TÜBİTAK

Turkish Journal of Engineering & Environmental Sciences

http://journals.tubitak.gov.tr/engineering/

Research Article

Turkish J Eng Env Sci
(2014) 38: 217 – 230

doi:10.3906/muh-1409-2

Use of response surface methodology for the bioaccumulation of Violet 90 metal-complex dye by *Candida tropicalis*

Müjgan OKUR[1,*], Nurdan SARAÇOĞLU[1], Zümriye AKSU[2]
[1]Department of Chemical Engineering, Gazi University, Maltepe, Ankara, Turkey
[2]Department of Chemical Engineering, Hacettepe University, Beytepe, Ankara, Turkey

Received: 10.09.2014 • **Accepted/Published Online:** 27.12.2014 • **Printed:** 30.06.2015

Abstract: In this study, the combined effects of initial dye (40–700 mg/L) and initial reducing sugar concentration (1–15 g/L) on the bioaccumulation of Violet 90 metal-complex dye by growing cells of *Candida tropicalis* yeast were investigated in growth media containing sugar beet molasses as a carbon and energy source. The bioaccumulation experiments were performed at pH 3 at 30 °C and at 140 rpm agitation rate in a batch system. The highest uptake was obtained as 61.46% at 14.9 g/L reducing sugar concentration and at 38 mg/L dye concentration while the maximum uptake capacity was achieved as 56.28 mg/g at 3.2 g/L reducing sugar concentration and at 711.1 mg/L dye concentration. Higher uptakes were obtained at lower dye concentrations; higher uptake capacities were observed at higher initial dye concentrations. The combined effects of the initial dye (100–500 mg/L) and initial reducing sugar concentration (5–15 g/L) on dye uptake capacity and growth rate of *Candida tropicalis* yeast were also investigated by response surface methodology (RSM). Optimum design variables from RSM were calculated by numerical optimization with the Design Expert program. The optimum values of the variables to maximize uptake capacity were estimated as 5.1 g/L reducing sugar concentration and 499 mg/L dye concentration. The maximum uptake capacity was achieved as 41.3 mg/g at these optimized conditions.

Key words: Bioaccumulation, metal-complex dye, yeast, sugar beet molasses, *Candida tropicalis*

1. Introduction

Industrial sectors such as textiles, paper and wood-pulp, printing, iron and steel, petrol, pesticides, dyes, solvents, and pharmaceuticals consume large amounts of water and organic-based chemicals [1]. Synthetic dyestuffs are frequently utilized in the textile, dye, paper, and printing industries. Today more than 100,000 synthetic dyes are commercially used and more than 700,000 t of dye is produced annually [1–3]. The lack of effective coloring in textile dying processes results in the release of approximately 5%–10% of the used material into wastewater and the effluents of these industries become highly colored [4,5]. This loss increases up to 50% in reactive dyestuffs [5–8].

Colored wastewaters affect photosynthetic activities negatively by reducing the sunlight penetration into deeper layers when given to the received environments. The dye accumulated in some marine organisms also causes a risk of forming toxic and carcinogenic products. Treatment of these effluents by conventional methods is associated with many difficulties, mainly due to the complex aromatic nature of textile dyes, which makes them resistant to degradation. Their removal is therefore of great importance [9].

*Correspondence: mtelli@gazi.edu.tr

The dyestuffs used in the textile industry are classified either according to their chemical structures (azo-, anthraquinone, indigo, polymethane, carbonium, phthalocyanine, nitro, and sulfur dyestuffs) or their coloring features (direct, bearing sulfur, naphtholate, reactive, ingrain, oxidation, acid, basic, mordant, chrome, metal-complex, disperse, pigment dyestuffs). Metal-complex dyes show great affinity towards protein fibers. Generally it has been seen that metal-complex dyes are chromium and cobalt complexes [10]. Among the popular metal-complex dyes, a variety known as 1:2 metal-complex dyes, which have one metal ion for each dyestuff molecule, find application for dyeing polyamide fibers such as wool, nylon, and silk; they also provide high levels of fastness and paint without any shade differences on the fabrics [11,12]. Besides this, metal-complex dyestuffs can also be applied to cotton without requiring any auxiliary chemicals [11].

The textile industry frequently uses physical, chemical, and biological methods for the elimination of colored substances in waste waters. Physical methods are classified as adsorption with materials such as active carbon, grass, wood shavings, volatile ash and charcoal, silicate gel, clay, and corncob, and as membrane filtration, ion exchange, electronic coagulation, and radiation. Chemical methods are H_2O_2-Fe(II) salts (Fenton reagents), ozonide, photochemical methods, sodium hydrochloride (NaOCl), electrochemical methods, and oxidation with cucurbituril [13–15]. While the utilization of physiochemical methods in the decolorization of textile wastewaters is very efficient, these methods are very expensive, are incapable of being applied to all dyestuffs, produce large amounts of sludge after the decolorization process [12,16,17], require large amounts of chemicals [18], form secondary pollutants, and have regeneration problems [8]. Biological methods such as bioaccumulation and biosorption with living and nonliving microorganisms for dyestuff decolorization may be alternative methods to physicochemical methods [13–15]. The utilization of microorganisms in the decolorization process of dyestuffs in industrial wastewaters has many advantages. Such a process is very cheap and the operational expenses are low. Additionally, the final products are not toxic when they are fully biodegraded [19].

