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Application of response surface methodology for the biosorption of Acid Blue 25 dye using raw and HCl-treated macroalgae

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ABSTRACT

The present study was conducted to optimize the various experimental conditions, such as biomass loading, initial C.I. Acid Blue 25 (AB25) dye concentration, and initial solution pH for biosorption of dye on raw and HCl-treated brown alga, *Padina australis* and red alga, *Jania adhaerens*. Biosorption process was optimized in a batch system under Box–Behnken design. Second-order polynomial equation was successfully used to describe the effects of studied variables on response. The quadratic models exhibited higher R^2 values, significant *p*-values, and insignificant lack-of-fit *p*-values showed high adequacy for predicting the response. Chemically modified red alga exhibited better AB25 dye biosorption capacity as compared to modified brown alga. Maximum dye removal efficiencies of 77.34, 71.28, 50.56, and 85.19% for *P. australis*, HCl-treated *P. australis*, *J. adhaerens*, and HCl-treated *J. adhaerens*, respectively, were obtained at optimal conditions. The surface modification on tested algal biomass was found to be strongly dependent on their cell wall constituents.

Keywords: Response surface methodology; Acid Blue 25 dye; Biosorption; Chemical modification; Macroalgae

1. Introduction

Dyes are extensively used in textile dyeing, paper printing, color photography, pharmaceutical, food, cosmetic, and leather industries [1,2]. Wastewaters containing dye molecules are difficult to treat, since the dyes are recalcitrant pollutants, resistant to aerobic digestion, and stable when exposed to oxidizing agents. Low concentrations of dyes also make the dye wastewater treatment difficult. In practice, no single process provides adequate treatment and a combination of different processes is often used to achieve the desired water quality in the most economical way [3].

There is an urgent need to develop an economic and effective treatment method for dyeing

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wastewaters in the current scenario of the growing production activities [4]. Presently, different methods are available for the treatment of dye wastewaters. These include physicochemical methods, like adsorption, chemical oxidation, precipitation, coagulation, filtration, electrolysis, photo-degradation, and biological/microbiological methods. The major disadvantage of physicochemical methods has been largely due to the high cost, low efficiency, limited versatility, interference by other wastewater constituents, and lack of waste management [5].

Adsorption in comparison with the other listed processes for the treatment of polluted aqueous effluents, allows flexibility in design and operation, and produces aesthetically superior effluent sometimes suitable for reuse. In addition, as adsorption is sometimes reversible, the regeneration of the sorbent might be possible, which makes the process economically feasible [6]. Use of activated carbon has been found to be effective, but it is very expensive. Therefore, many studies have been undertaken previously by various researchers to investigate the use of inexpensive adsorbents, such as peat, bentonite, steel-plant slag, fly ash, china clay, maize cob, wood shavings, and silica [7–9], or bacteria, fungi, yeasts, and algae [5,10,11] for color removal from dye wastewater. Biological removal of AB25 in the presence of some organisms has been reported previously [12,13] and has shown promising results.

Algae are biological resources, which are easily available in considerable quantities in both fresh and saltwater. Algal cell wall constitutes several functional groups, e.g. hydroxyl, carboxylate, amino, and phosphate, which played an important role in pollutant removal from wastewater [14]. In this study, *Padina australis* and *Jania adhaerens* biomasses have been used for the treatment of a dye solution containing C.I. Acid Blue 25 (AB25) dye. *P. australis* is common in tide pools and reef flats glued to solid substrates and are relatively widespread. This species prospers chiefly in tropical and subtropical waters [15]. The marine red alga *J. adhaerens* inhabit in a broad range of marine environments in the Pacific, Caribbean, and Gulf of Mexico coasts of North and Central America [16]. This work therefore aimed to optimize the biosorption of AB25 dye onto raw and HCl-treated brown/red macroalgae using the response surface methodology. The effects of different experimental parameters viz. initial solution pH, initial dye concentration, and initial biosorbent dosage were investigated by applying Box–Behnken design under response surface methodology using Design Expert software (Version 8.0.4).

2. Materials and methods

2.1. Biomass preparation

P. australis and *J. adhaerens* were used as biosorbents for the biosorption of AB25 dye. Samples of marine algae were collected from the Oman Sea coast of Chabahar, Iran. The algae were first washed with tap water, followed by several washings with distilled water to remove extraneous materials and salts. The biomass was then sun-dried for three days followed by drying at 70°C for 24 h in an oven (Lab Tech, LDO-060 E, USA). The dried algal biomass was cut, sieved, and the particles in range of 106–250 μm were used for the experiments.

Modification of biomass was carried out with HCl. This treatment results in esterification of carboxylic acids present on the biomass and reduces the expulsive electrostatic effects between carboxylic groups and anionic dye molecules. Five grams of biomass was suspended in 500 mL of 1 M hydrochloric acid.

Table 1
General characteristics of C.I. Acid Blue 25 dye

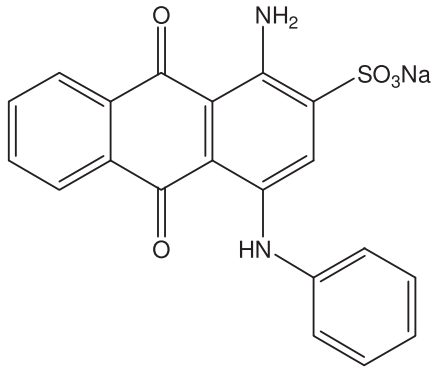
Chemical structure	
C.I. number	62065
C.I. name	Acid Blue 25
Class	Anthraquinone
Ionization	Acidic
λmax (nm)	600

Table 2
Experimental ranges and levels in the response surface design

	Range and level		
Factors	−1	0	+1
x_1: Biomass loading, g/L	0.2	0.8	1.4
x_2: Initial AB25 dye concentration, mg/L	20	60	100
x_3: Initial solution pH	2	4	6

Table 3
Experimental design of real values along with experimental and predicted values for removal efficiency (%) of AB25 dye by untreated and HCl-treated *P. australis* and *J. adhaerens* biomasses

Run	Real values			AB25 removal efficiency (%)							
				P. australis				*J. adhaerens*			
				Untreated		HCl treated		Untreated		HCl treated	
	x_1	x_2	x_3	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted	Experimental	Predicted
1	0.8	60	4	20.80	16.47	30.31	29.59	11.05	10.29	23.33	23.71
2	0.8	60	4	3.87	2.47	12.15	10.15	9.61	10.29	23.69	23.71
3	0.8	60	4	13.10	12.53	17.74	18.81	11.17	10.29	26.10	23.71
4	1.4	20	4	47.14	48.47	42.23	43.20	15.75	16.06	41.96	43.73
5	0.8	20	6	26.07	27.46	54.23	56.23	18.08	17.01	11.11	12.16
6	0.8	60	4	77.97	77.34	71.23	71.28	11.17	10.29	28.63	23.71
7	0.2	20	4	17.43	16.47	32.36	29.59	6.47	8.02	20.73	19.09
8	1.4	60	2	18.41	18.34	36.32	35.29	34.64	35.13	85.80	85.20
9	0.2	60	2	16.35	16.47	27.90	29.59	34.76	34.00	50.77	53.59
10	0.2	60	6	14.78	16.47	30.91	29.59	4.79	4.31	5.03	5.63
11	0.8	100	2	73.77	73.02	74.46	72.41	39.28	40.34	60.10	59.06
12	0.8	60	4	15.63	16.47	26.22	29.59	10.21	10.29	21.64	23.71
13	1.4	100	4	61.09	61.16	51.59	52.61	12.75	11.20	31.15	32.79
14	0.8	60	4	13.82	16.47	29.83	29.59	8.53	10.29	18.88	23.71
15	0.8	100	6	20.89	21.45	35.25	34.18	21.96	22.75	17.19	18.36
16	0.8	20	2	15.89	14.56	42.23	41.25	51.17	50.38	85.40	84.22
17	1.4	60	6	8.89	9.52	7.92	7.88	13.10	13.86	23.21	20.39
18	0.2	100	4	13.36	14.12	22.33	24.38	8.88	8.57	12.83	11.06

