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Use of response surface methodology (RSM) in the evaluation of growth and copper(II) bioaccumulation properties of *Candida utilis* in molasses medium

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Abstract

The influential factors on simultaneous growth and copper(II) bioaccumulation by growing cells of *Candida utilis* yeast under various ambient conditions, such as changing concentrations of molasses sucrose and copper(II) were tested. The highest growth rate of 0.133 h^{-1} was obtained at an initial sucrose concentration of 15 g l^{-1} in absence of copper(II). For each constant sucrose concentration chosen between 5 and 15 g l^{-1}, the increase in initial copper(II) concentration up to 500 mg l^{-1} resulted in a decrease in the percentage uptake of copper(II) and moreover all copper(II) concentrations tested inhibited the yeast growth. On the other hand, at each constant copper(II) concentration studied, both the growth and copper(II) uptake yield enhanced with raising sucrose concentration up to 15 g l^{-1}. Maximum uptake yield of 34.2% was observed in 15 g l^{-1} sucrose and 50 mg l^{-1} copper(II) containing growth medium. The binary effects of initial sucrose and copper(II) concentrations on the specific growth rate and copper(II) uptake yield of yeast were analyzed by experimental design method and two model equations for predicting the growth rate and copper(II) uptake yield of yeast due to arbitrarily chosen sucrose and copper(II) concentrations were developed by using response surface methodology (RSM).

Keywords: *Candida utilis*; Bioaccumulation; Copper(II); Molasses; Response surface methodology

1. Introduction

Rapid urbanization, industrialization and technological innovations in various walks of life have lead to the problem of environmental pollution. Heavy metal pollution of water bodies is a major environmental problem the modern world faces. Although, some amount of heavy metals is required by all life forms, however, there is a threshold limit to this requirement [1,3,4,5]. At high concentrations, heavy metal ions react to form toxic compounds in cells [6–8]. Another major problem with metals is their persistence as they tend to persist indefinitely in the food chain [2,9,10]. Traditional metal removal processes such as chemical oxidation–reduction, precipitation, adsorption, solidification, electrolytic recovery and ion exchange are some of the physicochemical wastewater treatments. Application of such processes, however, is restricted because of technical or economical constrains. Therefore, there is a need to develop rapid, economical and environmentally benign technology for the removal of metals from industrial effluents [2,11].

Bioremediation involves potential application of microorganisms in removal of heavy metals and has been recognized as a potential alternative to the conventional methods for treatment of metal contaminated wastewaters. The growing, resting and non-living cells of microorganisms are reported to remove metal ions from aqueous solutions [12,13–18]. However, most of the works to remove metal ions have been carried out using non-living cells and a very little information is available on use of growing and resting cells. The use of non-living cells has advantages over growing and resting cells due to the absence of both toxicity limitations and requirements of growth media and nutrients. Moreover, adsorbed metal ion can be easily desorbed and regenerated biomass can be reused. However, the most important limitation with non-living biomass is that biochemical cell energetic reactions are no longer continued as the cells

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are dried, whereas both growing and resting cells can be maintained biochemically active. Moreover, the growing systems have the advantages over the non-living and resting cells that the simultaneous removal of metal is obtained during growth of the organism and separate biomass production processes, e.g., cultivation, harvesting, drying, processing and storage can be avoided. However, the major limitations of using growing systems for biosorption of metals are that the nutrient media is required for the growth of the organisms and cell growth is inhibited when the metal concentration is high. This problem can be overcome by the use of metal tolerant microorganisms which can survive in high concentrations of metals and have the potential to accumulate different metals. Compared to bacteria and filamentous fungi, yeasts exhibit attractive features. Though not as fast as bacteria, yeasts can grow faster than most filamentous fungi, and like them, they have the ability to resist unfavorable environments. Yeasts can adapt and grow under various extreme conditions of pH, temperature and nutrient availability as well as high metal concentrations. Heavy metal bioaccumulation by the yeast is achieved by the virtue of covalent interaction of metal at cell surface or within the cell by different processes [19,20,21,22].