The accumulation of pollutants from waste waters by actively growing cells is called bioaccumulation [6]. Most bacteria, fungi, and algae are capable of bioaccumulating or reducing a major portion of dyestuffs [4,17]. It is known that many strains of anaerobic bacteria are able to degrade some dyes, but the disadvantage of this method is the production of carcinogenic aromatic amines by these organisms [20]. On the other hand, the slow growth of filamentous fungi compared with most single-cell microorganism, low pH requirements, and long hydraulic retention time for complete decolorization limit the large-scale application of filamentous fungi for decolorization [21]. Compared to bacteria and filamentous fungi, yeasts have many advantages. They not only grow rapidly like bacteria, but like filamentous fungi they also have the ability to resist unfavorable environments. Yeasts are also a cheap, easily obtainable biomass source and are capable of bioaccumulating dyes with low pH levels [6,16]. However, little work has been carried out investigating the ability of yeast to act as a bioaccumulator for textile dyes in wastewater effluents [6,7]. *Candida oleophila* [22], *Candida zeylanoides* [23,24], *Candida tropicalis* and *Debaryomyces polymorphus* [17,25], *Kluyveromyces marxianus* IMB3 [18], *Candida tropicalis* [4,7,16,26], *Saccharomyces cerevisiae* [6], *Candida utilis* [27], *Trichosporon beigelii* NCIM-3326 [28], *Rhodotorula mucilaginosa* [8], and *Pichia fermentans* [29] yeasts have been used for the removal of different dyes. On the other hand, even though metal-complex dyestuffs are frequently utilized in the textile industry for coloring wool, nylon, cotton, and silk, studies about their bioaccumulation with microorganisms from waste waters are very few. *Cladosporium cladosporioides* [12] and *Trametes versicolor* [30,31] have been used in studies with regard to the biodegradation of metal-complex dyestuffs with microorganisms, but yeasts have never been used before for metal-complex dyestuff bioaccumulation.

In this study, the bioaccumulation of C.I. Violet 90 metal-complex dye from wastewaters with *Candida tropicalis*, which has great potential for effectively removing some reactive dyes from wastewaters, was investigated at batch-scale level. Molasses was used as the carbon and energy source for *Candida tropicalis*. Due to its high sugar content, molasses is a highly desired and consumed raw material and is used in many areas (http://www.turkseker.gov.tr/Urunler.aspx). It also serves as a good nutritional source for *Candida tropicalis* and is preferred because of its cheap price, easy accessibility, and easy storage features [7].

2. Materials and methods

2.1. Preparation of substrate

In this study, sugar beet molasses, which contains approximately 50% (w/v) sucrose, was used as a carbon source for *Candida tropicalis* yeast. The sucrose in the sugar beet molasses was hydrolyzed to glucose and fructose before using it in the experiments. A solution of molasses (1/3 solid-liquid) was prepared and acidified with concentrated H_2SO_4 until pH 2. It was then boiled at 90 °C for 5 min and centrifuged for another 5 min at 2000 rpm. Later, the hydrolyzed solution was neutralized (pH 6) with 10 M NaOH and centrifuged again at 2000 rpm for 5 min. The prepared hydrolyzed molasses solution containing 138 g/L reducing sugar was used as a stock solution.

2.2. Microorganism and growth medium

Candida tropicalis was maintained at 4 °C on slant agar. The medium consisted of (g/L) 3 yeast extract, 3 malt extract, 5 peptone, 10 glucose, and 20 agar [32] until use. Before each bioaccumulation experiment, one loopful from a slant was used to inoculate a 250-mL Erlenmeyer flask containing 100 mL of sterile growth medium (at 121 °C, 30 min). The growth medium was composed of 10 g/L reducing sugar, 1 g/L KH_2PO_4, and 1 g/L $(NH_4)_2SO_4$. The plugged flasks were incubated on a magnetic stirrer at 30 °C and 220 rpm for 15–20 h.

2.3. Bioaccumulation

In order to produce yeast cultures tolerant to dye ions, acclimatization of cells to progressively increased concentrations of the dye was performed. For this purpose, *Candida tropicalis* cultures were serially subcultured in molasses growth media supplemented with different dye concentrations in the range of 40–700 mg/L [27]. Adaptation studies were carried out on a magnetic stirrer at 220 rpm at 30 °C in 250-mL flasks containing 100 mL of sterile growth medium.

The effect of reducing sugar concentration and initial dye concentration on the bioaccumulation properties of adapted *C. tropicalis* was also studied. After sterilization, a defined amount of dye solution was mixed with the sterilized growth medium including molasses solution with varying reducing sugar concentrations of 1, 2.5, 5, 10, and 15 g/L. The final dye concentration at each reducing sugar concentration was changed to 40, 100, 300, 500, and 700 mg/L. An inoculum (1% v/v) of adapted yeast from the exponential growth phase was transferred to 150 mL of bioaccumulation media supplemented with different dye concentrations at each constant reducing sugar concentration. The bioaccumulation experiments were carried out with 250-mL Erlenmeyer flasks at 30 °C in a shaking bath (140 rpm) for 15 days because the bioaccumulation process must be dependent on cell biomass and on actively growing cells [7].

2.4. Textile dye

The C.I. Violet 90 1:2 metal-complex dye used in the experiments was provided by ERSA COLOR. The dye stock solutions were prepared by dissolving the powdered dye in distilled water to 2% (w/v). The structure of C.I. Violet 90 1:2 metal-complex dye is shown in Figure 1 [11].

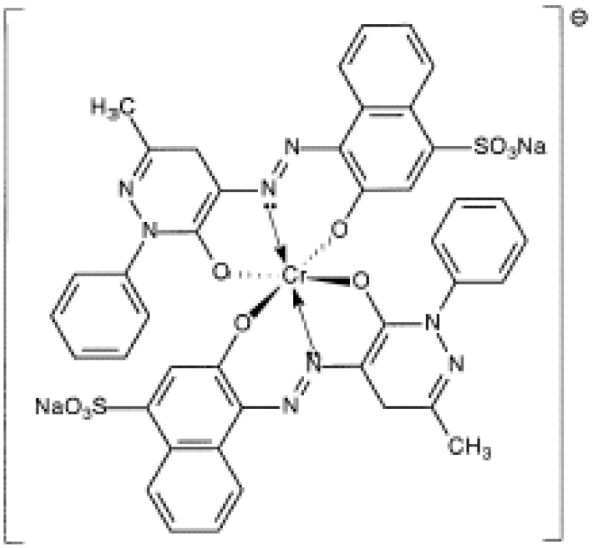

Figure 1. C.I. Violet 90.

2.5. Analytical methods

Samples of 3 mL were drawn daily from each flask. The pH level of samples were adjusted to 6 with 0.1 M HCl or 0.1 M NaOH to prevent the precipitation [33] of dye during centrifuging. Samples were then centrifuged at 3700 rpm for 5 min to remove suspended biomass. The remaining supernatant was used for measuring the dye and reducing sugar concentrations. The dye concentration in samples was determined by reading UV absorbance at 523 nm for Violet 90 dye. Molasses medium without dye was used as the blank. The reducing sugar analysis in samples was performed using the DNS method [34]. For the measurement of yeast growth, the precipitated biomass after centrifugation was diluted, and the turbidity of diluted samples at 600 nm was measured using a standard curve of absorbance against dry weight.