Note: x_1: Biomass loading, g/L; x_2: Initial AB25 concentration, mg/L; x_3: Initial solution pH.

The mixture was shaken on a rotary shaker (Labcon, FSIM-SPO16, USA) for 3 h at 130 rpm, and was allowed to stand for 24 h. The suspension was then filtered, followed by distilled water washing. Biomass was dried at 70˚C for 24 h and then sieved for particle size of 106–250 μm. All experiments were conducted at room temperature (25 ± 2˚C).

2.2. Analytical methods

All chemicals used in this study were of analytical grade. C.I. Acid Blue 25 dye [1-amino-9,10-dihydro-9,10-dioxo-4-(phenyl amino)-2-anthracenesulfonic acid, monosodium salt] (AlvanSabet Co. Iran) was used as received (Table 1). An accurately weighed quantity of the dye was dissolved in distilled water to prepare a stock solution (1,000 mg/L) and the solutions for all tests were prepared from this solution for the desired concentrations through successive dilutions. The initial solution pH was adjusted by diluted HCl or NaOH. The pH measurements were made using pH meter (Metrohm, 620, Switzerland). The resulting solutions were filtered through 0.2 μm membrane filters and analyzed quantitatively. For the absorbance measurement of samples, UV–vis spectrophotometer (Hach, DR/4000 Spectrophotometer, USA) was used. The maximum wavelength used for determination of residual concentration of AB25 dye in supernatant solution was 600 nm.

2.3. Experimental design

To employ Box–Behnken design, we need prior knowledge on the upper and lower limits of the parameters and awareness of the sorption process and its factors. So, we started an initial experiment to determine upper and lower limits of the effective parameters. The effects of initial dye concentration, initial solution pH, and biomass loading on the biosorption process were investigated from these experiments. Therefore, these three variables were chosen to obtain the optimum levels. A three level Box–Behnken design was used to determine the optimal values for these factors [17]. Three variables namely initial dye concentration (20–100 mg/L), initial solution pH (2–6), and biomass loading (0.2–1.4 g/L) were used and their lowest and the highest levels were fixed (Table 2). The design was employed to investigate the performance of the biosorption percentage as response variables. Each independent variable had three levels: −1, 0, and 1. A Box–Behnken design with a total of 18 different combinations was constructed by Design Expert software (Version 8.0.4, Stat-Ease, Inc., Minneapolis, USA) statistical package.

Table 4
ANOVA results for removal efficiency (%) of AB25 dye by untreated and HCl-treated *P. australis* and *J. adhaerens* biomasses

Biosorbent	Source	Sum of squares	DF	Mean square	*F*-value	*p*-value
P. australis	Model	8484.73	9	942.75	187.21	<0.0001
	Residual	40.29	8	5.04		
	Lack of fit	10.01	3	3.34	0.55	0.6693
	Pure error	30.28	5	6.06		
	Cor total	8525.02	17			
HCl treated *P. australis*	Model	5475.10	9	608.34	103.82	<0.0001
	Residual	46.88	8	5.86		
	Lack of fit	22.68	3	7.56	1.56	0.3086
	Pure error	24.19	5	4.84		
	Cor total	5521.98	17			
J. adhaerens	Model	2920.61	9	324.51	163.62	<0.0001
	Residual	15.87	8	1.98		
	Lack of fit	10.14	3	3.38	2.95	0.1372
	Pure error	5.73	5	1.15		
	Cor total	2936.48	17			
HCl treated *J. adhaerens*	Model	9313.61	9	1034.85	91.14	<0.0001
	Residual	90.83	8	11.35		
	Lack of fit	33.19	3	11.06	0.96	0.4797
	Pure error	57.64	5	11.53		
	Cor total	9404.44	17			

Note: DF: Degree of freedom.

Table 5
Regression analysis for acquired removal efficiencies (%) of AB25 dye by untreated and HCl-treated *P. australis* and *J. adhaerens* biomasses

		Coefficient estimate		Standard error		*F*-value		*p*-value (Probability > *F*)	
Biosorbent	Factor	Untreated	HCl treated	Untreated	HCl treated	Untreated	HCl treated	Untreated	HCl treated
P. australis	Intercept	16.47	29.59	0.92	0.99				
	x_1	8.48	15.36	0.79	0.86	114.15	322.21	<0.0001	<0.0001
	x_2	−4.02	−7.68	0.79	0.86	25.65	80.52	0.0010	<0.0001
	x_3	−25.43	−16.34	0.79	0.86	1027.31	364.48	<0.0001	<0.0001
	x_1x_2	1.01	−3.35	1.12	1.21	0.82	7.64	0.3927	0.0245
	x_1x_3	−5.96	1.32	1.12	1.21	28.20	1.20	0.0007	0.3058
	x_2x_3	1.91	2.22	1.12	1.21	2.90	3.37	0.1272	0.1038
	x_1^2	−2.34	−2.51	1.07	1.16	4.73	4.69	0.0613	0.0623
	x_2^2	1.85	2.76	1.07	1.16	2.96	5.69	0.1236	0.0442
	x_3^2	23.34	13.82	1.07	1.16	472.07	142.30	<0.0001	<0.0001
J. adhaerens	Intercept	10.29	23.71	0.57	1.38				
	x_1	2.67	11.59	0.50	1.19	28.72	94.69	0.0007	<0.0001
	x_2	−1.08	−4.74	0.50	1.19	4.66	15.84	0.0628	0.0041
	x_3	−12.74	−28.19	0.50	1.19	654.82	559.95	<0.0001	<0.0001
	x_1x_2	−1.35	−0.73	0.70	1.68	3.69	0.19	0.0910	0.6771
	x_1x_3	2.11	−4.21	0.70	1.68	8.95	6.25	0.0173	0.0369
	x_2x_3	3.94	7.84	0.70	1.68	31.38	21.67	0.0005	0.0016
	x_1^2	−5.06	0.35	0.67	1.61	56.37	0.05	<0.0001	0.8314
	x_2^2	5.74	2.60	0.67	1.61	72.36	2.60	<0.0001	0.1456
	x_3^2	16.60	17.14	0.67	1.61	605.99	112.88	<0.0001	<0.0001

Note: x_1: Biomass loading, g/L; x_2: Initial AB25 dye concentration, mg/L; x_3: Initial solution pH.