The classical method of studying with one variable at a time can be effective in some cases but it is not capable of representing the combined effects of all the factors involved. The response surface methodology (RSM) can be employed as an interesting strategy to implement process conditions which drive to optimal response by performing a minimum number of experiments. Response surface methodology is a combination of mathematical and statistical techniques used for developing, improving and optimizing the processes and used to evaluate the relative significance of several affecting factors even in the presence of complex interactions. This methodology can be used in treatment technology notably to show the effects of operational conditions on the removal process or to determine a region that satisfies the operating specifications [23,24]. RSM usually contains three steps: (1) design and experiments; (2) response surface modelling through regression; (3) optimization. The aim of RSM is to find a suitable approximation for the true functional relationship between the dependent variable (response) (Y) and the set of independent variables (factors) (X_1, X_2, ...). If knowledge concerning the shape of the true response surface is insufficient, first attempts generally try to approximate the shape by fitting a first-order model to the response values. However, if the first-order model suffers from lack of fit arising from existence of surface curvature, the first-order model is upgraded by adding higher order terms to it. The next higher order model is the second-order model and is given by Eq. (1) [25].

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} X_i X_j + \varepsilon \qquad (1)$$

where X_1, X_2, ..., X_k are the input variables, which influence the response Y; β_0 (independent term of regression equation), β_i ($i = 1, 2, \ldots, k$) (linear term of regression equation), β_{ii} ($i = 1, 2, \ldots, k$) (second-order term of regression equation), and β_{ij} ($i = 1, 2, \ldots, k$; $j = 1, 2, \ldots, k$) (interactive term of regression equation) are unknown parameters; ε is a random error. In developing this regression equation, the test variables were coded according to the following equation:

$$x_i = X_i - \left(\frac{X_i^*}{\Delta X_i} \right) \quad i = 1, 2, 3 \ldots \qquad (2)$$

where x_i is the coded value of the ith independent variable, X_i the uncoded real value of the ith independent variable, X_i^* the uncoded real value of the ith independent variable at the central point, and ΔX_i is the step change value. A 2^2 full factorial central composite experimental design for two independent variables was employed to fit a second-order polynomial model, which indicates that 13 data were required for this procedure.

The main purpose of the present study was twofold, firstly to investigate the growth and metal accumulation properties of *C. utilis* as a function of initial sucrose and copper(II) concentrations in a batch system, and secondly to see if the growth and copper(II) uptake by the yeast could be modelled by using RSM with respect to growth medium components of sucrose and copper(II) for developing more rational strategies of water pollution control. In the study copper(II) was chosen for bioaccumulation with regard to its wide use in industry and potential pollution impact. As *C. utilis* could not use the heavy metal ion as a carbon source for the cell growth under the experimental conditions, sucrose of molasses, a sugar industry waste, was chosen as main carbon source in this study. By this way a high heavy metal bioaccumulation was provided and the waste molasses was evaluated.

2. Materials and methods

2.1. Microorganism and growth conditions

C. utilis used in this study was kindly supplied by Dr. S. Dönmez, Faculty of Agriculture, Ankara University, Turkey. Molasses sucrose was the only carbon source supplied for cell growth. The strain was first grown in the medium composed of 10 ml molasses solution per liter (approximately equivalent to 5 g l^{-1} sucrose); 1 g l^{-1} $(NH_4)_2SO_4$ and 1 g l^{-1} KH_2PO_4. The pH of the growth medium was adjusted to 4.0 by dilute and concentrated sulphuric acid solutions. Precultures were performed in 250-ml Erlenmeyer flasks containing 150 ml of growth medium (sterilized at 121 °C, 0.99 bar for at least 15 min) inoculated with 1.5 ml of culture medium and incubated on a rotary shaker at 150 rpm at a temperature of 25 °C.