2.6. Statistical analysis

Response surface methodology (RSM) is a collection of mathematical and statistical techniques useful for developing, improving, and optimizing processes and it can be used to evaluate the relative significance of several affecting factors even in the presence of complex interactions [27,35]. The main objective of RSM is to determine the optimum operational conditions of the system or to determine a region that satisfies the operating specifications [35].

In this study, the experiments were based on a 3^2 three-level factorial design in order to study the combined effects of two independent variables (reducing sugar concentration and dye level) on bioaccumulation. The independent variables for RSM were the concentrations of dye (X_1) and reducing sugar concentration (X_2).

Each independent variable was coded as x_i according to the following relationship:

$$x_i = \left(\frac{X_i - X_o}{\Delta X} \right) \times 100, \tag{1}$$

where x_i is the coded value of the independent variable, X_i is the actual value of the independent variable, X_o is the actual value of independent variable at the center point, and ΔX is the step change value. The experimental design, experimental range, and levels of independent variables are presented in Table 1.

Table 1. Experimental design and levels of independent variables.

Independent variables	Factor	Range and levels		
	x_i	−1	0	+1
Dye concentration (mg/L)	X_1	100	300	500
Reducing sugar concentration (g/L)	X_2	5	10	15

The growth rate and uptake capacity were taken as the response of the design experiments. Thirteen experiments were performed to calculate the coefficients of second-order polynomial equations by fitting the experimental data to the response functions in the form of the following equation:

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{12} x_1 x_2, \tag{2}$$

where x_1, x_2, x_{12} are the input variables, which influence the response Y; β_0 is a constant term; β_1, β_2 are coefficients of the polynomial for linear effects; β_{11}, β_{22} are coefficients of the polynomial for quadratic effects; and β_{12} are coefficients of the polynomial for interactive effects [27]. The statistical software Design Expert 7.0 was used for numerical analysis and to estimate the responses of dye and initial sugar concentration. The statistical significance of the model equation and the goodness of fit was evaluated by R^2 and the F-test analysis of variance (ANOVA).

3. Results and discussion

The growth and bioaccumulation properties of adapted *C. tropicalis* were investigated as a function of initial sugar concentration and initial dye concentration at pH 3. The results are given as the specific growth rate of yeast μ (1/h), the dye uptake capacity (q_m, mg dye per unit of dry weight of cells), and uptake %. The dye uptake capacity (q_m) and the uptake % were calculated from Eqs. (4) and (**??**), respectively.

$$q_m = \left(\frac{C_o - C_f}{X_m} \right) \tag{3}$$

$$\text{Uptake } \% = \left(\frac{C_o - C_f}{C_o} \right) \times 100 \tag{4}$$

C_o : Initial dye concentration (mg/L),

C_f : Final dye concentration (mg/L),

X_m: Maximum cell concentration on dry basis (g/L).

3.1. Combined effects of initial reducing sugar and initial dye concentrations on growth of *C. tropicalis*

The reducing sugar concentration in bioaccumulation experiments was changed between 1 and 15 g/L, and its effects on *C. tropicalis* growth and bioaccumulation performance were examined. Controls without dye were also carried out. Growth curves in the absence and presence of dye (100 mg/L dye) for different initial sugar concentrations are depicted in Figures 2 and 3, respectively. In both the control experiments and dye-containing experiments, while the sugar concentration was increasing, an evident increase in maximum yeast concentration (X_m) was observed at a constant dye concentration. It was also confirmed that growth occurred quickly in low-sugar medium and no delay in the growth phase of *C. tropicalis* was observed, compared to no sugar or dye concentration as in Figures 2 and 3. Aksu and Dönmez [7] also reported no lag period for *Candida tropicalis* growth while Dönmez [16] and Das et al. [29] observed that increasing the dye concentration in growth medium caused a long lag period and growth inhibition in bioaccumulation of the textile dyes. Figure 4 depicts the *C. tropicalis* yeast growth curve for various dye concentrations (0–700 mg/L) while initial sugar concentration was held constant at 15 g/L for each experiment set at pH 3. The curve at the top demonstrates the control environment and the dye concentration increases on the way down. It can also be seen that the growth curves for the control and dye-containing assays (up to 100 mg/L) are close to each other. Above 100 mg/L dye, microbial growth significantly diminished with increasing dye concentration, which is thought to occur because of the dye inhibition. Higher dye levels lower the highest biomass reached (Figure 4).

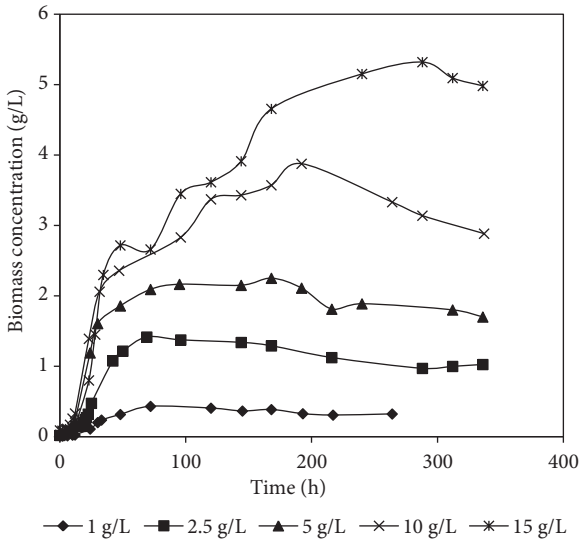

Figure 2. Effect of initial reducing sugar concentration on the growth of *Candida tropicalis* in control medium (140 rpm, 30 °C, pH 3).