All experiments were carried out in batch mode at 25 ± 2˚C on a rotary shaker at 130 rpm using 100 mL conical flasks.

3. Results and discussion

3.1. Fitting the process models

In the present study, the effect of three parameters, namely biomass loading, initial AB25 dye concentration, and initial solution pH on AB25 dye biosorption was studied. The subsequent statistical analysis was performed by ANOVA. Box–Behnken design matrix was used for real and coded values. The experimental and predicted values for removal efficiency (%) of AB25 dye by untreated *P. australis* and *J. adhaerens* and HCl-treated *P. australis* and *J. adhaerens* are shown in Table 3. The maximum dye removal efficiency (%) were found to be 77.97, 74.46, 51.17, and 85.8% for untreated *P. australis*, HCl-treated *P. australis*, untreated *J. adhaerens*, and HCl-treated *J. adhaerens*, respectively (Table 3).

The quadratic model was employed to find out the relationship between variables and responses. The tests for significance of regression model were evaluated and the results of ANOVA tests are presented in Table 4. Model validity and adequacy were tested through lack-of-fit, *F*-values, and *p*-values. The ANOVA for the systems confirms the adequacy of the quadratic model, since the probability > *F* was found to be < 0.0001, indicating the significance of the proposed model. The *p*-values of lack-of-fit for AB25 removal onto *P. australis*, *J. adhaerens*, HCl-treated *P. australis*, and *J. adhaerens* were 0.55, 2.95, 1.56, and 0.96, respectively. The lack of fit *p*-value was greater than 0.05 (Table 4). Nonsignificant lack-of-fit explained that quadratic model was adequate to describe for the sorption AB25 onto all biomasses.

The calculated *p*-value of 0.0001 was found to be below the chosen 95% significance level and so the null hypothesis might be rejected (i.e. 95% confidence), indicating that the models can be considered significant. The high-model *F*-value (i.e. 187.21 for *P. australis*, 103.82 for HCl-treated *P. australis*, 163.62 for *J. adhaerens*, and 91.14 for HCl-treated *J. adhaerens*) demonstrate that the models are significant. Corresponding values of $p < 0.0001$ also indicated that all model terms are significant.

The regression analysis for suggested models is given in Table 5. The large quantity of the *F*-value and the smaller *p*-value indicates the significance of the corresponding coefficient [18]. As can be seen in Table 5, the quadratic model main, square and

Table 6
Final equations and their regression results

Final equations[a]	R^2	Adjusted R^2	Predicted R^2	Adequate Precision	SD[b]	CV[c]	PRESS[d]
$Y_1 = 16.47 + 8.48x_1 - 4.02x_2 - 25.43x_3 + 1.01x_1x_2 - 5.96x_1x_3 + 1.91x_2x_3 - 2.34x_1^2 + 1.85x_2^2 + 23.34x_3^2$	0.9953	0.9900	0.9761	44.7589	2.2441	8.4283	203.7370
$Y_2 = 29.59 + 15.36x_1 - 7.68x_2 - 16.34x_3 - 3.35x_1x_2 + 1.32x_1x_3 + 2.22x_2x_3 - 2.51x_1^2 + 2.76x_2^2 + 13.82_3^2$	0.9915	0.9820	0.9280	35.7695	2.4206	6.7531	397.7694
$Y_3 = 10.29 + 2.67x_1 - 1.08x_2 - 12.74x_3 - 1.35x_1x_2 + 2.11x_1x_3 + 3.94x_2x_3 - 5.06x_1^2 + 5.74x_2^2 + 16.6x_3^2$	0.9946	0.9885	0.9419	43.8928	1.4083	7.8388	170.4834
$Y_4 = 23.71 + 11.59x_1 - 4.74x_2 - 28.19x_3 - 0.73x_1x_2 - 4.21x_1x_3 + 7.84x_2x_3 + 0.35x_1^2 + 2.6x_2^2 + 17.41x_3^2$	0.9903	0.9795	0.9347	31.6805	3.3696	10.3232	614.0739

[a]x_1: Biomass loading, g/L; x_2: Initial AB25 dye concentration, mg/L; x_3: Initial solution pH.
[b]SD: Standard deviation.
[c]CV: Coefficient of variation.
[d]PRESS: Predicted residual error sum of squares.
(Y_1) *P. australis*; (Y_2) HCl-treated *P. australis*; (Y_3) *J. adhaerens*; (Y_4) HCl-treated *J. adhaerens*.

interaction effects of biomass loading, initial AB25 dye concentration, and initial solution pH are significant model terms.

The second order equation response was used to correlate the dependent and independent variables (Eq. (1)):

$$Y = b_0 + b_1x_1 + b_2x_2 + b_3x_3 + b_{12}x_1x_2 + b_{13}x_1x_3 + b_{23}x_2x_3 + b_{11}x_1^2 + b_{22}x_2^2 + b_{33}x_3^2 \quad (1)$$

where Y is the response variable of removal efficiency, b_0 is the constant, b_1, b_2, and b_3 are the linear coefficients, b_{12}, b_{13}, and b_{23} are the cross product coefficients, b_{11}, b_{22}, and b_{33} are the quadratic coefficients, x_1 is the biomass loading, x_2 is the dye concentration, and x_3 is the solution pH. The removal efficiencies have been predicted using the equations in Table 6. A positive sign in the equations represents a synergistic effect of the variables, while a negative sign indicates an antagonistic effect of the variables on the responses. The R^2 and adjusted R^2 values presented in Table 6 for *P. australis*, HCl-treated *P. australis*, *J. adhaerens*, and HCl-treated *J. adhaerens*, respectively, suggest a high correlation between the experimental and the predicted values (see Fig. 1). The predicted R^2 values in Table 6 were in acceptable agreement with the adjusted R^2.

Adequate precision is a measure of the range in predicted response relative to its associated error. Its desired value is four or more [19]. It means that regression models provide a good prediction of the relationship between the process variables and their response (AB25 dye removal efficiency). The large "Adequate Precision" values in Table 6 for each treatment indicates an adequate signal. Hence, the quadratic model can be used to navigate the design space. The coefficient of variation (CV) and standard deviation (SD) indicate the degree of precision. The low values of CV and SD exhibits the high reliability of model. The predicted residual sum of squares (PRESS) is a measure of how well the model fits each point in two models. The smaller PRESS shows that the model fits well with the data points. Therefore, these models were used for further analysis.