In order to produce more resistant and efficient strain, adaptation of the cells to progressively higher concentrations of copper(II) was performed. Microbial adaptation is defined as the ability of a microbial population to adjust itself to a changing environment [26]. If a yeast culture accomplishes growing in a toxic compound containing nutrient medium and can reach to exponential growth phase, it is assumed that yeast biomass is adapted to toxic compound. Adapted *C. utilis* strain was obtained during serial subcultures in growth medium supplemented with different concentrations of copper(II) changing between 50 and 500 mg l^{-1} at a constant sucrose concentration varied for each experimental set. The culture grown in the medium containing

copper(II) at the lowest level was transferred to the next medium supplemented with a higher concentration of copper(II)) and thus, acclimatized to higher concentrations of copper(II) at the same sucrose concentration. The presence and increase in copper(II) concentration caused a long lag period changing from 4 to 8 h in the incubation of yeast at 10 g l^{-1} sucrose concentration. The adaptation studies were repeated two times for each copper(II) and sucrose concentration. These adaptation studies were also carried out at 25 °C with rotational shaking at 150 rpm in 250 ml flasks containing 100 ml of the growth medium.

2.2. Copper(II) accumulation experiments

In order to prepare the bioaccumulation medium, heavy metal solution autoclaved separately, was mixed with the sterilized growth medium including molasses sucrose in varying concentrations from 5 to 15 g l^{-1} and 1.0 g l^{-1} $(NH_4)_2SO_4$ and 0.5 g l^{-1} KH_2PO_4. An aliquot (1%, v/v) of an adapted preculture harvested from the exponential growth phase was transferred to fresh media (150 ml) supplemented with copper(II) ions in concentrations varying from 50 to 500 mg l^{-1} at a constant sucrose concentration. Cultures were grown at 25 °C on a rotary shaker at 150 rpm for a minimum period of 10 days. Samples (5 ml) were taken at fixed time intervals for the analysis of microorganism and residual copper(II) concentrations in the culture media. Before analysis the samples were centrifuged at 4000 rpm for 3 min and the supernatant fraction was analyzed for copper(II). The centrifuged cells were washed and resuspended in distilled water and resuspension diluted to 5 ml again. All the experiments were carried out at least two times. The values used in calculations were mostly the arithmetic average of the experimental data.

Copper(II) uptake values were determined as the difference between the initial metal ion concentration and that in the supernatant.

2.3. Analytical methods

The residual copper(II) concentration in the medium was analyzed spectrophotometrically at 460 nm using sodium diethyl dithiocarbamate as the complexing agent for copper(II) [27]. 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.

3. Results and discussion

3.1. Effect of initial copper(II) concentration on the growth and copper(II) bioaccumulation of C. utilis at a constant concentration of sucrose

For this part of study, while initial copper(II) concentration was changed from 0 to 500 mg l^{-1}, initial sucrose concentration was held constant at 5, 10, or 15 g l^{-1} at an initial pH value of pH 4.0 for each experiment set. The dependence of specific *C. utilis* growth rate on the initial concentration of copper(II) was shown in Fig. 1. As seen from the figure maximum specific growth rate values were obtained in the absence of copper(II) ions. The presence of copper (II) ions in the growth medium inhibited the growth of the microorganism irreversibly and this inhibition effect increased with copper(II) concentration at each constant sucrose concentration. In 5 g l^{-1} sucrose containing growth medium with raising the copper(II) concentration from 0 to 500 mg l^{-1}, the growth rate of yeast diminished from 0.097 to 0.056 h^{-1} resulting in a 42.3% reduction. The phenomenon was substantially result from intense copper(II) inhibition at high copper(II) concentration. It is clear that sucrose concentration has a major role in the growth of yeast and decreases the inhibitory effects of copper(II) ions on the yeast growth rate. When sucrose concentration was kept constant at 15 g l^{-1} and copper(II) concentration was changed from 0 to 500 mg l^{-1}, growth rate of yeast diminished from 0.133 to 0.088 h^{-1}. The decrease in the growth rate was only 33.8% in this case.

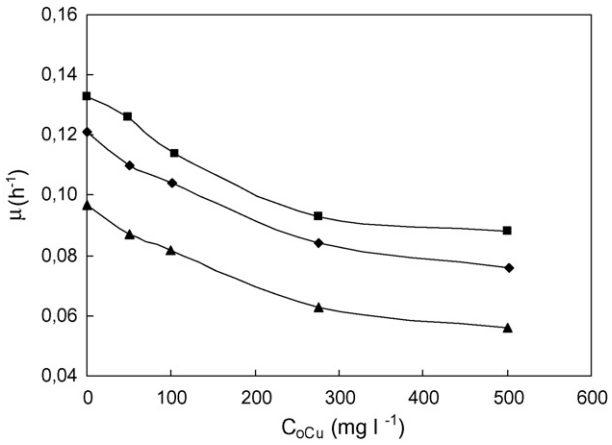

Fig. 1. Effect of initial copper(II) concentration on the growth rate of *C. utilis* at a constant sucrose concentration (T = 25 °C, SR = 150 rpm, -▲- S_0 = 5 g l^{-1}, -◆- S_0 = 10 g l^{-1}, -■- S_0 = 15 g l^{-1}).

