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# Combined effects of sugarcane bagasse extract and synthetic dyes on the growth and bioaccumulation properties of *Pichia fermentans* MTCC 189

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ABSTRACT

Bioaccumulation of synthetic dyes viz. Acid Blue 93, Direct Red 28 and Basic Violet 3 by growing cells of yeast, *Pichia fermentans* MTCC 189 was investigated in growth media prepared from sugarcane bagasse extract. The maximum dye bioaccumulation was determined at pH 5.0 for all the dyes tested. Two kinetic models viz. Noncompetitive and Uncompetitive models were tested in order to determine the toxic effects of dyes on the specific growth rate of *P. fermentans* MTCC 189. Basic Violet 3 was found to be more toxic than the other two dyes. The combined effects of sugarcane bagasse extract and initial Basic Violet 3 dye concentrations on the specific growth rate and dye bioaccumulation efficiency of *P. fermentans* MTCC 189 was investigated and optimized using Response Surface Methodology (RSM). A $2^2$ full factorial central composite design was successfully used for analysis of results. The optimum combination predicted via RSM confirmed that *P. fermentans* MTCC 189 was capable of bioaccumulating Basic Violet 3 dye upto 69.8% in the medium containing 10 mg/L of dye and 24 g/L sugar extracted from sugarcane bagasse.



## 1. Introduction

Synthetic dyes are widely used in textile, food, paper and cosmetic industries. The effluents of these industries are highly colored and the disposal of these wastes into receiving waters causes damage to the environment. They may also be toxic to some aquatic life due to the presence of metals, chlorides, etc., in them [1]. Existing physico-chemical methods of dye removal from effluents suffer from severe constraints like low efficiency, high operational cost and large amount of sludge generation [2]. Innovative technologies, such as bioremediation are needed as alternatives to conventional methods to find inexpensive ways of removing dyes from large volumes of effluents [3]. For the last two decades, considerable work has been done with the goal of using microorganisms as bioremediation agents in the treatment of dye containing wastewaters. Microorganisms are potent bioremediators, removing dyes via bioaccumulation or biodegradation mechanisms. The growing, resting and non-living cells of microorganisms are reported to bioremove reactive dyes from aqueous solutions [3–6]. Removal of pollutants by actively growing cells by metabolism-and temperature-independent and metabolism-dependent mechanism steps is defined as bioaccumulation [7–9]. In the bioaccumulative processes, there is an initial rapid accumulation step, i.e. temperature and metabolism independent which is thought to involve contaminants binding on the biomass surface. This step is followed by a second process, i.e. metabolism-dependent and can accumulate large quantities of pollutants than the first process. Bioaccumulation can be accomplished for the removal of different kinds of textile dyes if the growing cells find sufficient quantity of easily used carbon and nitrogen sources instead of dyes in the growth medium. Using growing cultures in bioremoval has the advantage over the non-living and resting cells that the simultaneous removal of dye is obtained during growth of the organism and separate biomass production can be avoided. However, the major limitations of using growing systems for bioaccumulation of dyes are that the nutrient media is required for the growth of the organism and cell growth is inhibited when dye concentration is too high. Moreover, other components or constraints of the wastewater may also be toxic to living cells, e.g. extremes of pH and high salt concentration. If the dye toxicity problem to the growing cells is overcome by the use of dye resistant organisms, the self-replenishing system can run continuously for extended periods [6,10–12].

Yeasts has long been known to be capable of rapid bioaccumulation of metal ions from solution, but very little work has been carried out investigating the ability of yeast to act as a bioaccumulator for textile dyes in wastewaters [12–14]. Yeasts have many advantages as compared to bacteria and filamentous fungi. Yeasts are an inexpensive, readily available source of biomass. Yeasts can adapt and grow under various extreme conditions of pH, temperature and nutrient availability as well as high pollutant concentrations. They not only grow rapidly like bacteria, but like filamentous fungi they also have the ability to resist unfavourable environment [15].

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Up to date, bioaccumulation of azo type and anthraquinone reactive dyes has been extensively documented [6,10,16–18] while studies on another important type of reactive dyes, triphenylmethane dyes have seldom been reported.

The objectives of the present study were: (1) to identify the combined effects of sugarcane bagasse extract and the synthetic dyes viz. Acid Blue 93 and Basic Violet 3 (triphenylmethane group) and Direct Red 28 (azo group) on the growth and bioaccumulation properties of the yeast *Pichia fermentans* MTCC 189 in a batch system; (2) to typify the inhibition of Basic Violet 3 dye on yeast growth; and (3) to see if the specific growth rate and Basic Violet 3 dye uptake by the yeast *P. fermentans* MTCC 189 could be modelled by using RSM with respect to bagasse extract concentration and Basic Violet 3 dye at different levels for developing rational strategies for remediation of dye bearing wastewater. There seems to be no literature correlating the bioaccumulation of synthetic dyes by adapted growing yeast *P. fermentans* MTCC 189 in the medium containing sugarcane bagasse extract as a sole carbon source.

### 1.1. Kinetic approach

The relationship between the specific growth rate and substrate concentration in absence of inhibitory substance such as dyes can be described by the Monod equation as given below.

$$\mu = \frac{\mu_m}{1 + (K_s/S)} \tag{1}$$

where $S$ is the substrate concentration (g/L), $\mu$ and $\mu_m$ are the specific growth rate and the maximum specific growth rate of microorganism ($h^{-1}$) respectively, and $K_s$ is the saturation constant (g/L), Eq. (1) can be linearized in double-reciprocal form:

$$\frac{1}{\mu} = \frac{1}{\mu_m} + \frac{K_s}{\mu_m}\frac{1}{S} \tag{2}$$

$K_s$ and $\mu_m$ values can be determined from the plot of $1/\mu$ versus $1/S_0$ (assuming $S = S_0$ at the beginning of exponential growth) yields a linear line with a slope of $K_s/\mu_m$ and $y$-axis intercept of $1/\mu_m$. In presence of inhibitory (toxic) substances such as dyes in nutrient media, microbial growth becomes inhibited, and specific growth rate depends on inhibitor concentration. Inhibitions models viz. Noncompetitive and Uncompetitive are classified according to the effects of toxic compounds on the specific growth rate and saturation constant $K_s$ and the rate expressions of the models are expressed as follows:

Noncompetitive inhibition:

$$\mu = \frac{\mu_m}{[1 + (K_s/S)]\,[1 + (C_0/K_I)]} \tag{3}$$

or

$$\mu = \frac{\mu_{m,app}}{1 + (K_s/S)} \tag{4}$$

where

$$\mu_{m,app} = \frac{\mu_m}{1 + [C_0/K_I]} \tag{5}$$

where $C_0$ and $K_I$ are the initial dye concentration (mg/L) and inhibition constants of dye ions (mg/L) respectively. Eq. (3) can be linearized as similar to Eq. (2):

$$\frac{1}{\mu} = \frac{1}{\mu_{m,app}} + \frac{K_s}{\mu_{m,app}}\frac{1}{S} \tag{6}$$

The $\mu_{m,app}$ values can be determined from the linear plot of $1/\mu$ versus $1/S$ at different initial dye concentrations and then an average value $K_I$ can be calculated from Eq. (5).