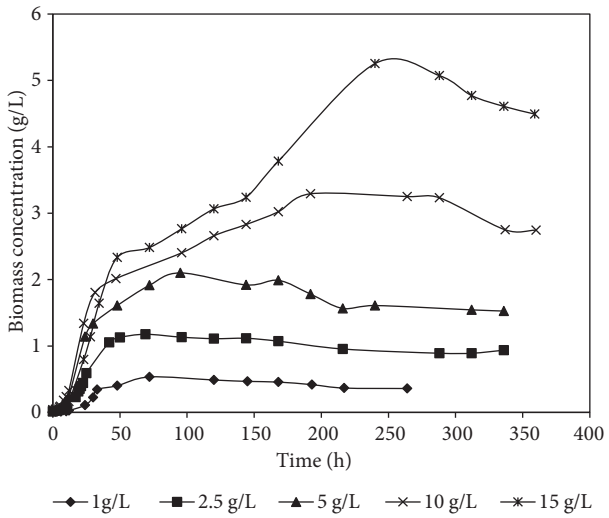

Figure 3. Effect of initial reducing sugar concentration on the growth of *Candida tropicalis* in medium containing 100 mg/L dye (140 rpm, 30 °C, pH 3).

Figure 5 shows the inhibition kinetics of growth of *C. tropicalis* by sugar concentrations in the control and various dye-containing media. Increasing the concentration of sugar in the growth medium first caused an increase in specific growth rates of *C. tropicalis* with up to 10 g/L sugar concentration in all runs. For example, maximum specific growth rate increased from 0.1185 1/h to 0.2023 1/h when initial reducing sugar concentration increased from 0.95 g/L to 9.67 g/L with 100 mg/L dye. Thereafter, it decreases to 0.144 1/h with a further

increase in initial sugar concentration to 14.58 g/L. The relation between reducing sugar concentration and growth rate in dye bioaccumulation of *Candida tropicalis* clearly indicates the presence of substrate inhibition. In addition to this, when sugar concentration was kept constant and initial dye concentration was changed from 40 to 700 mg/L, the growth rate of yeast was reduced. The presence of dyes in the growth medium negatively influenced the growth of the *Candida tropicalis*. Das et al. [29] observed an increased growth rate of yeast *P. fermentans* MTCC-189 with raised initial sugar concentration at constant dye concentration. This increase was related to the cell defense mechanism.

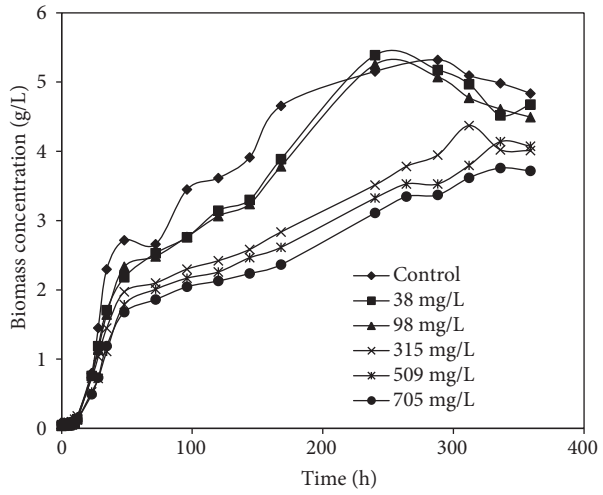

Figure 4. Effect of initial dye concentration on the growth of *Candida tropicalis* in medium with constant reducing sugar concentration (140 rpm, 30 °C, pH 3, sugar = 15 g/L).

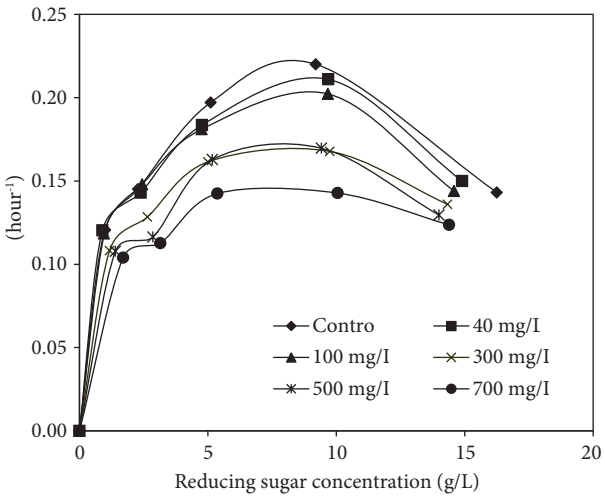

Figure 5. Combined effect of initial reducing sugar and dye concentrations on the growth rate of *Candida tropicalis* (140 rpm, 30 °C, pH 3).

3.2. The effect of initial reducing sugar concentration and initial dye concentration on dye bioaccumulation

The combined effects of initial reducing sugar concentration and initial dye concentration on dye bioaccumulation performance of *Candida tropicalis* was investigated. Table 2 shows uptake %, bioaccumulated dye concentrations (C_{acc}), and specific dye uptake capacity (q, mg g^{-1}). As seen in Table 2, the uptake % and bioaccumulated dye concentrations increased with initial sugar concentration when initial dye concentration was 40 and 100 mg/L. However, at 300 mg/L dye concentration and those above this level, an increase in sugar concentration had an adverse effect on the uptake % and bioaccumulated dye concentration. This trend is likely to be the result of the combined effect of dye and substrate inhibition. Chen et al. [36], Kumar et al. [37], and Shahvali et al. [38] reported a decrease in dye uptake efficiency in the presence of high glucose concentrations.

While the highest uptake % for 38 mg/L and 98 mg/L initial dye concentrations was respectively obtained as 61.5% and 28.4% in media containing 14.9 g/L and 14.58 g/L initial sugar concentrations, for higher dye concentrations above 300 mg/L, the highest uptake % was observed in the medium containing 10 g/L initial sugar concentration. On the other hand, dye uptake capacity (q) increased with the increasing initial dye concentration at a constant reducing sugar concentration in all runs. Although higher removal yields were obtained at lower dye concentrations, higher bioaccumulation capacities were observed at higher initial dye concentrations (Table

2), which could be due to an important driving force provided by initial pollutant concentration to overcome mass transfer resistances between the aqueous and solid phases [27]. Otherwise, dye uptake capacity increased with the increase of initial reducing sugar concentration up to 2.5 g/L and then it decreased with the increase of initial reducing sugar concentration (from 2.5 to 15 g/L) at all various initial dye concentrations. The high initial reducing sugar concentration after 2.5 g/L had a negative effect on dye uptake capacity (q). Similar results were also reported by Aksu and Dönmez [7] on bioaccumulation of Remazol Black dye with *Candida tropicalis*.