Evaluating the bioaccumulation data, maximum bioaccumulated copper(II) concentrations and uptake yields of copper(II) obtained at changing copper(II) concentrations in the presence of same sucrose levels are presented in Table 1. Initial

Table 1
Comparison of maximum bioaccumulated copper(II) concentrations and copper(II) uptake yields obtained at increasing concentrations of sucrose in the presence of constant copper(II) concentration

S_0 (g l^{-1})	C_{0Cu} (mg l^{-1})	$C_{acc,m}$ (mg l^{-1})	Copper(II) uptake yield (%)
5.0	50.9	8.9	17.5
10.0	51.2	13.7	26.7
15.1	48.9	16.7	34.2
5.1	99.8	17.2	17.2
11.2	101.3	27.3	27.0
15.3	103.8	36.4	35.1
5.1	276.2	32.1	12.1
10.7	275.1	51.7	19.3
15.2	274.8	66.7	25.1
5.1	500.6	39.0	7.8
9.9	502.5	63.0	12.5
14.9	501.4	91.2	18.2

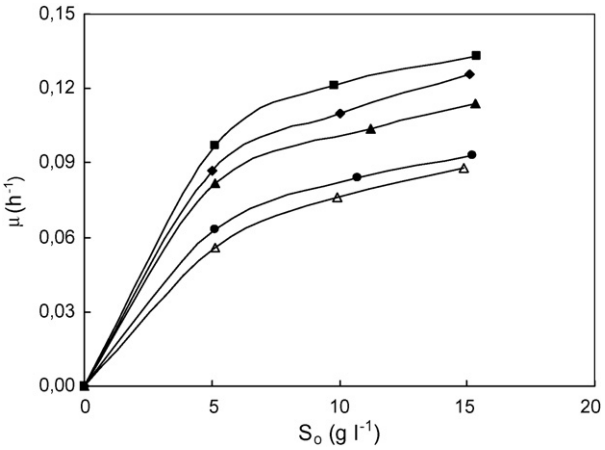

Fig. 2. Effect of initial sucrose concentration on the growth rate of *C. utilis* at a constant copper(II) concentration (T = 25 °C, SR = 150 rpm, -■- C_{0Cu} = 0 mg l^{-1}, -◆- C_{0Cu} = 50 mg l^{-1},-▲-C_{0Cu} = 100 mg l^{-1}, -●- C_{0Cu} = 275 mg l^{-1}, -△- C_{0Cu} = 500 mg l^{-1}).

copper(II) concentration also highly affected the metal uptake properties of *C. utilis*. Bioaccumulated copper(II) concentration enhanced with initial copper(II) concentration up to 500 mg l^{-1}, while uptake yields of copper(II) showed opposite trend. For example, at a 5 g l^{-1} of constant sucrose containing growth medium, in the presence of 50 mg l^{-1} copper(II), copper(II) removal and copper(II) removal yield were found as 8.9 mg l^{-1} and 17.5%, respectively. When initial copper(II) concentration changed to 500 mg l^{-1} at the same sucrose concentration, copper(II) uptake raised to 39.0 mg l^{-1} and uptake yield decreased to 7.8%. The phenomena of higher copper(II) bioaccumulation observed at higher initial copper(II) concentrations seems simple to explain. Initial concentration provides an important driving force to overcome all mass transfer resistances of the copper(II) between the aqueous and solid phases. Hence, a higher initial concentration of metal ion enhanced the bioaccumulation.