Noncompetitive inhibition model describing the dye component inhibition was selected for assessing the dynamic behaviour of yeast cells [19].

Uncompetitive inhibition:

$$\mu = \frac{\mu_m S}{[K_s/[1 + (C_0/K_I)] + S][1 + (C_0/K_I)]} \tag{7}$$

This equation can be linearized as follows:

$$\frac{1}{\mu} = \frac{1}{\mu_{m,app}} + \frac{K_s}{\mu_m}\frac{1}{S} \tag{8}$$

where

$$\mu_{m,app} = \frac{\mu_m}{1 + [C_0/K_I]} \tag{9}$$

## 2. Materials and methods

### 2.1. Microorganism and growth media

Microorganism used for bioaccumulation, *P. fermentans* MTCC 189 was obtained from IMTECH, Chandigarh, India. The pure cultures were maintained in Yeast Extract Peptone Dextrose (YEPD) media composed of glucose – 20 g/L, peptone – 20 g/L, yeast extract – 10 g/L and agar – 15 g/L. Then the strain was grown in the mineral media composed of glucose – 10 g, $KH_2PO_4$ – 1 g, $(NH_4)_2SO_4$ – 1 g, $MgSO_4{\cdot}7H_2O$ – 500 mg, yeast extract – 200 mg, distilled water – 1 L. For bioaccumulation assay, the pregrown culture from aforementioned media was used to inoculate the aqueous extract of sugarcane bagasse. The extract was prepared by boiling desired amount of sugarcane bagasse in 100 mL of distilled water. Various concentrations of extract (10%, 20%, and 30%) were prepared by boiling different amount of bagasse (10, 20, 30 g) in 100 mL of distilled water respectively. The total sugar contents were analysed as 8 g/L, 16 g/L and 24 g/L for 10%, 20%, and 30% respectively by anthrone method [20]. This aqueous extract was used as sole carbon source for yeast growth.

### 2.2. Dyestuff and chemicals

Triphenylmethane dyes (Acid Blue 93 and Basic Violet 3) and Azo dye (Direct Red 28) which are commonly used in textile industries were purchased from Hi Media (Mumbai, India). Fig. 1 shows the structure of the synthetic dyes used for the present study. Dye stock solutions were prepared by dissolving 0.1 g of dye in 1 L of distilled water. The working solutions were prepared by diluting the stock solutions. All chemicals used were of highest purity available and of analytical grade.

### 2.3. Bioaccumulation assay

An aliquot of 2 mL of pregrown culture harvested from exponential phase was transferred to 100 mL of aqueous extract. The influences of pH on bioaccumulation of dyes were investigated by varying the pH (3, 5, 7 and 9) of the aqueous extract. The combined effect of media concentration and dye concentration was studied by varying the dye concentration from 10 to 30 mg/L at constant aqueous extract concentrations (8, 16 and 24 g/L). Cultures were grown at 28 °C on a rotary shaker at 120 rpm. Samples were withdrawn at regular intervals and subjected to centrifugation at 8000 rpm for 5 min. The pellet was dried at 40 °C to a constant weight for biomass estimation and the supernatant was analysed for residual dye concentration. All the experiments were carried out in triplicates. The dye uptake values were determined as follows:

$$\text{Bioaccumulation}\,\% = \frac{C_0 - C_f}{C_f} \times 100 \tag{10}$$

where $C_0$ is the initial concentration of dye (mg/L). $C_f$ is the final concentration of dye (mg/L).

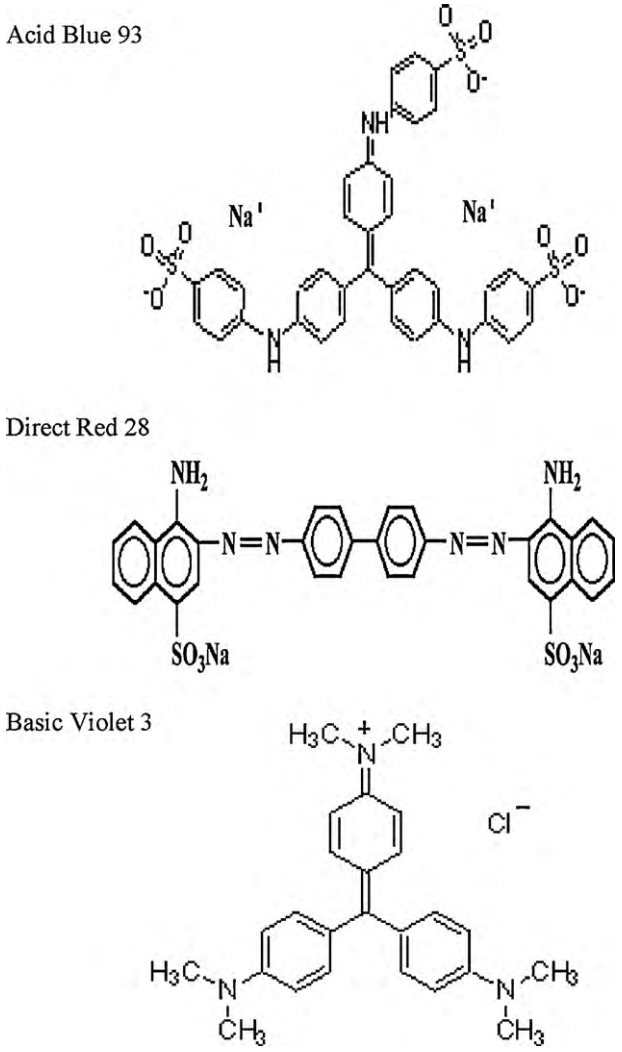


**Fig. 1.** Structure of the synthetic dyes used in batch assays.

### 2.4. Analytical procedure

Residual dye concentration of the broth was measured spectrophotometrically by reading absorbance at 604, 584, 488 nm for Acid Blue 93, Basic Violet 3 and Direct Red 28 respectively. Dye containing bagasse medium was used as the blank. For the measurement of yeast growth, the biomass concentration was determined by measuring the turbidity of the diluted sample at 540 nm using a standard curve of absorbance against dry cell mass. Two control flasks were prepared. First control medium contained bagasse extract without any dye to examine the growth of the yeast and to detect the possible dye inhibitory effects. Second control medium contained both the dye and bagasse extract without any yeast growth to observe any reaction of bagasse extract with the dye.

### 2.5. Statistical analysis

A classical approach of changing one variable at a time to study the effects of other variables on a particular response is time consuming for multivariable systems. Hence, an alternate strategy involving statistical approaches, e.g. response surface methodology (RSM) has been adapted to solve this complexity. RSM involves three steps (1) design and experiments, (2) response surface modelling through regression and (3) optimization. Recently, several studies have described the use of RSM for optimization of process parameters such as pH, pollutant concentration, biosorbent dosage for metals [21] or dyes [22] from synthetic solution.