Table 2. Comparison of bioaccumulated dye concentration, dye uptake %, and dye uptake capacity at different initial dye and reducing sugar concentrations.

S_o (g/L)	C_o (mg/L)	C_{acc} (mg/L)	Uptake %	Uptake capacity (q, mg/g)
1.0	0.0	-	-	-
2.3	0.0	-	-	-
5.1	0.0	-	-	-
9.2	0.0	-	-	-
16.3	0.0	-	-	-
0.9	39.0	4.46	11.43	8.32
2.4	49.7	9.77	19.67	7.02
4.8	39.0	11.00	28.21	5.13
9.7	42.2	12.67	34.54	2.78
14.9	38.0	23.35	61.46	4.33
1.0	104.0	7.10	6.83	13.27
2.4	104.8	17.27	16.48	14.67
4.8	105.9	16.85	15.92	8.02
9.7	103.0	20.08	19.51	6.10
14.6	98.0	28.00	28.41	5.33
1.2	305.0	11.45	3.75	21.28
2.6	316.3	41.26	13.05	38.10
5.0	319.6	43.55	13.63	24.89
9.8	313.0	59.58	19.03	21.90
14.3	315.0	49.84	15.82	11.40
1.4	503.0	8.03	1.60	15.84
2.9	528.3	56.55	10.70	52.51
5.2	525.8	70.80	13.47	41.02
9.4	516.0	85.79	16.63	33.60
14.0	509.4	82.69	16.23	19.97
1.7	720.0	12.33	3.01	24.36
3.2	711.1	60.05	8.45	56.28
5.4	680.0	71.78	10.56	39.23
10.1	685.9	122.33	17.84	49.29
14.4	705.2	102.38	14.52	27.23

Microbial uptake of dyes is mainly due to biosorption and biodegradation mechanisms [28,39]. In the biosorption mechanism, the absorption spectrum of dye decreases approximately in proportion to the dye adsorbed as well as the cell becoming deeply colored, whereas in biodegradation, either the major visible light absorbance peak will completely disappear or a new peak will appear [28]. The dye bioaccumulation of *Candida tropicalis* demonstrated that the biomass during the experiments turned to color Violet 90. The

color transformation of the yeast strengthens the existence of dyestuffs' bioaccumulation or, in other words, bioabsorption [17,24].

In this study, the highest uptake % with *C. tropicalis* yeast was obtained at 38 mg/L initial dye concentration as 61.5% in medium containing 14.9 g/L initial reducing sugar concentration. The highest uptake values for 313, 516, and 685.85 mg/L dye concentrations were achieved as 19%, 16.6%, and 17.8%, respectively at 10 g/L initial reducing sugar concentration. The uptake % for *C. tropicalis* was reported between 30% and 97% for bioaccumulation of different reactive dyes (100 mg/L dye concentration and 5 g/L glucose concentration) by Yang et al. [17]. Aksu and Dönmez [7] also measured the bioaccumulation uptake % of Remazol Black B and Remazol Blue dyes (271.2 and 237.8 mg/L dye concentrations and 10 g/L sucrose concentration) between 97.4% and 98.3%. For bioaccumulation of mono azo disperse dye (50 mg/L dye concentration and 1.3 g/L glucose concentration), uptake % values of 80%–82% were reported by Arora et al. [4]. These differences between the reported uptake % for different dye types with *C. tropicalis* clearly indicate that the effectiveness of uptake depends on the structure and complexity of each dye. Relatively small structural differences can markedly influence the uptake achieved. Steric factors, electron distribution, and charge density may also contribute to differences [12].

3.3. Response surface methodology for bioaccumulation

In this study, the cumulative effect of initial dye and reducing sugar concentrations on responses of uptake capacity and growth rate was also investigated by RSM using the Design Expert 7.0 statistical program in dye bioaccumulation. For the combined effects of independent variables, 100–500 mg/L (step change of 200 mg/L) and 5–15 g/L (step change of 5 g/L) were chosen for initial dye and reducing sugar concentrations, respectively. Table 3 shows the actual (Xi) values of independent variables and also the experimental and predicted responses corresponding to these actual variables. A 3^2 factorial design was used to develop correlations between independent variables and responses.

Table 3. Comparison of experimental and predicted responses for growth rate and dye uptake capacity.

Run	Dye concentration (mg/L)	Reducing sugar concentration (g/L)	Uptake capacity (experimental) (mg/g)	Growth rate (experimental) (1/h)	Uptake capacity (experimental) (mg/g)	Growth rate (predicted) (1/h)
1	100	15	5.3	0.144	4.0	0.151
2	300	15	11.4	0.136	12.8	0.129
3	300	10	21.9	0.168	21.6	0.169
4	300	10	22.5	0.167	21.6	0.169
5	100	10	6.1	0.202	8.2	0.192
6	300	10	21.5	0.166	21.6	0.169
7	300	5	24.9	0.162	25.2	0.161
8	500	5	41.0	0.163	41.5	0.161
9	500	10	33.6	0.168	33.3	0.170
10	100	5	8.0	0.181	7.2	0.185
11	300	10	21.8	0.165	21.6	0.169
12	300	10	22.0	0.170	21.6	0.169
13	500	15	20.0	0.130	19.9	0.131

Functions of uptake capacity and growth rate based on experimental results were evaluated and are given in Eqs. (5) and (6).

$$Uptakecapacity = -10.809 + 0.121X_1 + 2.193X_2 - 4.58210^{-3}X_1X_2 - 2.18110^{-5}X_1^2 - 0.103X_2^2 \quad (5)$$

$$Growthrate = 0.1505 - 2.42910^{-4}X_1 + 0.0158X_2 + 110^{-6}X_1X_2 + 2.96510^{-7}X_1^2 - 9.65510^{-4}X_2^2 \quad (6)$$

ANOVA results of the quadratic models of Eqs. (5) and (6) are presented in Tables 4 and 5. Table 4 indicates that the model equation can adequately be used to describe the uptake capacity of *C. tropicalis* under a wide range of operating conditions. The F-value of 174.34 ($P < 0.0001$) implies that the model is significant for uptake capacity. A P-value is the indicator of the significance of the test. Values of less than 0.05 ($P < 0.05$) indicate the significance of the model terms, while $P > 0.05$ indicates that the model terms are not significant. In this case, each term except X_1^2 for uptake capacity is statistically significant at the 5% level ($P < 0.0001$). The value of the coefficient of determination R^2 was equal to 0.992, which ensures a satisfactory adjustment of the model to the experimental data. The main effects of reducing sugar and dye concentrations were the most significant parameters ($P < 0.0001$) for dye uptake capacity.