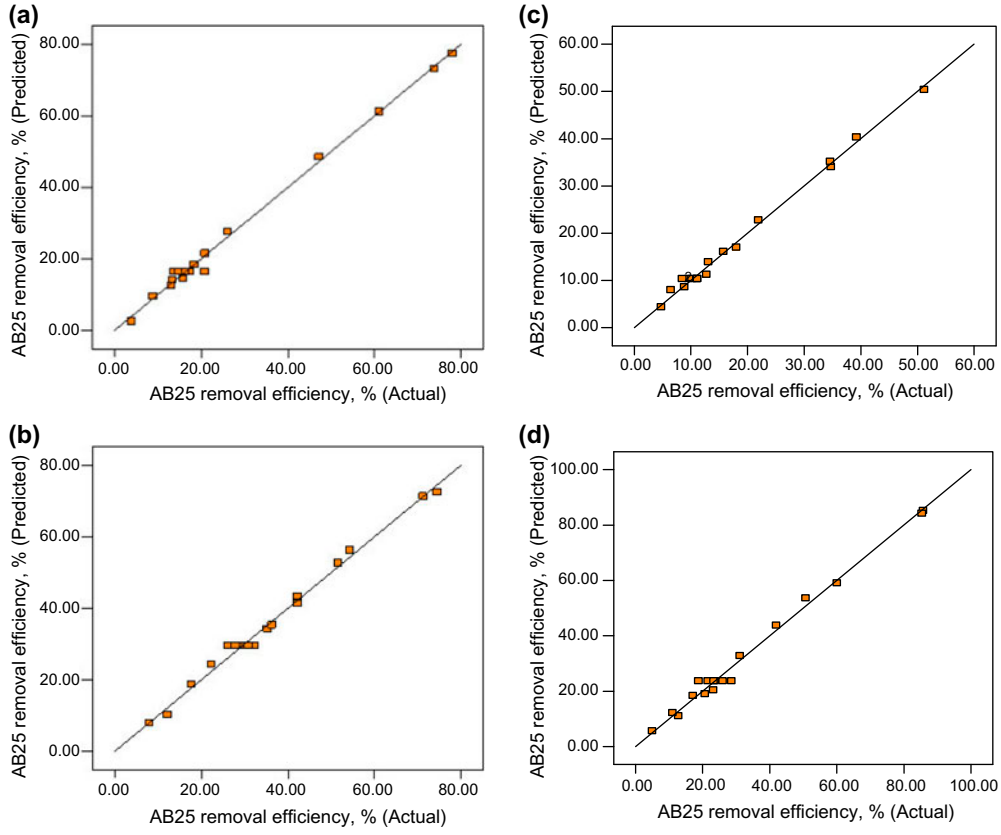


Fig. 1. Actual vs. predicted response plots for AB25 dye removal efficiency onto (a) *P. australis*, (b) HCl-treated *P. australis*, (c) *J. adhaerens*, and (d) HCl-treated *J. adhaerens*.

3.2. Effects of process variables

The investigated parameters which have a considerable influence on the removal of AB25 dye are biomass loading, initial AB25 dye concentration, and initial solution pH. Biosorbent dosage is an important parameter as it determines the percentage of decolorization and might also be used to predict the cost of biomass per unit of the dye solution to be treated. The initial dye concentration is the driving force to overcome the mass transfer resistance of all molecules between sorbent and sorbate. The initial solution pH significantly influences the overall biosorption process. Other variables, such as the temperature, speed of agitation, and reaction time were kept constant (25 ± 2°C, 130 rpm and 60 min, respectively). The contour plots are graphical representations of the regression equation. These plots show the type of interaction between the tested variables and hence indicate the optimum conditions. The contour plots of the second-order polynomial equation with one variable kept constant and the other two varying within the determined experimental ranges are given in Figs. 2–4. The maximum predicted value is represented by the surface confined in the smallest ellipse in the contour diagram [20].

Fig. 2(a)–(d) depict the effect of biomass loading (0.2–1.4 g/L) and initial AB25 dye concentration (20–100 mg/L) on the sorption potential of *P. australis*, HCl-treated *P. australis*, *J. adhaerens*, and HCl-treated *J. adhaerens*, for dye removal in an aqueous solution. The contour plots of the RSM have been presented as a function of two variables (i.e. biomass loading and dye concentration 0), while the pH was kept constant at 4.0. Fig. 2(a), (b) and (d) exhibit the effect of *P. australis*, HCl-treated *P. australis*, and HCl-treated *J. adhaerens* biomass loading on the removal efficiency of AB25 dye. As can be seen in these figures, it seems that removal efficiency of AB25 dye was influenced with the increase in the biomass loading. The AB25 dye removal efficiency was increased with an increase in biomass loading from 0.2 to 1.4 g/L, when initial AB25 dye concentration decreased from 100 to 20 mg/L. Therefore, a maximum removal efficiency of 27.46% for *P. australis*, 56.23% for HCl-treated *P. australis*, and 43.72 for HCl-treated *J. adhaerens* was

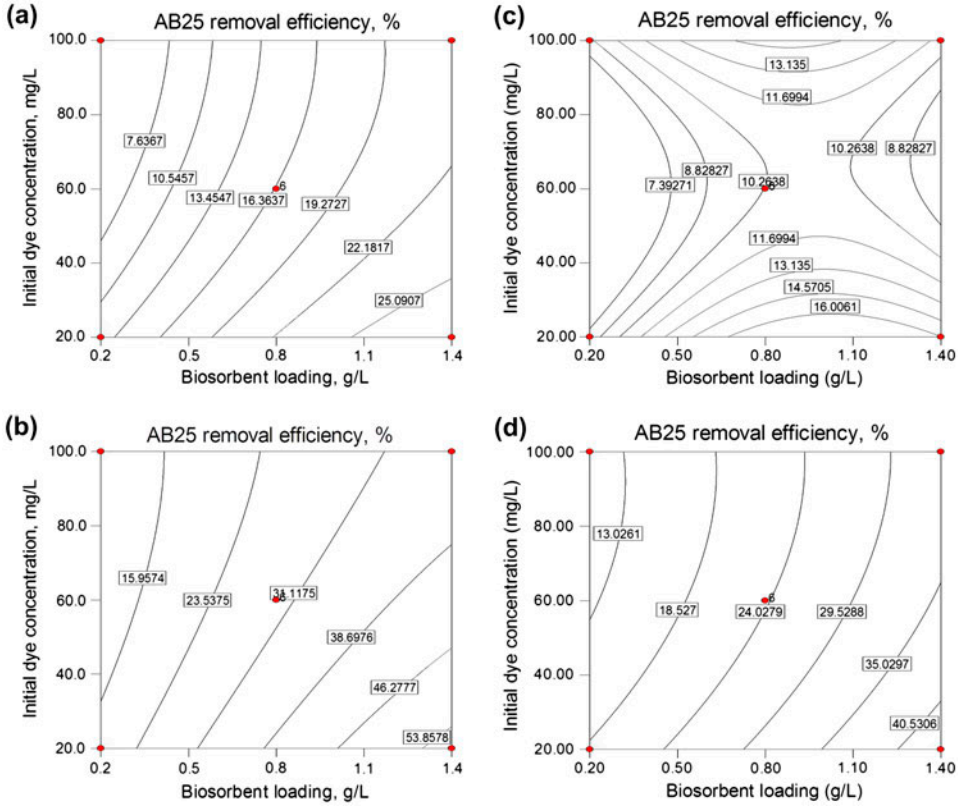


Fig. 2. Contour plot showing the combined effects of biomass loading and initial dye concentration on the removal % of AB25 dye: (a) *P. australis*, (b) HCl-treated *P. australis*, (c) *J. adhaerens*, and (d) HCl-treated *J. adhaerens*.

obtained at initial dye concentration of 20 mg/L and biomass loading of 1.4 g/L. This observation can attributed to the fact that increase of algal biomass loading provides more surface area and more binding sites for sorption of the AB25 dye on the surface of algae [11]. The finding was in agreement with literature where higher biomass loading would result in higher removal efficiency [21]. Fig. 2(c) exhibits that AB25 removal efficiency onto untreated *J. adhaerens* biomass increased with an increase in biomass loading from 0.2 to 1.04 g/L, and then decreased with an increase in biomass loading to 1.4 g/L. A maximum removal efficiency of AB25 dye 17.89% was achieved when biomass loading and dye concentration were 1.04 g/L and 20.03 mg/L, respectively. On the other hand, the decrease in dye removal efficiency observed at biomass levels higher than the optimum values was suggested to be the result of increasing particle interaction and aggregation, leading to a reduction of total biosorbent surface area [22]. Such aggregation would lead to decrease in total surface area of the biosorbent and an increase in diffusional path length [23]. Similar observation was reported when higher biomass loading of *Cystoseira indica* and *Gracilaria persica* biomasses resulted in lower Acid Black 1 removal efficiency [24].