**Table 1**
Experimental ranges and levels of independent variables.

| Independent variables | Design variables | Range and level | | | | |
|---|---|---|---|---|---|---|
| | | $-\alpha$ | −1 | 0 | +1 | $+\alpha$ |
| $S_0$ (g/L) | A | 4.69 | 8 | 16 | 24 | 27.31 |
| $C_{0B.V}$ (mg/L) | B | 5.86 | 10 | 20 | 30 | 34.14 |

The present study was carried out not only to investigate the combined effects of sugarcane bagasse extract and Basic Violet 3 dye concentrations on growth and bioaccumulation of *P. fermentans* MTCC 189, but also to find out the model equations representing growth and bioaccumulation percentage. The optimization procedure involved studying the response of statistically designed combinations, estimating the coefficients by fitting the experimental data to response functions thereby predicting the response of the model and checking the adequacy of the model in terms of $R^2$ values. Initial sugar concentration in bagasse extract and Basic Violet 3 dye concentrations (mg/L) in the bioaccumulation medium were chosen as independent variables (*A* and *B*). The levels of each variable were varied in the range −1.4 to +1.4 respectively as shown in Table 1. Initial sugar concentration was varied over two main levels denoted by −1 and +1 (8 and 24 g/L) relative to the centre point denoted by 0 (16 g/L). Basic Violet 3 dye concentrations were varied over two levels (10 and 30 mg/L) with respect to the centre point (20 mg/L). 13 sets of experiments were carried out with appropriate combinations of dye and bagasse concentrations which included the concentrations of both bagasse extract and dye coded by $\alpha$ value as shown in Table 2. Data were analysed using full factorial central composite design with respect to the coded and uncoded values as listed in Table 2. Numerical analysis for estimating responses of specific growth and Basic Violet 3 dye accumulation percent and the graphical analysis were done by using Design Expert Package (Version 8 Stat-ease Inc., Minneapolis, MN, USA). The statistical significance of the model was evaluated by the coefficient determination $R^2$ and by the *F* test analysis of variance (ANOVA). According to ANOVA, a large value of *F* indicates that most of the variation in response can be explained by regression equation. The associated *p*-value is used to estimate whether *F* is large enough to hold a statistical significance. A value of *p* lower than 0.05 indicates the model is statistically significant [21–25]. Adequate precision compares the range of predicted values at the design points to the average prediction error. A ratio greater than 4 is desirable and indicates model discrimination. The lack of fit *F* test describes the variation of data around the fitted model. If the model fits the data well, lack of fit will be non-significant.

**Table 2**
Full factorial central composite design matrix of two variables in coded and uncoded values.

| Run | $S_0$ (g/L) | $C_0$ (mg/L) | $S_0$ (g/L) | $C_0$ (g/L) |
|---|---|---|---|---|
| 1 | 0 | 1.41 | 16 | 34.14 |
| 2 | 1 | 1 | 24 | 30 |
| 3 | 0 | 0 | 16 | 20 |
| 4 | −1 | 1 | 8 | 30 |
| 5 | 0 | 0 | 16 | 20 |
| 6 | 0 | 0 | 16 | 20 |
| 7 | −1.41 | 0 | 4.69 | 20 |
| 8 | 0 | −1.41 | 16 | 5.86 |
| 9 | 0 | 0 | 16 | 20 |
| 10 | 1.41 | 0 | 27.31 | 20 |
| 11 | −1 | −1 | 8 | 10 |
| 12 | 1 | −1 | 24 | 10 |
| 13 | 0 | 0 | 16 | 20 |

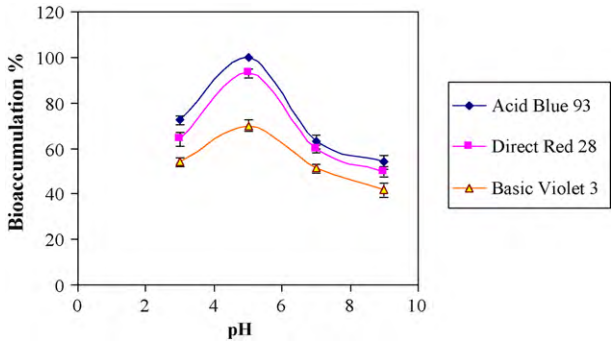


**Fig. 2.** Effect of pH on bioaccumulation of dyes by *Pichia fermentans* MTCC 189. $C_0$ – 10 mg/L; $S_0$ – 24 g/L.

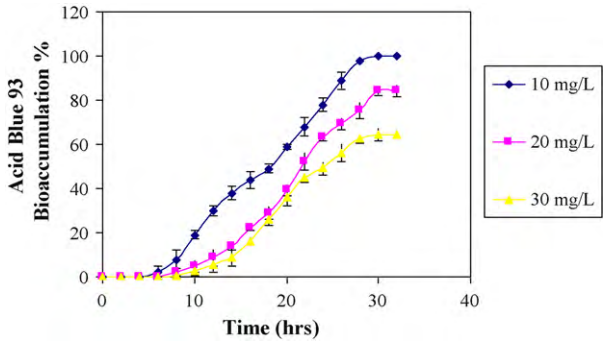


**Fig. 3.** Effect of initial dye concentration of Acid Blue 93 on bioaccumulation percentages of *Pichia fermentans* MTCC 189. $S_0$ – 24 g/L, pH 5.

## 3. Results and discussion

The growth and bioaccumulation properties of *P. fermentans* MTCC 189 were investigated as a function of initial pH, initial sugar concentration in bagasse extract and initial dye concentration.

### 3.1. Influence of pH on bioaccumulation

The influence of initial pH on bioaccumulation was tested taking 10 mg/L of each dye and 24 g/L of sugar extracted from bagasse in the medium varying the pH (3, 5, 7 and 9). The initial pH significantly influenced the dye bioaccumulation properties of *P. fermentans* MTCC 189 and maximum bioaccumulations were noted at pH 5 for all the dyes. Fig. 2 showed that *P. fermentans* MTCC 189 could accumulate dyes to a different extent at pH 5 and the bioaccumulation of three dyes differed significantly depending upon the dye. Acid Blue 93 and Direct Red 28 are anionic dyes which were accumulated at higher ranges, i.e. 100% and 93% whereas Basic Violet 3 being cationic in nature was found to be bioaccumulated in low range (69.8%). The difference in the bioaccumulation percentage of the dyes could be explained based on the structure of the dyes and the surface charges of the yeast biomass. Complexity in structure and more competition among the dye cations and $H^+$ ions on the yeast biomass surface may be the reason for minimum bioaccumulation of Basic Violet 3 at pH 5 compared to other two anionic dyes.

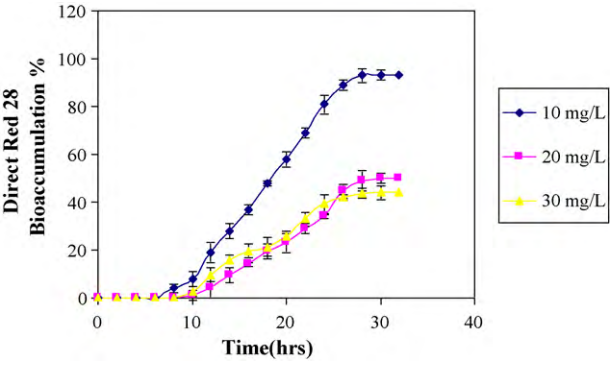


**Fig. 4.** Effect of initial dye concentration of Direct Red 28 on bioaccumulation percentages of *Pichia fermentans* MTCC 189. $S_0$ – 24 g/L, pH 5.