Table 4. Analysis of variance (ANOVA) for dye uptake capacity.

Source	Sum of squares	df	Mean square	F-value	P-value
Model	1285.38	5	257.08	174.34	< 0.0001
X_1	941.75	1	941.75	638.68	< 0.0001
X_2	230.27	1	230.27	156.16	< 0.0001
X_1 X_2	84.00	1	84.00	56.97	0.0001
X_1^2	2.10	1	2.10	1.43	0.2715
X_2^2	18.27	1	18.27	12.39	0.0097
Residual	10.32	7	1.47		
Lack of fit	9.79	3	3.26	24.54	0.0049
Pure error	0.53	4	0.13		

Table 5. Analysis of variance (ANOVA) for growth rate.

Source	Sum of squares	df	Mean square	F-value	P-value
Model	3.898E-003	5	7.796E-004	21.47	0.0004
X_1	7.260E-004	1	7.260E-004	20.00	0.0029
X_2	1.536E-003	1	1.536E-003	42.31	0.0003
X_1 X_2	4.000E-006	1	4.000E-006	0.11	0.7497
X_1^2	3.886E-004	1	3.886E-004	10.70	0.0136
X_2^2	1.609E-003	1	1.609E-003	44.32	0.0003
Residual	2.541E-004	7	3.631E-005		
Lack of Fit	2.393E-004	3	7.978E-005	21.56	0.0062
Pure Error	1.480E-005	4	3.700E-006		

Table 5 shows the ANOVA of the model for growth rate. The F-value was 21.47 (P-value of 0.0004), which indicated that the model was statistically significant. As can be seen from Table 5, it was observed that the each term except X_1X_2 had significant effects on growth rate. The values of R^2 and adj. R^2 were equal to 0.94 and 0.895, which is very high and has a high correlation between the experimental values and predicted values. Figure 6 shows the three-dimensional response surfaces and the combined effect of initial

dye and initial reducing sugar concentrations on dye uptake capacity. Increasing dye concentration from 100 to 500 mg/L enhanced the uptake capacity of the dye. The increase in dye uptake capacity with increasing dye concentration is due to an important driving force provided by initial dye concentration to overcome all mass transfer resistances of each pollutant between the aqueous and solid cell phases [27]. On the other hand, it can be said that the reducing sugar concentration adversely affected dye uptake capacity while initial dye concentration had a positive effect on dye uptake capacity (Figure 6).

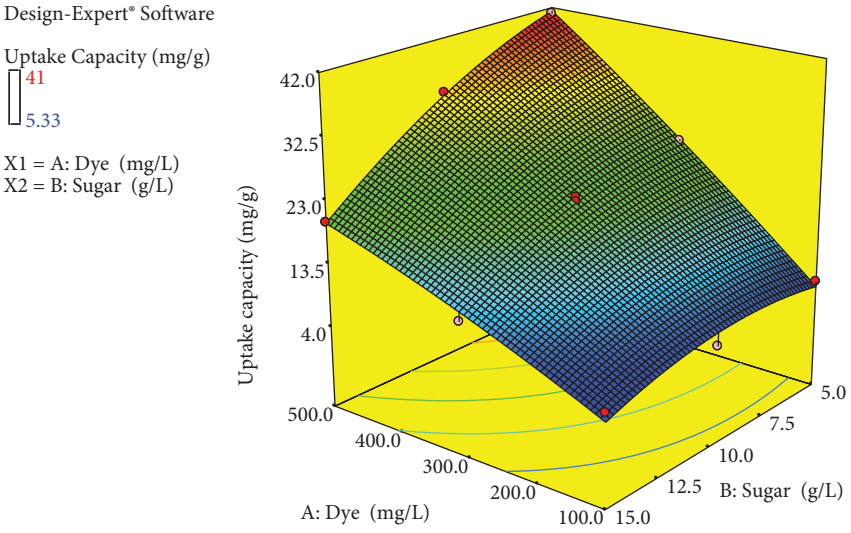

Figure 6. 3D surface plot for dye uptake capacity as a function of dye and reducing sugar concentrations.

Figure 7 shows a three-dimensional response graph given to illustrate the combined effect of initial dye and initial reducing sugar concentrations on growth rate. It shows that growth rate was enhanced with increasing reducing sugar concentrations between 5 and 10 g/L and afterwards showed a slight decrease. Otherwise, increasing dye concentrations from 100 to 500 mg/L decreased the growth rate of *Candida tropicalis* due to the toxicity of the dye at higher concentrations.

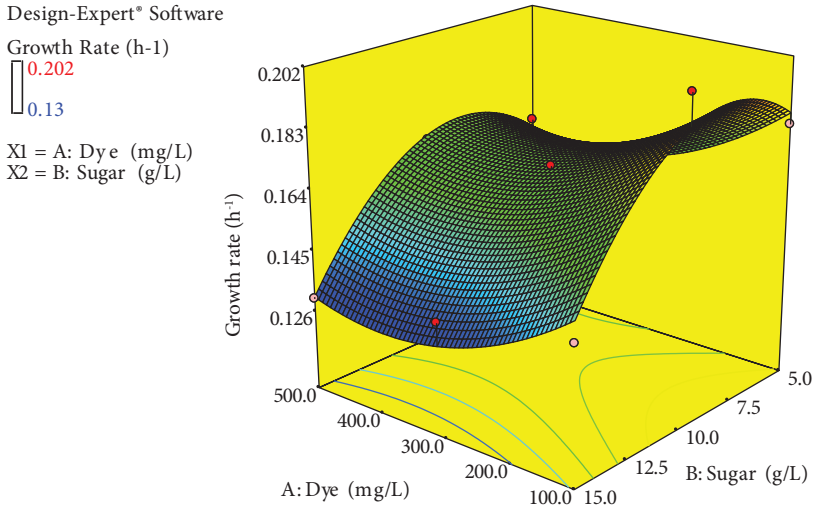

Figure 7. 3D surface plot for growth rate as a function of dye and reducing sugar concentrations.