3.2. Effect of initial sucrose concentration on the growth and copper(II) bioaccumulation of C. utilis at a constant concentration of copper(II)

At this part of the bioaccumulation studies, this time, the growth and copper(II) accumulation behaviour of *C. utilis* was investigated at an initial pH value of 4.0 at increasing initial sucrose concentrations in the presence of a constant copper(II) level. While initial sucrose concentration was changed from 5 to 15 g l^{-1}, initial copper(II) concentration was kept constant between 50 and 500 mg l^{-1} for each experiment set. Fig. 2 shows the variation of microbial growth rate with sucrose concentration at a constant copper(II) level. From Fig. 2 the growth rate of *C. utilis* increased with raising initial sucrose concentration up to 15 g l^{-1} both in the absence and in the presence of constant copper(II) concentration at changing level. In the absence of copper(II) ions, the specific growth rate enhanced from 0.097 to 0.133 h^{-1} by varying sucrose concentration from 5 to 15 g l^{-1}. However, the presence of copper(II) in growth medium caused a reduction in yeast growth rate. At a constant 500 mg l^{-1} of copper(II) concentration with raising sucrose concentration from 5 to 15 g l^{-1} the cell growth rate increased from 0.056 to 0.088 h^{-1}. The increase in growth rate with increasing initial sucrose concentration could be due to cell defense mechanisms such as acclimation to toxicity.

The results in Table 1 also indicate the effect of initial sucrose concentration on the copper(II) uptake and percent copper(II) removal at each constant copper(II) concentration. Both copper(II) accumulation and copper(II) uptake yield was also affected by initial sucrose concentration and increased slightly up to 15 g l^{-1} sucrose concentration for each constant copper(II) concentration studied. For example, at a constant copper(II) concentration of 50 mg l^{-1}, with changing initial sucrose concentration from 5 to 15 g l^{-1}, accumulated copper(II) and copper(II) uptake yield by *C. utilis* increased from 8.9 to 16.7 mg l^{-1} and increased from 17.5 to 34.2%, respectively. When initial copper(II) concentration was kept constant at 500 mg l^{-1}, with raising the initial sucrose concentration from 5 to 15 g l^{-1}, accumulated copper(II) concentration and copper(II) removal yield enhanced from 39.0 to 91.2 mg l^{-1} and from 7.8 to 18.2%, respectively.

3.3. Response surface estimation for the combined effects of initial sucrose and copper(II) concentrations on the growth and bioaccumulation properties of C. utilis

The need to ensure the effectiveness of the biological wastewater treatment with growing cells has stimulated interest in the use of mathematical models for predicting microbial behaviour. The main problem in most predictive models has been the difficulty of acquiring sufficient reproducible data, suitable for modelling. The objective of this study, is not only to investigate the combined effects of sucrose and copper(II) concentration on growth and bioaccumulation properties of the yeast, but also to find the best models represent growth and bioaccumulation process.

In this analysis, initial molasses sucrose and copper(II) concentrations in the bioaccumulation medium were chosen as independent variables and the specific growth rate and copper(II) uptake yield of the yeast as dependent output response variables. In order to study the combined effects of these variables on the responses, 13 set of experiments with appropriate combinations of copper(II) and sucrose concentrations were conducted using Box-Wilson statistical method. The first independent variable (initial sucrose concentration) was varied over two levels (5 and 15 g l^{-1}) relative to the center point (10 g l^{-1}) while the second independent variable (initial copper(II) concentration) was changed over two levels (50 and 500 mg l^{-1}) relative to the center point (275 mg l^{-1}) (Table 2). The full factorial central composite design matrix of two variables with respect to their uncoded and coded values were also listed in Table 3. For the numerical analysis for estimating the responses of μ and % copper(II) uptake and for the graphical analysis of the data the statistical software package Design Expert® 7.1 was used. The goodness of fit of the model was evaluated by the coefficient determination (R^2) and the analysis of variances (ANOVA). The experimental

Table 2
Experimental range and levels of independent variables

Independent variables	Design variables	Range and levels		
		−1	0	+1
S_0 (g l^{-1})	X_1	5	10	15
C_{0Cu} (mg l^{-1})	X_1	50	275	500

Table 3
Full factorial central composite design matrix of two variables in coded and uncoded values