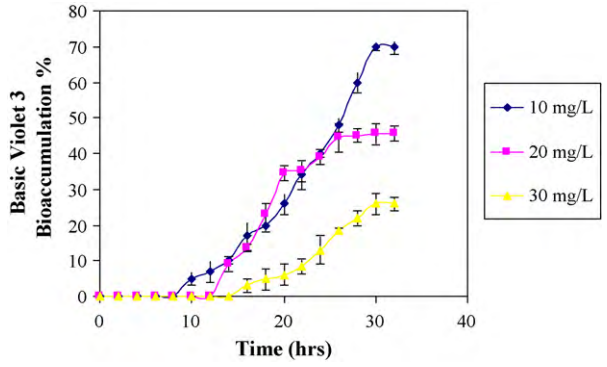


**Fig. 5.** Effect of initial dye concentration of Basic Violet 3 on bioaccumulation percentages of *Pichia fermentans* MTCC 189. $S_0$ – 24 g/L, pH 5.

### 3.2. Influence of initial dye concentration on bioaccumulation and growth properties of P. fermentans MTCC 189

The influence of initial dye concentration on bioaccumulation was carried out by varying the dye concentration (10, 20, 30 mg/L) in 24 g/L bagasse extract with optimized pH values. Bioaccumulation % of the dyes Acid Blue 93, Direct Red 28 and Basic Violet 3 by the yeast species at different initial dye concentrations is shown in Figs. 3–5 respectively. For all the three dyes, bioaccumulation percentages in *P. fermentans* MTCC 189 decreased as the dye concentration increased from 10 to 30 mg/L. The decrease in bioaccumulation percentage was caused due to the toxicity of dyes at higher concentrations which reduced the biomass yield.

Yeast growth curves in the absence and presence of increasing concentrations of dyes (10, 20, 30 mg/L) in 24 g/L sugar extracted from bagasse are shown in Figs. 6–8. Maximum bioaccumulation percentages were noted in the exponential growth phase of the yeast. There was an increase in lag phases with the increasing concentrations of dyes.

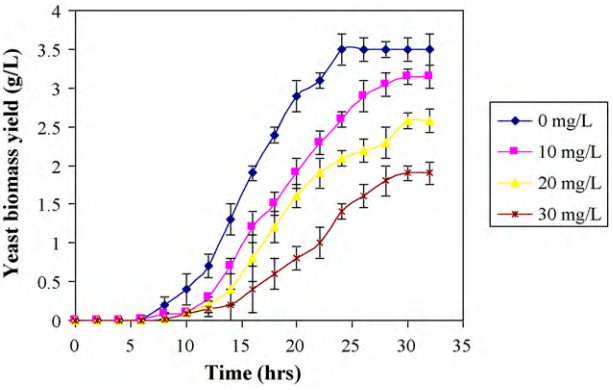


**Fig. 6.** Growth of *P. fermentans* MTCC 189 in absence and presence of increasing concentrations of Acid Blue 93.

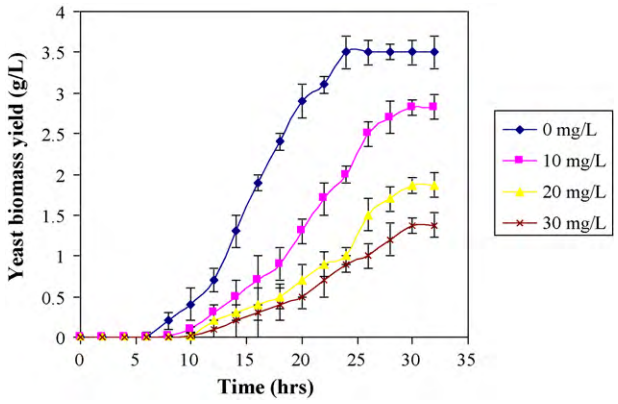


**Fig. 7.** Growth of *P. fermentans* MTCC 189 in absence and presence of increasing concentrations of Direct Red 28.

### 3.3. Decolorization kinetics of synthetic dyes

To determine the decolorization kinetics of synthetic dyes, experiments with constant initial substrate (24 g/L of sugar extracted from bagasse) and different initial dye concentrations varying between 10 and 30 mg/L were performed in order to detect the residual dye concentrations and bioaccumulation percentages throughout 32 h of the incubation period. The bioaccumulation percentages for different initial dye concentrations are depicted in Figs. 3–5 which indicated that the color was not completely removed in every dye concentration except Acid Blue 93.

Experimental data obtained from the batch tests were plotted in forms $C_t$ versus time, ln $C_t$ versus time and $1/C_t$ versus time respectively following Eqs. (11)–(13):

$$C_t = C_0 - K_0 t \quad (11)$$

$$C_t = C_0 e^{-K_1 t} \quad (12)$$

$$\frac{1}{C_t} = \frac{1}{C_0} + K_2 t \quad (13)$$

In the following step, the color removal rate constants were calculated for zero, first and second order reaction kinetics from the slopes of the best fit lines respectively. The zero order, first order and second order rate constants ($K_0$, $K_1$ and $K_2$) are listed in Table 3. Among the data obtained, the rate constants with the highest regression coefficients were accepted as the most suitable kinetic constants. In other words, a high degree linear relationship ($R^2 > 97$) between dye concentration ($C_t$) and time showed that the color was removed according to zero order kinetics in batch decolorization tests. As seen in Table 3, the decolorization rate constants increased with the increase in dye concentrations at zero order.