As a result both the responses were highly affected by initial dye and reducing sugar concentrations under the operating conditions. One of the main aims of this study was to find the optimum design parameters to maximize the dye uptake capacity from the obtained model equations. In order to maximize the dye uptake capacity of *Candida tropicalis*, a numerical optimization was performed using the Design Expert 7.0 statistical program, which is using a quadratic model equation in the experimental range. The optimum values of the design variables for maximizing dye uptake capacity were 499 mg/L dye concentration and 5.1 g/L reducing sugar concentration with a desirability of 1.0. The corresponding responses were 41.3 mg/g uptake capacity and 0.161 1/h growth rate. The model's suitability was determined as optimum levels of the design parameters to achieve the maximum dye uptake capacity compared with the experimental values (Table 6).

Table 6. Model suitability.

	Dye conc., X_1 (mg/L)	Reducing sugar conc., X_1 (mg/L)	Growth rate (1/h)	Uptake capacity (mg/g)
Experimental	500	5.0	0.163	41.0
Predicted	499	5.1	0.161	41.3

4. Conclusion

In this study we examined the combined effect of initial reducing sugar and dye concentrations on growth rate, uptake %, and dye uptake capacity of *Candida tropicalis* in a batch system using molasses as a growth medium. Initial reducing sugar and dye concentrations in bioaccumulation experiments at pH 3 were changed between 1 and 15 g/L and 40 and 700 mg/L, respectively. It was observed that increasing reducing sugar concentration after 5 g/L had a negative effect on dye uptake capacity while it had a positive effect on growth rate until 10 g/L reducing sugar concentration. In addition to this, studies of the effect of sugar concentration on dye removal indicated that sugar source might have a synergistic effect as well as an antagonistic effect on dye bioremoval due to the microbial species employed and different metabolic characteristics. On the other hand, increasing the initial dye concentration from 40 to 700 mg/L decreased the growth rate of *C. tropicalis* and increased the dye uptake capacity. The highest uptake capacity was obtained as 56.28 mg/g in medium containing 3.2 g/L initial reducing sugar concentration and 711.1 mg/L initial dye concentration.

While the highest uptakes at low dye concentrations (40 and 100 mg/L) were obtained at 15 g/L initial reducing sugar concentration, 10 g/L initial reducing sugar concentration provided the highest uptake at high dye concentrations (300 mg/L and above). The higher dye uptake values were always obtained at lower dye levels. The highest dye uptake % was observed as 61.46% in the medium containing 14.9 g/L initial reducing sugar concentration and 38 mg/L initial dye concentration.

The combined effect of initial reducing sugar (5–15 g/L) and initial dye concentration (100–500 mg/L) on growth rate and dye uptake capacity of *Candida tropicalis* was also investigated by RSM using Design Expert 7.0. It was demonstrated that the effect of design variables on responses was fitted by a second-order quadratic equation. The design variables had a significant effect on bioaccumulation by *C. tropicalis* according to ANOVA. The optimum design variables for maximizing the uptake capacity were obtained by numerical optimization using Design Expert and these variables were 5.1 g/L initial reducing sugar concentration and 499 mg/L dye concentration. The corresponding experimental and predicted responses to these variables were 41 mg/g and 41.3 mg/g uptake capacity, respectively.

As a result, the reducing sugar and dye concentrations were the most significant parameters for dye

uptake capacity of the microorganism, and it is not easy to reach a general conclusion. The studies of the effect of sugar concentration on dye removal indicated that sugar source might have a synergistic effect as well as an antagonistic effect on dye bioremoval due to the microbial species employed and different metabolic characteristics.

Increasing initial dye concentration always decreased the growth rate of yeast while it caused an increase in dye uptake capacity. The optimization of variables is always necessary for effective dye removal. The optimization procedure carried out in this study points out that low or mild sugar concentrations with high dye concentrations leads to high uptake.

Acknowledgment

The financial support provided by the Gazi University Scientific Research Projects Unit (BAP-06/2007-56 coded project) is gratefully acknowledged.

References

[1] Aksu Z. Application of biosorption for the removal of organic pollutants: a review. Process Biochem 2005; 40: 997–1026.

[2] Karapınar Kapdan I, Kargı F. Removal of textile dyestuffs from wastewater by adsorptive biodegradation. Turk J Engin Environ Sci 2000; 24: 161–169.

[3] Fu Y, Viraraghavan T. Fungal decolorization of dye wastewaters: a review. Bioresource Technol 2001; 79: 251–262.

[4] Arora S, Saini SH, Singh K. Decolorisation of a monoazo disperse dye with *Candida tropicalis*. Color Technol 2005; 121: 298–303.

[5] Rai HS, Bhattacharyya MS, Singh J, Bansal TK, Vats P, Banerjee UC. Removal of dyes from the effluent of textile and dyestuff manufacturing industry: a review of emerging techniques with reference to biological treatment. Crit Rev Env Sci Tec 2005; 35: 219–238.

[6] Aksu Z. Reactive dye bioaccumulation by *Saccharomyces cerevisiae*. Process Biochem 2003; 38: 1437–1444.

[7] Aksu Z, Dönmez G. Combined effects of molasses sucrose and reactive dye on the growth and dye bioaccumulation properties of *Candida tropicalis*. Process Biochem 2005; 40: 2443–2454.

[8] Ertuğrul S, San NO, Dönmez G. Treatment of dye (Remazol Blue) and heavy metals using yeast cells with the purpose of managing polluted textile wastewaters. Ecol Eng 2009; 35: 128–134.

[9] Kocaer FO, Alkan U. Treatment alternatives for textile effluents containing dyes. Uludağ Üniversitesi Mühendislik-Mimarlık Fakültesi Dergisi 2002; 7: 47–55 (in Turkish with English abstract).

[10] Hunger K. Industrial Dyes: Chemistry, Properties, Applications. Weinheim, Germany: Wiley-VCH; 2003.