Run	x_1	x_2	S_0 (g l^{-1})	C_{0Cu} (mg l^{-1})
1	+1	−1	15	50
2	−1	0	5	275
3	+1	0	15	275
4	−1	+1	5	500
5	0	0	10	275
6	0	0	10	275
7	0	−1	10	50
8	0	0	10	275
9	−1	−1	5	50
10	0	0	10	275
11	0	+1	10	500
12	+1	+1	15	500
13	0	0	10	275

results of growth rate and copper(II) uptake yield were fitted to a second-order quadratic equation, giving two numerical correlations to estimate the responses of specific growth rate and copper(II) uptake yield,

$$\mu = +0.062 + 7.725\text{E} - 003X_1 - 1.703\text{E} - 004X_2 - 1.555\text{E} - 006X_1X_2 - 1.965\text{E} - 004X_2^1 + 1.992\text{E} - 007X_2^2 \quad (3)$$

$$\%\ \text{copper(II) uptake} = +9.149 + 2.098X_1 - 2.192\text{E} - 002X_2 - 1.400\text{E} - 003X_1X_2 - 1.883\text{E} - 002X_1^2 + 1.144\text{E} - 005X_2^2 \quad (4)$$

where X_1 and X_2 are initial sucrose concentration (g l^{-1}) and initial copper(II) concentration (mg l^{-1}), respectively. The values of μ and % copper(II) uptake experimentally obtained and predicted from the related empirical models listed in Table 4 indicated that for both independent variables, the calculated values of μ and % copper(II) uptake agreed very well with the predicted values of μ and % copper(II) uptake at all concentration combinations studied.

The statistical significance of the ratio of mean square variation due to regression and mean square residual error was tested using analysis of variance. ANOVA is a statistical technique that subdivides the total variation in a set of data into component parts associated with specific sources of variation for the purpose of testing hypotheses on the parameters of the model [28]. The results of the quadratic model for growth rate of the yeast and percentage uptake of copper(II) in the form of analysis of variance (ANOVA) are given in Table 5 and Table 6. The associated Prob. >F value for the each model (0.0001 for μ and 0.0001 for % copper(II) uptake) is lower than 0.05 (i.e.

Table 4
Comparison of the values of μ and copper(II) uptake yields experimentally obtained and predicted from RSM

S_0 (g l^{-1})	C_{0Cu} (mg l^{-1})	$\mu_{experimental}$ (h^{-1})	$\mu_{predicted}$ (h^{-1})	Copper(II) uptake yield$_{experimental}$ (%)	Copper(II) uptake yield$_{predicted}$ (%)
15.1	48.9	0.126	0.125	34.2	34.3
5.1	276.2	0.063	0.062	12.1	12.1
15.2	274.8	0.093	0.096	25.1	25.4
5.1	500.6	0.056	0.057	7.8	7.6
10.7	275.1	0.084	0.084	19.3	19.2
10.7	275.1	0.084	0.084	19.3	19.2
10.0	51.2	0.110	0.111	26.8	26.5
10.7	275.1	0.084	0.084	19.3	19.2
10.7	275.1	0.087	0.087	17.5	17.8
10.7	275.1	0.084	0.084	19.3	19.2
9.9	502.5	0.076	0.077	12.5	13.1
14.9	501.4	0.088	0.087	18.2	17.8
10.7	275.1	0.084	0.084	19.3	19.2

Table 5
Analysis of variance (ANOVA) for quadratic model for the specific growth rate of yeast *C. utilis*

Sources of variation	Sum of squares	Degree of freedom	Mean square	F-value	Probability >F
Model	3.765E-003	5	7.531E-004	385.95	<0.0001
Residual	1.366E-005	7	1.951E-006		
Lack of fit	1.366E-005	3	4.553E-006		
Pure error	0.000	4	0.000		
Total	3.779E-003	12			

$R^2 = 0.9964$; CV = 1.62%.

Table 6
Analysis of variance (ANOVA) for quadratic model for the copper(II) uptake yield

Sources of variation	Sum of squares	Degree of freedom	Mean square	F-value	Probability $>F$
Model	545.72	5	109.14	798.11	<0.0001
Residual	0.96	7	0.14		
Lack of fit	0.96	3	0.32		
Pure error	0.000	4	0.000		
Total	546.68	12			

$R^2 = 0.9982$; CV = 1.92%.