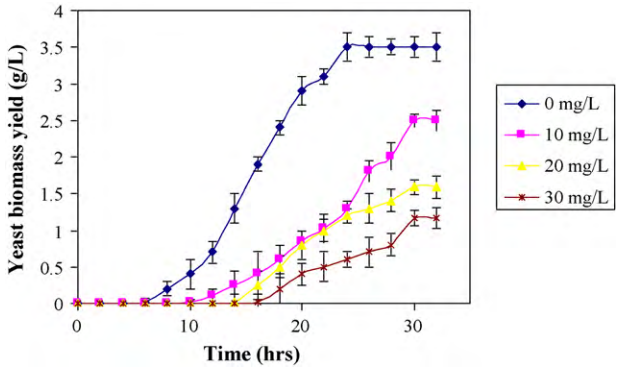


**Fig. 8.** Growth of *P. fermentans* MTCC 189 in absence and presence of increasing concentrations of Basic Violet 3.

**Table 3**
Zero, first and second order kinetic constants obtained in dye decolorization.

| Kinetics | Constant | 10 mg/L | 20 mg/L | 30 mg/L |
|---|---|---|---|---|
| **Acid Blue 93** | | | | |
| Zero order | $k_0$ (mg L$^{-1}$ h$^{-1}$) | 0.3700 | 0.6202 | 0.7456 |
| | $R^2$ | 0.9776 | 0.9268 | 0.9198 |
| First order | $k_1$ (h$^{-1}$) | 0.1004 | 0.0605 | 0.0380 |
| | $R^2$ | 0.7034 | 0.8437 | 0.8904 |
| Second order | $k_2$ (L mg$^{-1}$ h$^{-1}$) | 0.0756 | 0.0075 | 0.0021 |
| | $R^2$ | 0.2932 | 0.6896 | 0.8447 |
| **Direct Red 28** | | | | |
| Zero order | $k_0$ (mg L$^{-1}$ h$^{-1}$) | 0.3662 | 0.3761 | 0.5221 |
| | $R^2$ | 0.9527 | 0.9188 | 0.9478 |
| First order | $k_1$ (h$^{-1}$) | 0.0960 | 0.0253 | 0.0226 |
| | $R^2$ | 0.8656 | 0.8899 | 0.9382 |
| Second order | $k_2$ (L mg$^{-1}$ h$^{-1}$) | 0.0426 | 0.0018 | 0.0010 |
| | $R^2$ | 0.6926 | 0.8490 | 0.9250 |
| **Basic Violet 3** | | | | |
| Zero order | $k_0$ (mg L$^{-1}$ h$^{-1}$) | 0.2384 | 0.3487 | 0.4452 |
| | $R^2$ | 0.9046 | 0.8899 | 0.8049 |
| First order | $k_1$ (h$^{-1}$) | 0.0372 | 0.0224 | 0.0098 |
| | $R^2$ | 0.8210 | 0.8901 | 0.8831 |
| Second order | $k_2$ (L mg$^{-1}$ h$^{-1}$) | 0.0065 | 0.0015 | 0.0004 |
| | $R^2$ | 0.7130 | 0.8851 | 0.7884 |

These results are in agreement with the findings obtained in some batch decolorization studies [26,27].

### 3.4. Combined effects of sugarcane bagasse extract and initial dye concentration on growth and bioaccumulation properties of yeast

The growth behaviour of the yeast was investigated at an initial pH value of 5.0 with increasing bagasse extract concentrations in the absence and in the presence of a constant dye concentration for all the three dyes separately. Initial concentration of sugar extracted from bagasse was changed from 8 to 24 g/L keeping the initial dye concentration constant between 10 and 30 mg/L. Comparison of bioaccumulation %, biomass yield, and specific growth rate at different concentration of dyes with increasing substrate concentration is given in Table 4. When sugar concentration was kept constant and initial dye concentration was changed from 10 to 30 mg/L, growth rate of yeast was reduced. The presence of dyes in the growth medium repressed the growth of the microorganism irreversibly and this inhibition effect increased with the dye concentrations for all initial sugar concentration. For all three dyes, the growth rate of yeast *P. fermentans* MTCC 189 increased with raising initial sugar concentration up to 24 g/L both in presence and absence of constant dye concentrations. Higher growth rates were observed in media without dyes. The increase in growth rate with increasing initial sugar concentration at constant dye concentration could be due to cell defence mechanisms such as acclimation to toxicity. Similar results of significant reduction in specific growth rate with increasing Remazol blue concentration were also reported in case of *Candida tropicalis* [10]. Gonen and Aksu [28] also reported that the increase in sugar concentration resulted in an increment in cell concentration and also in specific growth rate. It is clear from the results of the present study that the concentration of sugar extracted from bagasse played a major role in the growth of yeast and decreased the inhibitory effects of dye on the yeast growth. Moreover, the residual sugar concentration in the broth was found to be very low which will not cause any disposal problem. As the bioaccumulation is dependent on growth of yeast, the increase in sugar concentration at constant dye concentration increased the biomass yield and bioaccumulation percentages reducing the spe-

**Table 4**
Comparison of bioaccumulation %, biomass yield and specific growth rate at different concentration of dyes with increasing sugar concentration in bagasse extract.

| $C_0$ (mg/L) | $S_0$ (g/L) | Bioaccumulation % | $X$ (g/L) | $\mu$ ($h^{-1}$) |
|---|---|---|---|---|
| 0 | 8 | – | 1.870 | 0.254 |
| 0 | 16 | – | 2.635 | 0.712 |
| 0 | 24 | – | 3.504 | 0.287 |
| Acid Blue 93 | | | | |
| 10 | 8 | 70 | 1.675 | 0.246 |
| 10 | 16 | 85 | 2.584 | 0.267 |
| 10 | 24 | 100 | 3.145 | 0.281 |
| 20 | 8 | 61 | 1.587 | 0.243 |
| 20 | 16 | 70 | 1.934 | 0.254 |
| 20 | 24 | 85 | 2.580 | 0.270 |
| 30 | 8 | 46 | 1.190 | 0.227 |
| 30 | 16 | 51 | 1.399 | 0.236 |
| 30 | 24 | 65 | 1.900 | 0.253 |
| Direct Red 28 | | | | |
| 10 | 8 | 57 | 1.503 | 0.240 |
| 10 | 16 | 78 | 2.316 | 0.264 |
| 10 | 24 | 93 | 2.823 | 0.275 |
| 20 | 8 | 35 | 1.049 | 0.220 |
| 20 | 16 | 45 | 1.616 | 0.244 |
| 20 | 24 | 50 | 1.866 | 0.254 |
| 30 | 8 | 25 | 0.908 | 0.212 |
| 30 | 16 | 37 | 1.233 | 0.229 |
| 30 | 24 | 44 | 1.374 | 0.235 |
| Basic Violet 3 | | | | |
| 10 | 8 | 40 | 1.127 | 0.224 |
| 10 | 16 | 50 | 1.645 | 0.245 |
| 10 | 24 | 70 | 2.489 | 0.230 |
| 20 | 8 | 22 | 0.731 | 0.200 |
| 20 | 16 | 35 | 1.256 | 0.230 |
| 20 | 24 | 45 | 1.587 | 0.243 |
| 30 | 8 | 16 | 0.657 | 0.194 |
| 30 | 16 | 23 | 1.012 | 0.218 |
| 30 | 24 | 26 | 1.168 | 0.226 |

cific dye uptake. But the increased dye concentrations at constant sugar concentration decreased the biomass yield and bioaccumulation percentages.

### 3.5. Inhibition models

In absence of dyes, the values of $\mu_m$ and $K_s$ for *P. fermentans* MTCC 189 were determined as 0.301 $h^{-1}$ and 1.532 g/L respectively from the Monod equation by linear regression method. As the Monod expression for the growth kinetics did not represent the inhibitory effects of toxic dyes, Noncompetitive inhibition model and Uncompetitive model were tested to characterize the dye inhibition kinetics of *P. fermentans* MTCC 189. The $\mu_{m,app}$ values could be determined from the intercept of linear plot of $1/\mu$ versus $1/S$ at different initial dye concentrations (Figs. 9–11). The values of inhibition constant, $K_I$ was also estimated from found $\mu_{m,app}$ and known initial dye concentrations values. Tables 5 and 6 showed the values of Noncompetitive and Uncompetitive model parameters. The values of inhibition constant indicate the tolerance limit of yeast species. At higher concentration, the inhibition constants for Acid Blue 93, Direct Red 28 and Basic Violet 3 were obtained as 195.18, 142.3 and 137.20 mg/L for Noncompetitive model and 195.52, 142.33 and 137.31 mg/L for Uncompetitive model respectively which confirmed the inhibition of yeast growth by the dyes. As the parameters of both the models (Noncompetitive and Uncompetitive) showed almost similar response in terms of $K_I$, $\mu_{m,app}$ and $K_s$ values, Noncompetitive model was selected. From the experimental data, it is clear that the Basic Violet 3 is more toxic than the other two dyes.

