removal of acid dyes, namely CR and IC, in a batch system. The following conclusions are made based on the results of present study:

- The biosorption efficiency increases with increasing temperature while it decreases with increasing solution pH and initial dye concentration.

- The Langmuir isotherm adequately describes the biosorption equilibrium suggesting monolayer biosorption on a homogeneous surface.
- The results of kinetics study show that the pseudo-second-order model fit well to the experimental biosorption data of CR and IC.
- Thermodynamic studies demonstrate feasible, spontaneous, and endothermic nature of biosorption of CR and IC by dried *Dietzia* sp. PD1 biomass.
- The results suggest that the dried native biomass of *Dietzia* sp. PD1 can be used as an efficient low-cost biosorbent for removal of acid dyes from wastewater.
- To date, it is the first *Dietzia* strain reported so far for biosorption of dyes from aqueous medium.

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