[11] Blackburn RS, Burkinshaw SM. A greener approach to cotton dyeings. Part 2: Application of 1:2 metal complex acid dyes. Green Chem 2002; 4: 261–265.

[12] Vijaykumar MH, Veeranagouda Y, Neelakanteshwar K, Karegoudar TB. Decolorization of 1:2 metal complex dye Acid blue 193 by a newly isolated fungus *Cladosporium cladosporioides*. World J Microb Biot 2006; 22: 157–162.

[13] Slokar YM, Marechal AML. Methods of decoloration of textile wastewaters. Dyes Pigments 1998; 37: 335–356.

[14] Robinson T, McMullan G, Marchant R, Nigam P. Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative. Bioresource Technol 2001; 77: 247–255.

[15] Doble M, Kumar A. Biotreatment of Industrial Effluents. Amsterdam, the Netherlands: Elsevier; 2005.

[16] Dönmez G. Bioaccumulation of the reactive textile dyes by *Candida tropicalis* growing in molasses medium. Enzyme Microb Tech 2002; 30: 363–366.

[17] Yang Q, Yang M, Pritsch K, Yediler A, Hagn A, Schloter M, Kettrup A. Decolorization of synthetic dyes and production of manganese-dependent peroxidase by new fungal isolates. Biotechnol Lett 2003; 25: 709–713.

[18] Meehan C, Banat IM, McMullan G, Nigam P, Smyth F, Marchant R. Decolorization of Remazol Black-B using thermotolerant yeast *Kluyveromyces marxianus* IMB3. Environ Int 2000; 26: 75–79.

[19] Forgacs E, Cserhati T, Oros G. Removal of synthetic dyes from wastewaters: a review. Environ Int 2004; 30: 953–971.

[20] Meyer U. Biodegradation of synthetic organic colorants. In: Leisinger T, Cook AM, Hunter R, Nuesch J, editors. Microbial Degradation of Xenobiotic and Recalcitrant Compounds. FEMS Symposium 12. London, UK: Academic Press; 1981. pp. 371–385.

[21] Yu Z, Wen X. Screening and identification of yeasts for decolorizing synthetic dyes in industrial wastewater. Int Biodeter Biodegr 2005; 56: 109–114.

[22] Lucas MS, Amaral C, Sampaio A, Peres JA, Dias AA. Biodegradation of the diazo dye Reactive Black 5 by a wild isolate of *Candida oleophila*. Enzyme Microb Tech 2006; 39: 51–55.

[23] Martins MAM, Cardoso MH, Queiroz MJ, Ramalho MT, Campos AMO. Biodegradation of azo dyes by the yeast *Candida zeylanoides* in batch aerated cultures. Chemosphere 1999; 38: 2455–2460.

[24] Ramalho PA, Scholze H, Cardoso MH, Ramalho MT, Oliveira-Campos AM. Improved conditions for the aerobic reductive decolorisation of azo dyes by *Candida zeylanoides*. Enzyme Microb Tech 2002; 31: 848–854.

[25] Yang Q, Tao L, Yang M, Zhang H. Effects of glucose on the decolorization of Reactive Black 5 by yeast isolates. J Environ Sci 2008; 20: 105–108.

[26] Das D, Charumathi D, Das N. Bioaccumulation of the synthetic dye Basic Violet 3 and heavy metals in single and binary systems by *Candida tropicalis* grown in a sugarcane bagasse extract medium modelling optimal conditions using response surface methodology (RSM) and inhibition kinetics. J Hazard Mater 2011; 186: 1541–1552.

[27] Gönen F, Aksu Z. Use of response surface methodology (RSM) in the evaluation of growth and copper(II) bioaccumulation properties of *Candida utilis* in molasses medium. J Hazard Mater 2008; 154: 731–738.

[28] Saratale RG, Saratale GD, Chang JS, Govindwr SP. Decolorization and biodegradation of textile dye Navy blue HER by *T beigelii* NCIM-3326. J Hazard Mater 2009; 166: 1421–1428.

[29] Das D, Charumathi D, Das N. Combined effects of sugarcane bagasse extract and synthetic dyes on the growth and bioaccumulation properties of *Pichia fermentans* MTCC 189. J Hazard Mater 2010; 183: 497–505.

[30] Blanquez P, Casa N, Font X, Gabarrell X, Sarra M, Caminal G, Vicent T. Mechanism of textile metal dye biotransformation by *Trametes versicolor*. Water Res 2004; 38: 2166–2172.

[31] Blanquez P, Caminal G, Sarra M, Vicent T. The effect of HRT on the dye decolorisation of the Grey Lanaset G textile dye by *Trametes versicolor*. Chem Eng J 2007; 126: 163–169.

[32] Varma RJ, Gaikwad BG. Rapid and high biodegradation of phenols catalyzed by *Candida tropicalis* NCIM 3556 cells. Enzyme Microb Tech 2008; 43: 431–435.

[33] Netpradit S, Thiravetyan P, Towprayoon S. Application of waste metal hydroxide sludge for adsorption of azo reactive dyes. Water Res 2003; 37: 763–772.

[34] Miller GL. Use of dinitrosalicylic acid reagent for reducing sugar. Anal Chem 1959; 31: 426–430.

[35] Srinivasan SV, Murthy DVS. Statistical optimization for decolorization of textile dyes using *Trametes versicolor*. J Hazard Mater 2009; 165: 909–914.

[36] Chen KC, Wua JY, Liou DJ, Hwang SCJ. Decolorization of the textile dyes by newly isolated bacterial strains. J Biotechnol 2003; 101: 57–68.

[37] Kumar V, Wati L, FitzGibbon F, Nigam P, Banat IM, Singh D, Marchant R. Bioremediation and decolorization of anaerobically digested distillery spent wash. Biotechnol Lett 1997; 19: 311–313.

[38] Shahvali M, Assadi MM, Rostami K. Effect of environmental parameters on decolorization of textile wastewater using Phanerochaete chrysosporium. Bioprocess Eng 2000; 23: 721–726.

[39] Park C, Lee M, Lee B, Kim SW, Chase HA, Lee J, Kim S. Biodegradation and biosorption for decolorization of synthetic dyes by *Funalia trogii*. Biochem Eng J 2007; 36: 59-65.