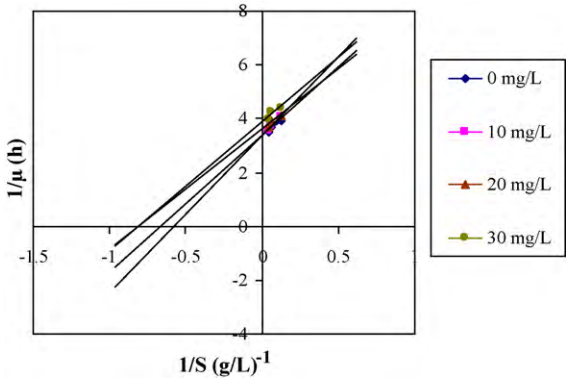


**Fig. 9.** Lineweaver–Burk plots for Acid Blue 93.

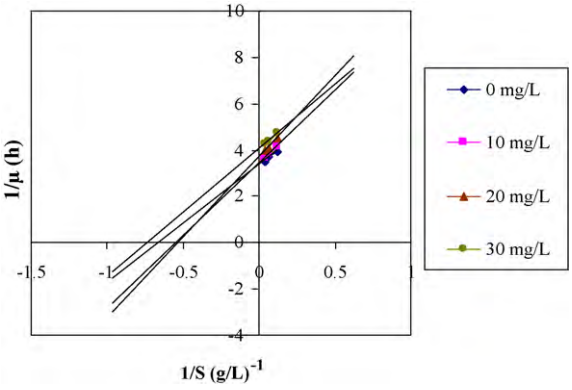


**Fig. 10.** Lineweaver–Burk plots for Direct Red 28.

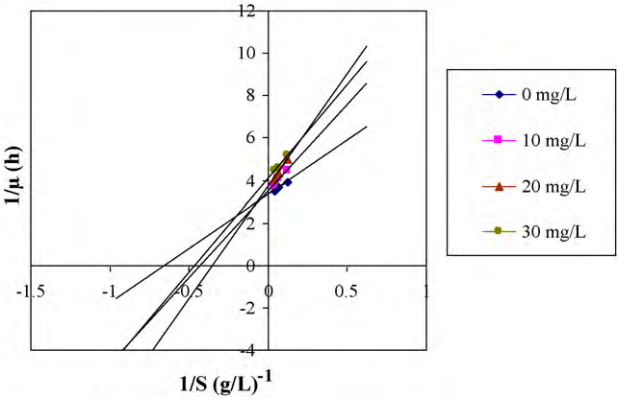


**Fig. 11.** Lineweaver–Burk plots for Basic Violet 3.

**Table 5**
Noncompetitive inhibition model parameters.

| Dyes | $C_0$ (mg/L) | Noncompetitive inhibition model parameters | | |
|---|---|---|---|---|
| | | $K_1$ (mg/L) | $\mu_{m,app}$ ($h^{-1}$) | $K_s$ (g/L) |
| Acid Blue 93 | 10 | 592.00 | 0.296 | 1.686 |
| | 20 | 279.50 | 0.281 | 1.283 |
| | 30 | 195.18 | 0.261 | 1.252 |
| Direct Red 28 | 10 | 238.76 | 0.289 | 1.648 |
| | 20 | 187.58 | 0.276 | 1.921 |
| | 30 | 142.30 | 0.249 | 1.380 |
| Basic Violet 3 | 10 | 227.00 | 0.288 | 2.357 |
| | 20 | 180.66 | 0.271 | 2.864 |
| | 30 | 137.20 | 0.247 | 1.906 |

**Table 6**
Uncompetitive inhibition model parameters.

| Dyes | $C_0$ (mg/L) | Noncompetitive inhibition model parameters | | |
|---|---|---|---|---|
| | | $K_1$ (mg/L) | $\mu_{m,app}$ ($h^{-1}$) | $K_s$ (g/L) |
| Acid Blue 93 | 10 | 710.78 | 0.297 | 1.714 |
| | 20 | 280.07 | 0.281 | 1.432 |
| | 30 | 195.52 | 0.261 | 1.444 |
| Direct Red 28 | 10 | 239.97 | 0.289 | 1.710 |
| | 20 | 190.31 | 0.272 | 1.701 |
| | 30 | 142.33 | 0.249 | 1.671 |
| Basic Violet 3 | 10 | 227.80 | 0.288 | 2.461 |
| | 20 | 185.42 | 0.272 | 2.384 |
| | 30 | 137.31 | 0.247 | 2.224 |

**Table 7**
Comparison of values of specific growth rate ($\mu$) and Basic Violet 3 bioaccumulation percentage experimentally obtained and predicted from RSM.

| Run | A | B | $\mu$-Exp | $\mu$-Pred | Bioaccumulation % (−exp) | Bioaccumulation % (−pred) |
|---|---|---|---|---|---|---|
| 1 | 0 | 1.41 | 0.219 | 0.220 | 15.5 | 13.7 |
| 2 | 1 | 1 | 0.236 | 0.235 | 26.7 | 29.6 |
| 3 | 0 | 0 | 0.230 | 0.230 | 35.0 | 35.0 |
| 4 | −1 | 1 | 0.189 | 0.186 | 16.0 | 17.4 |
| 5 | 0 | 0 | 0.230 | 0.230 | 35.0 | 35.0 |
| 6 | 0 | 0 | 0.230 | 0.230 | 35.0 | 35.0 |
| 7 | −1.41 | 0 | 0.177 | 0.180 | 25.9 | 26.7 |
| 8 | 0 | −1.41 | 0.251 | 0.252 | 53.4 | 56.2 |
| 9 | 0 | 0 | 0.230 | 0.230 | 35.0 | 35.0 |
| 10 | 1.41 | 0 | 0.229 | 0.229 | 58.7 | 57.6 |
| 11 | −1 | −1 | 0.224 | 0.222 | 39.7 | 38.0 |
| 12 | 1 | −1 | 0.242 | 0.242 | 69.8 | 69.4 |
| 13 | 0 | 0 | 0.230 | 0.230 | 35.0 | 35.0 |

**Table 8a**
Analysis of variance (ANOVA) for specific growth rate ($\mu$).

| Source | Sum of squares | DF | Mean square | $F$ value | $p$-Value Prob > $F$ | |
|---|---|---|---|---|---|---|
| Model | 0.00482 | 5 | 0.000964 | 170.81 | <0.0001 | Significant |
| $A$-$S_0$ | 0.00240 | 1 | 0.002399 | 425.14 | <0.0001 | |
| $B$-$C_{0\,B.V}$ | 0.00093 | 1 | 0.000930 | 164.80 | <0.0001 | |
| $AB$ | 0.00021 | 1 | 0.000210 | 37.26 | 0.0005 | |
| $A^2$ | 0.00110 | 1 | 0.001098 | 194.54 | <0.0001 | |
| $B^2$ | 0.00008 | 1 | 0.000082 | 14.57 | 0.0066 | |
| Residual | 0.00004 | 7 | 0.000006 | | | |
| Lack of fit | 0.00004 | 3 | 0.000013 | | | Non-significant |