$a = 0.05$, or 95% confidence). This result indicated that it is statistically significant at 99.99% confidence level for both μ and % copper(II) uptake values. The values of R^2 found to be close to 1.0 also advocated a high correlation between the observed and predicted values. This means that regression model provides an excellent explanation of the relationship between the independent variables (sucrose and copper(II) concentrations) and the responses (μ and % copper(II) uptake). This implies that 99.64 and 99.82% of the sample variation for growth rate of the yeast and copper(II) uptake percentage are explained by the independent variables and this also means that the model did not explain only about 0.36 and 0.18% of sample variation for μ and % copper(II) uptake, respectively. The examination of the fit summaries output revealed that the quadratic models are statistically significant for the responses and therefore these equations may be used for further analysis.

Figs. 3 and 4 respectively illustrate the three-dimensional response surface graphs and two-dimensional contour plots of the quadratic model for the growth rate and % copper(II) removal. As shown in the figures, both the microbial growth and uptake yield of copper(II) enhanced with raising sucrose concentration up to 15 g l^{-1} and diminished with the increase in initial copper(II) concentration up to approximately 500 mg l^{-1}. Through these three-dimensional plots and their respective con-

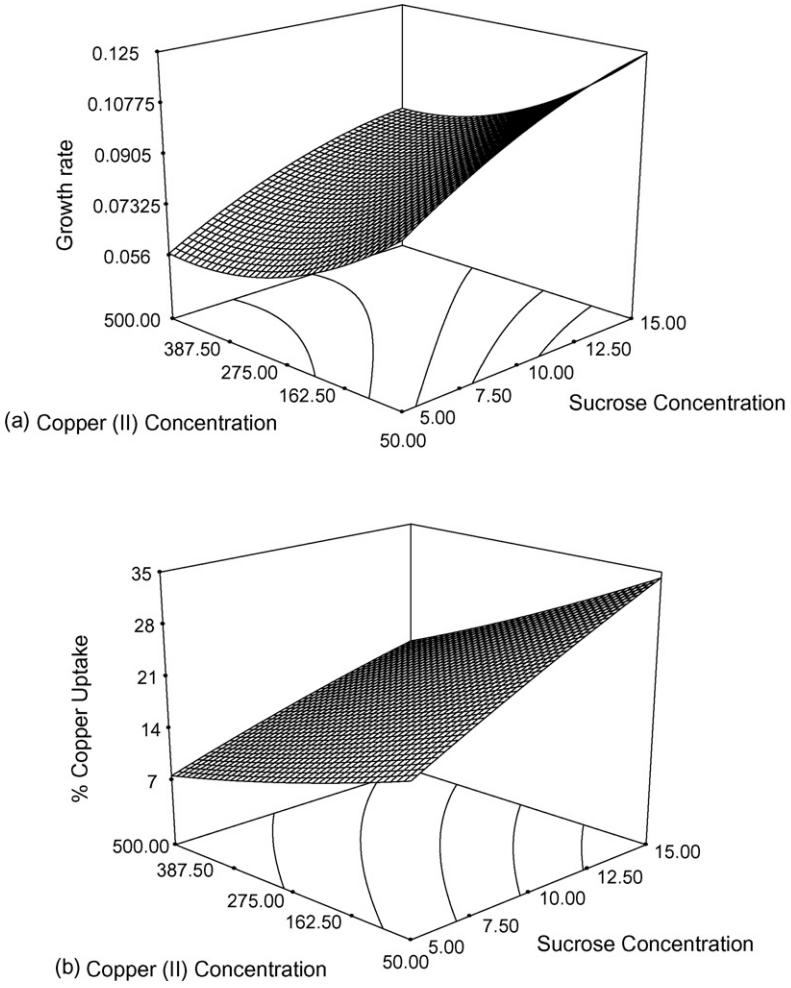

Fig. 3. Three-dimensional response surface graphs showing (a) combined effects of sucrose and copper(II) concentrations on the growth rate of *C. utilis* (b) combined effects of sucrose and copper(II) concentrations on percent copper(II) removal by *C. utilis*.