The simultaneous interactions between the biomass loading and initial solution pH are shown in Fig. 3(a)–(d). Experiments for 60 mg/L AB25 dye removal by *P. australis*, HCl-treated *P. australis*, *J. adhaerens*, and HCl-treated *J. adhaerens* biomasses were carried out in the pH range of 2–6. The removal efficiency of AB25 dye was influenced by the changes in solution pH. The AB25 dye removal efficiency increased with a decrease in pH from 6.0 to 2.0, when biomass loading was increased from 0.2 to 1.4 g/L for *P. australis*, HCl-treated *P. australis*, and HCl treated *J. adhaerens* and 0.2–1.04 g/L for *J. adhaerens*. In this case, maximum AB25 dye removal efficiency was obtained; 71.28% for *P. australis*, 77.34% for HCl-treated *P. australis*, and 85.19% for HCl-treated *J. adhaerens* at initial solution pH 2.0 and biomass loading of 1.4 g/L and 39.43% for untreated *J. adhaerens* with 1.04 g/L biomass loading and initial solution pH 2.0.

In general, isoelectric point of pH around 3.0–4.0 is determined for all algal species [25]. With decrease

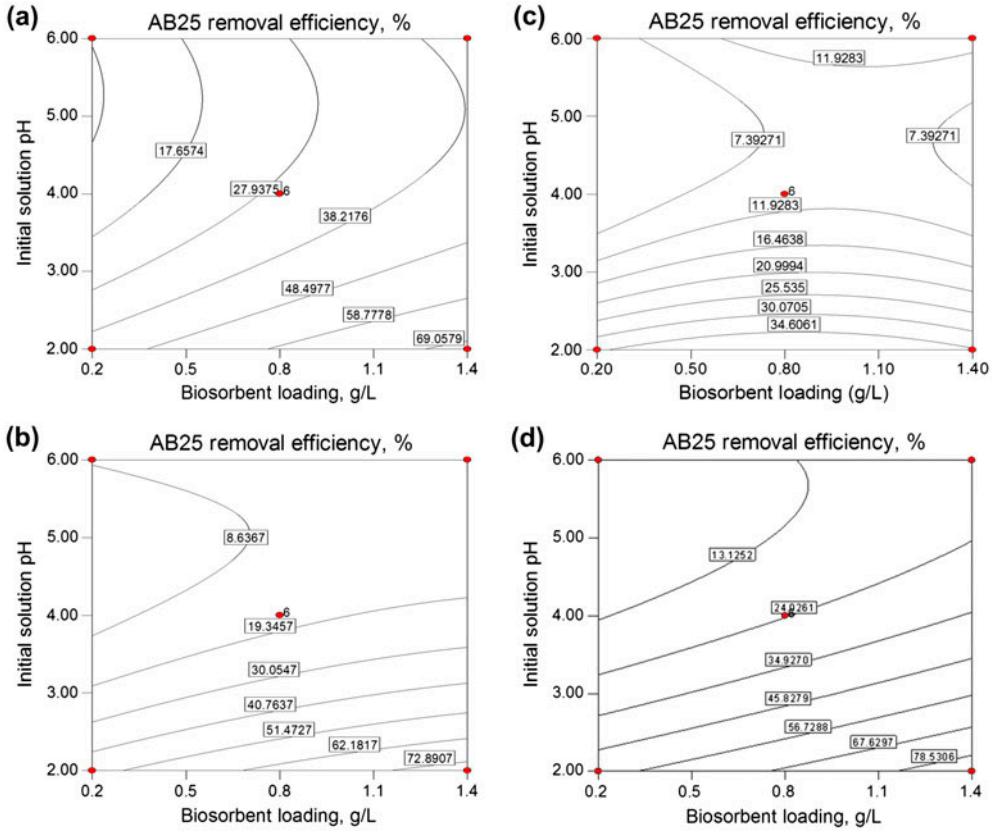


Fig. 3. Contour plot showing the interactive effect of biomass loading and initial solution pH on the removal % of AB25 dye: (a) *P. australis*, (b) HCl-treated *P. australis*, (c) *J. adhaerens*, and (d) HCl-treated *J. adhaerens*.

of pH, the H^+ ion concentration in the system increases and the surface of biomass achieves a positive charge by adsorbing H^+ ions. Positively charged surface sites on biomass favored the adsorption of the anionic dye molecules due to electrostatic interaction, leading to maximum AB25 dye removal efficiency.

On the other hand, when the pH of the solution increases, the number of negatively charged sites increases by absorbing hydroxyl ions. As the biomass surface is negatively charged at high pH, a significantly high electrostatic repulsion exists between the negatively charged surface of biomass and the anionic dye molecules, causing the decrease in AB25 dye removal efficiency. Similar observations were previously reported for biosorption of AB25 by the brown macroalga *Stoechospermum marginatum* [26] and diatomite [12].

As can be seen from Fig. 3(a) and (b), removal efficiency of AB25 on untreated and treated *P. australis* approximately is same, while AB25 removal efficiency for pretreated *J. adhaerens* with respect to untreated *J. adhaerens*, considerably increased. The success of chemical pretreatment strongly depends on the cellular components of the biomass itself [27]. *P. australis* and *J. adhaerens* belong to *Phaeophyta* and *Rhodophyta phylumes*, respectively. The *Phaeophyta* algal cell walls predominantly consist of alginic acid or alginate with a smaller loading of sulfated polysaccharide (fucoidan), whereas the *Rhodophyta* contains a number of sulfated galactans, such as agar, carregeenan, and porphyran [28]. Hence, the difference in the AB25 dye removal efficiency may be due to the difference in the adsorbent cell structure.

Fig. 3(c) and (d) shows the removal efficiency of AB25 dye onto *J. adhaerens* and HCl-treated *J. adhaerens*. Maximum removal efficiency of AB25 dye for *J. adhaerens* and HCl-treated *J. adhaerens* were obtained 39.43 and 85.19%, respectively. This is because some of the impurities and ions blocking the binding sites can be easily eliminated [29]. Hence, removal efficiency increased along with an increase of dye binding sites on adsorbent. A similar result for acid pretreatment was previously reported [30].