**Table 8b**
Analysis of variance (ANOVA) for Basic Violet 3 bioaccumulation %.

| Source | Sum of squares | DF | Mean square | $F$ value | $p$-Value Prob > $F$ | |
|---|---|---|---|---|---|---|
| Model | 2949.63 | 5 | 589.93 | 159.46 | <0.0001 | Significant |
| $A$-$S_0$ | 950.18 | 1 | 950.18 | 256.84 | <0.0001 | |
| $B$-$C_{0\,B.V}$ | 1811.98 | 1 | 1811.98 | 489.79 | <0.0001 | |
| $AB$ | 94.09 | 1 | 94.09 | 25.43 | 0.0005 | |
| $A^2$ | 88.60 | 1 | 88.60 | 23.95 | <0.0001 | |
| $B^2$ | 0.88 | 1 | 0.88 | 0.24 | 0.0066 | |
| Residual | 25.90 | 7 | 3.70 | | | |
| Lack of fit | 25.90 | 3 | 8.63 | | | Non-significant |

### 3.6. Response surface estimation for the combined effects of initial concentration of sugar extracted from bagasse and Basic Violet 3 dye concentrations on specific growth rate and bioaccumulation properties of P. fermentans MTCC 189

Binary effects of initial sugar and dye concentrations on specific growth rate and dye bioaccumulation percentage by *P. fermentans* MTCC 189 were studied and analysed by response surface methodology. The experimental results of specific growth rate and bioaccumulation percentage of Basic Violet 3 well fitted to a second order quadratic equation giving two numerical correlations to estimate responses of specific growth rate and dye bioaccumulation percentage.

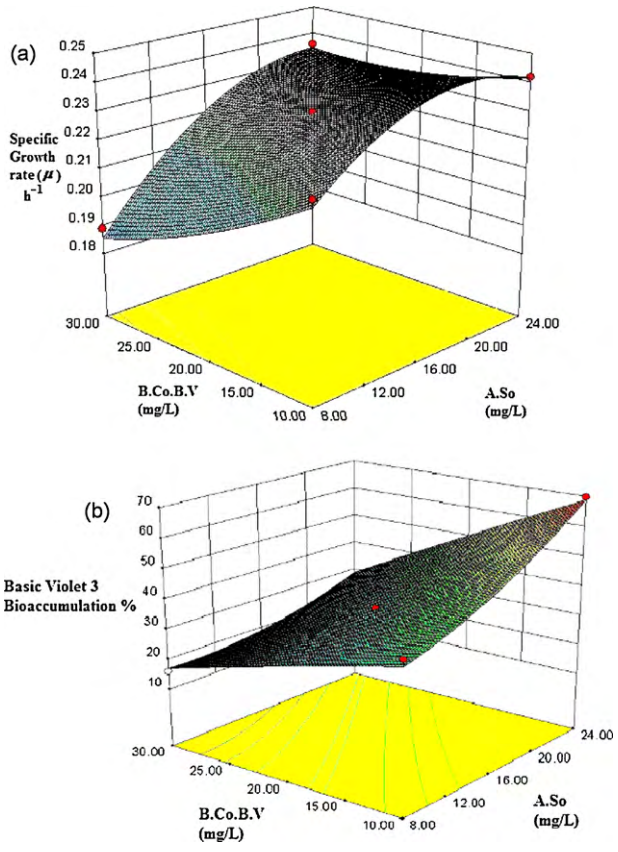


**Fig. 12.** (a) Three dimensional response surface graph showing the combined effects of initial sugar concentration in bagasse extract and Basic Violet 3 concentration on specific growth rate. (b) Three dimensional response surface graph showing the combined effects of initial sugar concentration in bagasse extract and Basic Violet 3 concentration on bioaccumulation percentage.

**Table 9a**
Summary of fit for specific growth rate ($\mu$).

| | |
|---|---|
| $R^2$ | 0.992 |
| $R^2$ adj. | 0.986 |
| Standard deviation | 0.002 |
| Mean of response | 0.224 |
| Adeq precision | 44.451 |

**Table 9b**
Summary of fit for Basic Violet 3 bioaccumulation %.

| | |
|---|---|
| $R^2$ | 0.991 |
| $R^2$ adj. | 0.985 |
| Standard deviation | 1.923 |
| Mean of response | 36.977 |
| Adeq precision | 42.861 |

$$\mu = 0.23 + 0.017A - 0.011B + 0.007AB - 0.013A^2 + 0.003B^2 \quad (14)$$

$$\text{Bioaccumulation}\,\% = 35 + 10.898A - 15.050B - 4.850AB + 3.569A^2 - 0.356B^2 \quad (15)$$

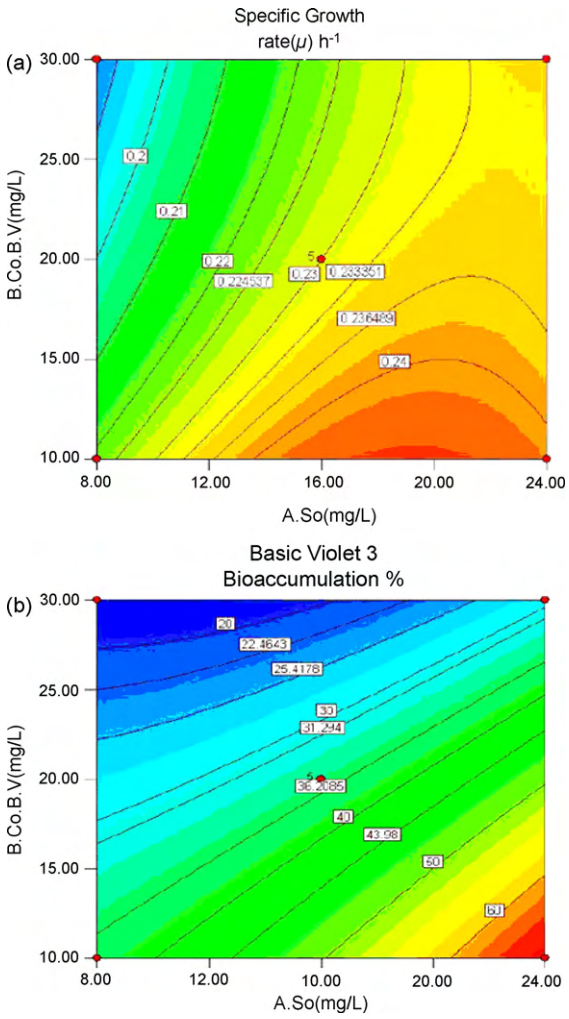


**Fig. 13.** (a) Two dimensional contour plots showing the combined effects of initial sugar concentration in bagasse extract and Basic Violet 3 concentration on specific growth rate. (b) Two dimensional contour plots showing the combined effects of initial sugar concentration in bagasse extract and Basic Violet 3 concentration on bioaccumulation percentage.