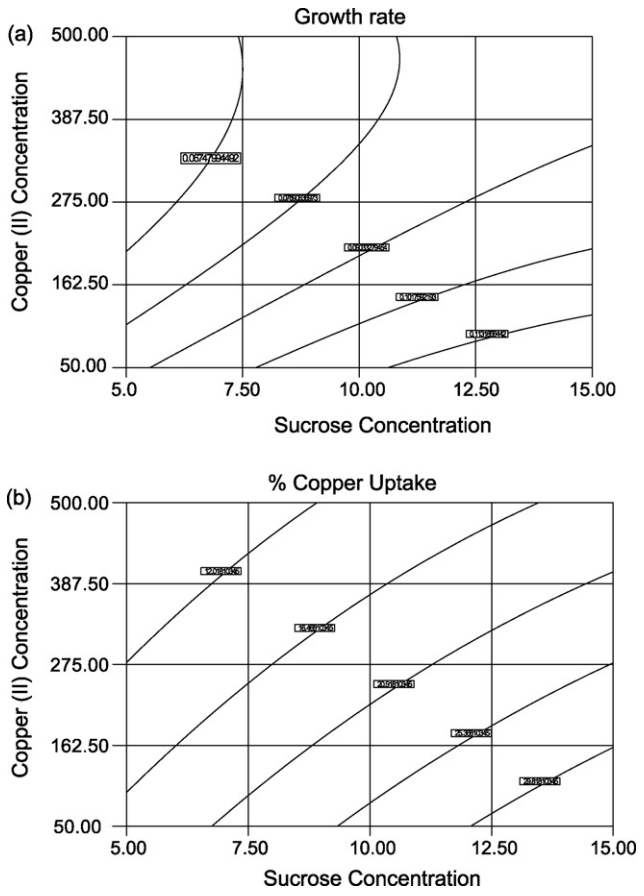

Fig. 4. Two-dimensional contour plots showing (a) combined effects of sucrose and copper(II) concentrations on the growth rate of *C. utilis* (b) combined effects of sucrose and copper(II) concentrations on percent copper (II) removal by *C. utilis*.

tour plots, it is also very easy and convenient to locate optimum levels of two variables. Again, the best value of growth and copper(II) removal occurred close to the upper point indicating the values of 50 mg l^{-1} copper(II) and 15 g l^{-1} sucrose.

4. Conclusion

It has been demonstrated previously that yeast cell offers interesting possibilities as metal resistant growing cells, showing high bioaccumulation capacity. In this study, the growth and metal accumulation properties of *C. utilis* were investigated as a function of initial sucrose and copper(II) concentrations in a batch system. The results indicated that, all concentrations of copper(II) caused an inhibition on the growth of yeast and delayed microbial growth (data not shown). The level of copper(II) accumulation was highly dependent on both sucrose and copper(II) concentrations. Copper(II) removal was maximum at the lowest concentration of copper(II) (50 mg l^{-1}) and the highest concentration of sucrose (15 g l^{-1}) due to higher cell density attained at higher concentrations of sucrose and lower concentrations of copper(II). It was found that although the inhibition effect of copper(II) ions on the growth of microorganism was significant, *C. utilis* cells was highly resistant to copper(II) ions and could accumulate this metal ion at high quantities.

The growth and % copper uptake capability of the *C. utilis* according to sucrose and copper(II) ions existing together in bioaccumulation medium, two major factors affecting the growth and uptake yield of the yeast, were modelled by means of the quadratic polynomial model (RSM). The models give a better idea of the growth properties of this yeast with respect to sucrose and copper(II) concentrations within the ranges studied and can be used to find growth rate and % copper uptake in mixture containing unstudied concentrations of sucrose and copper(II). Yet extrapolation of these models to actual treatment systems is inconvenient. Further investigations are underway for evaluation of bioaccumulation of copper(II) under various parameters, specifically in presence of competing ions. Then by taking account of the effects of these parameters, the most realistic models should be developed.

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Appendix A. Nomenclature

$C_{acc,m}$	maximum bioaccumulated copper(II) concentration at the end of microbial growth (mg l^{-1})
C_{0Cu}	initial copper(II) concentration (mg l^{-1})
S_0	initial sucrose concentration (g l^{-1})

Greek letter

μ	specific growth rate of yeast (h^{-1})

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