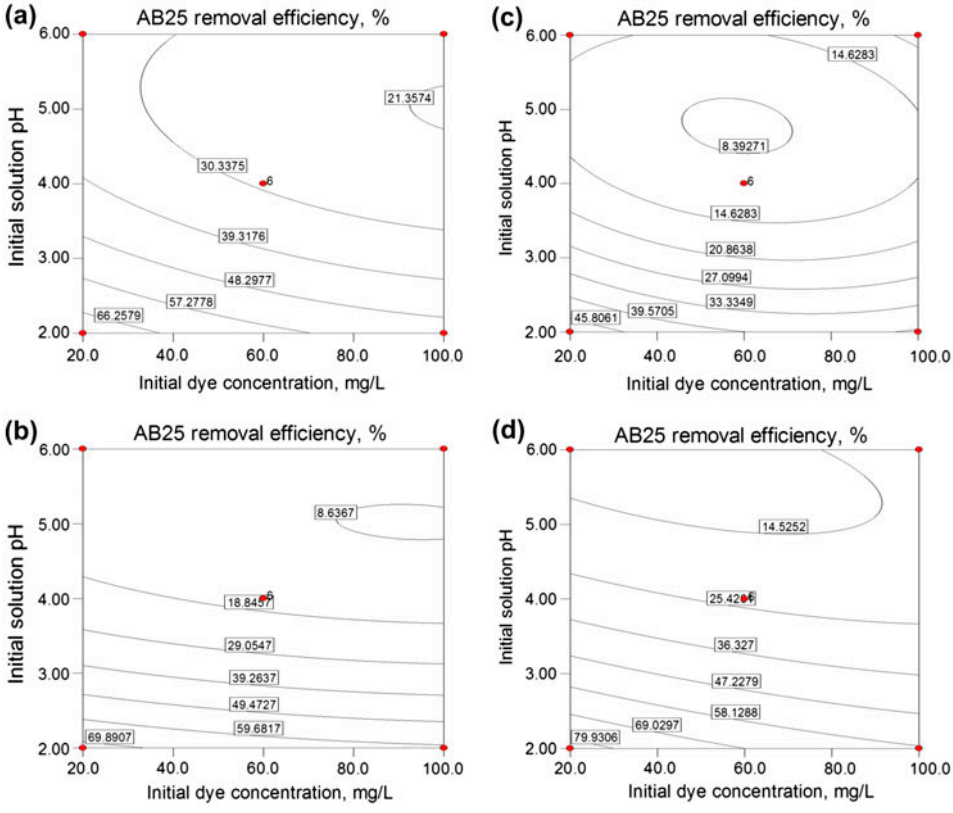


Fig. 4. Contour plot showing the interactive effect of initial dye concentration and initial solution pH on the removal % of AB25 dye: (a) *P. australis*, (b) HCl-treated *P. australis*, (c) *J. adhaerens*, and (d) HCl-treated *J. adhaerens*.

The AB25 dye removal as a function of initial AB25 dye concentration and initial solution pH for the untreated and HCl-treated nonliving biomasses were also studied that are depicted in Fig. 4(a)–(d). The removal efficiency of AB25 dye varied with the changes in the initial AB25 dye concentration. Fig. 4(a) and (b) demonstrates that the removal efficiency of AB25 dye was significantly increased with a decrease in initial solution pH from 6.0 to 2.0, when initial dye concentration was decreased from 100 to 20 mg/L. A maximum removal efficiency of 71.42% for *P. australis* and 73.02% for HCl-treated *P. australis* was obtained at initial AB25 dye concentration of 20 mg/L and initial solution pH 2.0. On the basis of Fig. 4(c) and (d), maximum removal efficiency of AB25 dye was obtained at minimum initial AB25 dye concentration (20 mg/L) and initial solution pH (2.0). In this case, dye removal efficiency was 46.38% for *J. adhaerens* and 84.22% for HCl-treated *J. adhaerens* at zero level (0.8 g/L) of biomass loading. The decrease in the AB25 dye removal efficiency with an increase in initial AB25 concentration can be attributed to the fact that all the sorbents had a limited number of active sites, which would have become saturated above a certain initial concentration [31]. Similar result was also reported by Kousha et al. [24].

3.3. Independent variables

The interactive effects of all process factors at same experimental temperature in the perturbation graphs are shown in Fig. 5(a)–(d). The perturbation plot of AB25 dye removal efficiency vs. all process variables depicts the contribution of each factor to the biosorption process. The perturbation plot illustrates removal efficiency of ions as each variable moves from the preferred reference with all other factors held constant at the zero level. Hence, the perturbation plots show the deviation of the factorial level from the adjusted reference point of all the variables [31]. As can be seen from Fig. 5, the three factors of the biomass loading (A), initial AB25 concentration (B), and initial solution pH (C) were influenced on AB25 dye removal

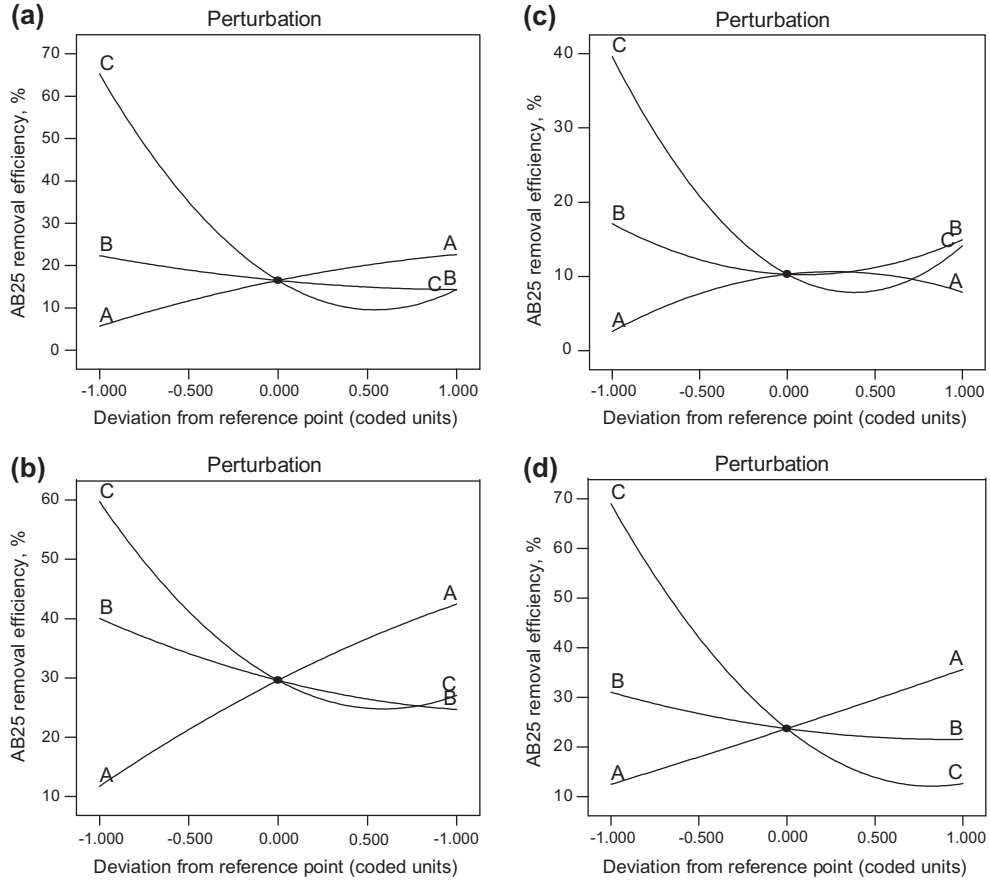


Fig. 5. Overlay plot of perturbation of all the variables: (a) *P. australis*, (b) HCl-treated *P. australis*, (c) *J. adhaerens*, and (d) HCl-treated *J. adhaerens*.