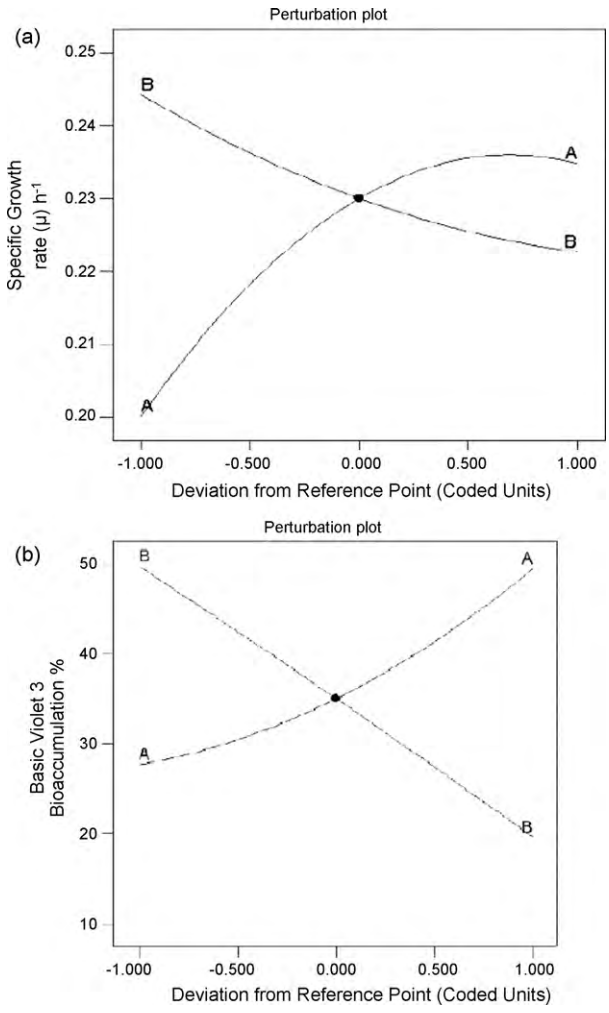


**Fig. 14.** (a) Perturbation plot showing the combined effects of initial sugar concentration in bagasse extract and Basic Violet 3 concentration on specific growth rate. (b) Perturbation plot showing the combined effects of initial sugar concentration in bagasse extract and Basic Violet 3 concentration on bioaccumulation percentage.

Since the term $B^2$ was not statistically significant with $p > F$ value was 0.640, Eq. (15) can be modified as:

$$\text{Bioaccumulation}\,\% = 35 + 10.898A - 15.050B - 4.850AB + 3.569A^2 \quad (16)$$

where $A$ and $B$ are initial sugar concentration in bagasse extract ($S_0$) and initial Basic Violet 3 dye concentration ($C_{0\,B.V}$). From the response function coefficients, it can be said that the initial Basic Violet 3 dye concentration adversely affected specific growth rate and bioaccumulation percentage. Effect of initial sugar concentration in bagasse extract was stronger as compared to initial dye concentration. The experimental and predicted values of specific growth rate and bioaccumulation percentage are listed in Table 7. The results indicate that experimental values agreed quite well with the predicted values.

The statistical significance of the model was tested using analysis of variance. ANOVA results (Tables 8a and 8b) of this quadratic models indicated that the models could be used to navigate the design space. The $F$ test results of ANOVA showed that (prob. > $F$ values) <0.0001 for $\mu$ and bioaccumulation % are lower than 0.05 implying a significant effect of corresponding variables on the responses. The statistical significance was also given by respective $R^2$ values. The results of summary of fit (Tables 9a and 9b) indicated that the values of $R^2$ were 0.992 for specific growth rate and 0.991 for bioaccumulation percentage. This implies that 99.2% and 99.1% of the sample variation for growth rate of yeast and bioaccumulation percentage are explained by independent variables. The adequate precision for $A$ (initial sugar concentration) and $B$ (initial dye concentration) was 44.451 and 42.861 respectively. The high values of adequate precision demonstrated that models are significant for the process.

The three dimensional response surface graphs (Fig. 12a and b), two dimensional contour plots (Fig. 13a and b) and perturbation plots (Fig. 14a and b) of the quadratic models are given to illustrate the combined effects of sugarcane bagasse extract and Basic Violet 3 dye on growth rate of *P. fermentans* MTCC 189 and bioaccumulation percentage. As shown in the figures, both yeast growth and bioaccumulation percentage enhanced with increasing concentration of sugar extracted from bagasse up to 24 g/L and diminished with the increase in initial Basic Violet 3 dye concentration up to 30 mg/L. In Fig. 12a and b, a steep uprising curve for sugar concentration confirmed the positive responses of the yeast species

towards specific growth rate and bioaccumulation percentage. The terms *A* and *B* denote initial sugar concentration in bagasse extract and dye concentrations. The effect of interaction (*AB*) on specific growth rate (Fig. 12a) depicted that there was a steady increase in specific growth rate with increasing $S_0$ and stabilized growth was noted after that. The 3-D mesh curve also denoted a steady downfall in specific growth rate with the increase in dye concentration along the axis of initial dye concentration. The effect of interaction (*AB*) on bioaccumulation showed that there was an increase in bioaccumulation % with increase in initial sugar concentration and decrease in dye concentrations which was shown by the upward rise of the 3-D mesh curve (Fig. 12b) along $S_0$ axis and a decreasing trend was noted along the axis of initial dye concentration.

Hence, it is clear that sugar extracted from sugarcane bagasse played a positive role for yeast growth as a carbon source and directly influenced the bioaccumulation process. Similarly the two dimensional contour plots (Fig. 13a and b) gave a clear idea that *P. fermentans* MTCC 189 showed a high growth rate and high bioaccumulation capacity with sugar concentrations ranging between 20 and 24 g/L indicated by the response flags having maximum value in this region. Perturbation plots (Fig. 14a and b) showed a steep uprising curve for sugar concentration and a down falling curve for Basic Violet 3 dye concentration thereby confirming a positive response of *P. fermentans* MTCC 189 towards specific growth rate and bioaccumulation percentage established by inversely relating these two parameters.

On the basis of the present study it can be said that RSM was successfully used as a fast and error free approach for the optimization of Basic Violet 3 accumulations by *P. fermentans* MTCC 189 with respect to parameters of dye and initial sugar concentrations as medium components. Besides, the interactive study between these two components provided an additional advantage of employing RSM. Moreover, Basic Violet 3 accumulations at intermediate levels, which were not experimentally studied, or the system performance at any experimental points with different combinations of variables may be estimated by using this method.

## 4. Conclusions

The obtained results indicate that the growing *P. fermentans* MTCC 189 was capable of accumulating synthetic dyes viz. Acid Blue 93, Direct Red 28 and Basic Violet 3 using extract of sugarcane bagasse as the sole carbon source in medium in a batch system. Use of sugarcane bagasse extract, an inexpensive waste, as nutrient source is one of the attractive features of this study. Moreover, bioaccumulation percentages of dyes could also be increased with increasing concentration of sugar extracted from sugarcane bagasse which reduced the inhibition effects of dyes on growth of yeast. Inhibition kinetics showed that Basic Violet 3 is more toxic than the other two dyes. This study also demonstrated that RSM can provide statistically reliable results for analysing the effect of various parameters on growth rate of *P. fermentans* MTCC 189 and Basic Violet 3 dye bioaccumulation.

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