Table 7
Dye removal efficiency at optimum values of the variables

Biomass	Biomass loading (g/L)	Initial AB25 dye concentration (mg/L)	Initial pH	Removal efficiency (%)	Desirability
P. australis	1.40	60	2.0	77.34	0.991
HCl treated *P. australis*	1.40	60	2.0	71.28	0.952
J. adhaerens	0.91	20	2.0	50.56	0.987
HCl treated *J. adhaerens*	1.40	60	2.0	85.19	0.933

efficiency. In the present study, the effects of initial solution pH and initial AB25 dye concentration on removal efficiency of AB25 dye were found to be negative, while the effects of biomass loading on removal efficiency of AB25 dye were positive. The reason for these observations has already been mentioned under section 3.2. This finding was in agreement with the one factor coefficient estimate, as presented in Table 5. Also, the initial solution pH (C) had the greatest effect as compared to biomass loading (A) and initial AB25 dye concentration (B) on removal efficiency of AB25 dye by algal biomass. Similar bservation was noted by Velis et al. [32]. On the other hand, perturbation plot indicated that the degree of effectiveness of pH on AB25 dye removal efficiency on HCl-treated *J. adhaerens* was more than for untreated *J. adhaerens*, and for untreated *P. australis* was more than for HCl-treated *P. australis*. This observation can be attributed to the fact that *J. adhaerens* and *P. Australis* have different cell wall structure.

3.4. Response optimization process

The effectiveness of treatment by algae depends not only on the properties of the adsorbent and adsorbate, but also on the following environmental conditions and variables used for the removal process: biomass loading, initial AB25 dye concentration, initial solution pH, etc. [7]. In numerical optimization process, the main objective is to determine the optimum values of variables for AB25 dye removal efficiency using *P. australis*, *J. adhaerens*, HCl-treated *P. australis* and HCl-treated *J. adhaerens* from the model obtained using experimental data. The desired

Table 8
Comparison of maximum uptake capacities or removal efficiency of Acid Blue 25 dye by various adsorbents with respect to different experimental conditions as reported in the literature

Sorbent	Initial dye concentration (mg/L)	pH	Sorbent dose (g/L)	Temperature (°C)	Time (min)	Removal efficiency (%)	q_e (mg/g)	Source
CSM	250	3–9	1	25	60	n.d	322	[3]
Organic clay (AMP)	n.d	9	0.4	n.d	30	79	n.d	[34]
Nizamuddin zanardini	10–50	2	1	27	90	n.d	29.79	[35]
Sphagnom moss peat	200	n.d	2	18	60	n.d	12.7	[36]
Egyptian bagasse pith	166	n.d	2	20	n.d	17.3	14.4	[6]
Eichhornia Crassipes	100	4	2.5	20	60	58.7	n.d	[13]
diatomite	50	2	0.9	25	300	72.81	n.d	[12]
Calcined diatomite	50	2	0.9	25	300	54.5	n.d	[12]
P. australis	60	2	1.40	25	60	77.34	33.15	Present study
HCl treated *P. australis*	60	2	1.40	25	60	71.28	30.55	Present study
J. adhaerens	20	2	0.91	25	60	50.56	11.11	Present study
HCl treated *J. adhaerens*	60	2	1.40	25	60	85.19	36.51	Present study

CSM: Cationized starch-based material; AMP: Aminopropyl-functionalized magnesium phyllosilicate; n.d: Not determined.

goal was to achieve highest treatment performance. A multiple response method was applied for optimization of any combination of four goals; biomass loading, initial AB25 dye concentration, initial solution pH, and removal efficiency of AB25 dye. Accordingly, in the numerical optimization, a point was found that maximizes the desirability function. The optimum values of the process variables for maximum AB25 dye removal efficiency are shown in Table 7.

3.5. Comparison of AB25 removal efficiencies by various adsorbents

The results show that the maximum sorption capacities of *P. australis*, *J. adhaerens*, HCl-treated *P. australis*, and HCl-treated *J. adhaerens* for AB25 dye were comparable to other corresponding sorbents reported in the literature. Optimum values of variables were used to achieve maximum removal efficiency of AB25 dye and, thereby maximum sorption capacity of AB25 dye by these biomasses was determined, and then compare with respect to other sorbents which have been previously used for removal of AB25 dye (Table 8). In Table 8, obtained maximum sorption capacities (mg/g) or maximum sorption percentage (%) in present study have been compared with the sorption capacity values of previously used sorbents with respect to different conditions reported in the literature. The differences in maximum sorption capacities on various sorbents might be due to dissimilar cell wall structure and surface functional groups of sorbents and experimental conditions [33].

4. Conclusions

The use of response surface design allowed for optimization of the AB25 dye removal efficiency of *P. australis*, *J. adhaerens*, HCl-treated *P. australis* and HCl-treated *J. adhaerens*. The high R^2 value, significant *p*-value, an insignificant lack-of-fit value, and low SD, CV and PRESS of models, verified successfully the validation of experimental data. The optimum values of independent variables for AB25 dye removal using raw and HCl-treated *P. australis* and *J. adhaerens* from the quadratic model were acquired. Maximum AB25 dye removal efficiency and maximum biosorption capacity and corresponding desirability values were obtained for each biomass at its optimum conditions. This study demonstrated that these biomasses could be used as efficient biosorbents for the removal of AB25 dye from aqueous solutions.

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References

[1] S.R. Couto, Dye removal by immobilized fungi, Biotechnol. Adv. 27 (2009) 227–235.
[2] R.C. Kuhad, N. Sood, K.K. Tripathi, A. Singh, O.P. Ward, Developments in microbial methods for the treatment of dye effluents, Adv. Appl. Microbiol. 56 (2004) 185–213.
[3] F. Renault, N. Morin-Crini, F. Gimbert, P.-M. Badot, G. Crini, Cationized starch-based material as a new ion-exchanger adsorbent for the removal of C.I. Acid Blue 25 from aqueous solutions, Biores. Technol. 99 (2008) 7573–7586.
[4] C. Park, M. Lee, B. Lee, S.-W. Kim, H.A. Chase, J. Lee, S. Kim, Biodegradation and biosorption for decolorization of synthetic dyes by *Funalia trogii*, Biochem. Eng. J. 36 (2007) 59–65.
[5] P. Kaushik, A. Malik, Fungal dye decolourization: Recent advances and future potential, Environ. Int. 35 (2009) 127–141.
[6] Y.S. Ho, G. McKay, Sorption of dyes and copper ions onto biosorbents, Process Biochem. 38 (2003) 1047–1061.
[7] G. Crini, Non-conventional low-cost adsorbents for dye removal: A review, Biores. Technol. 97 (2006) 1061–1085.
[8] V.K. Gupta, Suhas, Application of low-cost adsorbents for dye removal—A review, J. Environ. Manage. 90 (2009) 2313–2342.
[9] K.R. Ramakrishna, T. Viraraghavan, Dye removal using low cost adsorbents, Water Sci. Technol. 36 (1997) 189–196.
[10] S. Ertugrul, N.O. San, G. Dönmez, Treatment of dye (Remazol Blue) and heavy metals using yeast cells with the purpose of managing polluted textile wastewaters, Ecol. Eng. 35 (2009) 128–134.
[11] A.R. Khataee, M. Pourhassan, Biological decolorization of C.I. basic green 4 solution by *Chlorella* sp.: Effect of operational parameters, Chin. J. Appl. Environ. Biol. 15 (2009) 110–114.
[12] K. Badii, F.D. Ardejani, M.A. Saberi, N.Y. Limaee, S.Z.E.D. Shafaei, Adsorption of Acid Blue 25 dye on diatomite in aqueous solutions, Indian J. Chem. Techn. 17 (2010) 7–16.
[13] M.M. El Zawahry, M.M. Kamel, Removal of azo and anthraquinone dyes from aqueous solutions by *Eichhornia Crassipes*, Water Res. 38 (2004) 2967–2972.
[14] A. Srinivasan, T. Viraraghavan, Decolorization of dye wastewaters by biosorbents: A review, J. Environ. Manage. 91 (2010) 1915–1929.
[15] P.J.L. Geraldino, L.M. Liao, S.M. Boo, Morphological study of the marine algal genus *Padina* (Dictyotales,

Phaeophyceae) from southern philippines: 3 species new to philippines, Algae 20 (2005) 99–112.
[16] S. Enriquez, E. Avila, J.L. Carballo, Phenotypic plasticity induced in transplant experiments in a mutualistic association between the red alga *Jania adhaerens* (Rhodophyta, Corallinales) and the sponge *Haliclona caerulea* (Porifera: Haplosclerida): morphological responses of the alga, J. Phycol. 45 (2009) 81–90.
[17] M. Edrissi, N.R. Asl, B. Majidi, Interaction of mefenamic acid with cobalt (II) ions in aqeous media: Evaluation via classic and response surface methods, Turk. J. Chem. 32 (2008) 505–519.
[18] M. Zarei, A. Niaei, D. Salari, A. Khataee, Application of response surface methodology for optimization of peroxi-coagulation of textile dye solution using carbon nanotube–PTFE cathode, J. Hazard. Mater. 173 (2010) 544–551.
[19] N. Aghamohammadi, H.B.A. Aziz, M.H. Isa, A.A. Zinatizadeh, Powdered activated carbon augmented activated sludge process for treatment of semi-aerobic landfill leachate using response surface methodology, Biores. Technol. 98 (2007) 3570–3578.
[20] R.V. Muralidhar, R.R. Chirumamila, R. Marchant, P. Nigam, A response surface approach for the comparison of lipase production by *Candida cylindracea* using two different carbon sources, Biochem. Eng. J. 9 (2001) 17–23.
[21] M. Kousha, E. Daneshvar, M.S. Sohrabi, M. Jokar, A. Bhatnagar, Adsorption of acid orange II dye by raw and chemically modified brown macroalga *Stoechospermum marginatum*, Chem. Eng. J. 192 (2012) 67–76.
[22] A. Özer, G. Gürbüz, A. Çalimli, B.K. Körbahti, Biosorption of copper(II) ions on *Enteromorpha prolifera*: Application of response surface methodology (RSM), Chem. Eng. J. 146 (2009) 377–387.
[23] S.R. Shukla, R.S. Pai, Adsorption of Cu(II), Ni(II) and Zn(II) on dye loaded groundnut shells and sawdust, Sep. Purif. Technol. 43 (2005) 1–8.
[24] M. Kousha, E. Daneshvar, M.S. Sohrabi, N. Koutahzadeh, A.R. Khataee, Optimization of C.I. Acid Black 1 biosorption by *Cystoseira indica* and *Gracilaria persica* biomasses from aqueous solutions, Int. Biodeter. Biodegr. 67 (2012) 56–63.
[25] G. Çetinkaya Dönmez, Z. Aksu, A. Öztürk, T. Kutsal, A comparative study on heavy metal biosorption characteristics of some algae, Process Biochem. 34 (1999) 885–892.
[26] E. Daneshvar, M. Kousha, M.S. Sohrabi, A. Khataee, A. Converti, Biosorption of three acid dyes by the brown macroalga *Stoechospermum marginatum*: Isotherm, kinetic and thermodynamic studies, Chem. Eng. J. 195–196 (2012) 297–306.
[27] K. Vijayaraghavan, Y.-S. Yun, Bacterial biosorbents and biosorption, Biotechnol. Adv. 26 (2008) 266–291.
[28] T.A. Davis, B. Volesky, A. Mucci, A review of the biochemistry of heavy metal biosorption by brown algae, Water Res. 37 (2003) 4311–4330.
[29] K. Vijayaraghavan, Y.-S. Yun, Chemical modification and immobilization of C*orynebacterium glutamicum* for biosorption of reactive Black 5 from aqueous solution, Ind. Eng. Chem. Res. 46 (2006) 608–617.
[30] M. Kousha, E. Daneshvar, A.R. Esmaeli, M. Jokar, A.R. Khataee, Optimization of Acid Blue 25 removal from aqueous solutions by raw, esterified and protonated Jania adhaerens biomass, Int. Biodeter. Biodegr. 69 (2012) 97–105.
[31] R. Singh, R. Chadetrik, R. Kumar, K. Bishnoi, D. Bhatia, A. Kumar, N.R. Bishnoi, N. Singh, Biosorption optimization of lead(II), cadmium(II) and copper(II) using response surface methodology and applicability in isotherms and thermodynamics modeling, J. Hazard. Mater. 174 (2010) 623–634.
[32] C.A. Velis, P.J. Longhurst, G.H. Drew, R. Smith, S.J.T. Pollard, Biodrying for mechanical–biological treatment of wastes: A review of process science and engineering, Biores. Technol. 100 (2009) 2747–2761.
[33] M. Amini, H. Younesi, Biosorption of Cd(II), Ni(II) and Pb(II) from aqueous solution by dried biomass of Aspergillus niger: Application of response surface methodology to the optimization of process parameters, CLEAN—Soil, Air, Water 37 (2009) 776–786.
[34] H.-J. Shin, B.-M. Nam, Removal of acid blue 25 from aqueous solutions by organic clay, J. Biosci. Bioeng. 108(Supplement 1) (2009) S85.
[35] M. Kousha, E. Daneshvar, H. Dopeikar, D. Taghavi, A. Bhatnagar, Box–Behnken design optimization of Acid Black 1 dye biosorption by different brown macroalgae, Chem. Eng. J. 179 (2012) 158–168.
[36] Y.S. Ho, G. McKay, Sorption of dye from aqueous solution by peat, Chem. Eng. J. 70 (1998) 115–124.