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Application of Response Surface Methodology for Maximizing Dextransucrase Production from *Leuconostoc mesenteroides* NRRL B-640 in a Bioreactor

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Abstract The production of dextransucrase from *Leuconostoc mesenteroides* NRRL B-640 was investigated using statistical approaches. Plackett–Burman design with six variables, viz. sucrose, yeast extract, K_2HPO_4, peptone, beef extract, and Tween 80, was used to screen the nutrients that significantly affected the dextransucrase production. 2^4-Central composite design with four selected variables (sucrose, K_2HPO_4, yeast extract, and beef extract) was used for response surface methodology (RSM) for optimizing the enzyme production. The culture was grown under flask culture with 100 ml optimized medium containing 30 g/l sucrose, 18.5 g/l yeast extract, 15.3 g/l K_2HPO_4, and 5 g/l beef extract at 25 °C and shaking at 200 rpm gave dextransucrase with specific activity of 0.68 U/mg. Whereas the same optimized medium in a 3.0-l bioreactor (1.4 l working volume) gave an experimentally determined value of specific activity of 0.70 U/mg, which was in perfect agreement with the predicted value of 0.65 U/mg by the statistical model.

Keywords Dextransucrase · *Leuconostoc mesenteroides* · RSM · Plackett–Burman · Central composite design

Introduction

Dextran is synthesized from sucrose by dextransucrases, which belongs to a class of glucosyltransferases. Glucosyltransferases are produced mainly by two bacterial genera *Leuconostoc* and *Streptococcus* [1]. The major glucosyltransferases are dextransucrases, alternansucrases, and mutansucrases, which produce dextran, alternan, and mutan, respectively, and which differ in their linkage pattern of the D-glucose residues [2]. Dextrans are a class of homopolysaccharides composed of D-glucans with contiguous α-(1→6) glycosidic linkages in the main chains and α-(1→2), α-(1→3), or α-(1→4)-

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branched glycosidic linkages [3]. Dextrans have several applications in pharmaceutical, food, photochemical, and fine chemical industries [4–7]. Low molecular weight dextrans are used as a blood plasma substitute. High molecular weight dextrans are used to increase the puffiness of bakery products [4]. In addition to catalyzing the synthesis of dextran from sucrose, dextransucrase also catalyzes the transfer of a D-glucopyranosyl group from sucrose to other acceptor molecules resulting in the generation of oligosaccharides [6, 8]. Maltose, isomaltose, and galactose are the known acceptor molecules for dextransucrase which in the presence of sucrose synthesize oligosaccharides such as maltooligosaccharides, isomaltooligosaccharides, and galactooligosaccharides, respectively [9]. Oligosaccharides are used in food, feed, pharmaceutical, or cosmetics as stabilizers, anticarcinogenic agents, antioxidants, immunostimulating agents, and prebiotic compounds [6, 8, 9, 10]. As there are enormous applications of dextran, dextransucrase is required in higher amounts at the expense of optimum levels of nutrients.

Several authors have described the effect of nutrients and culture conditions for dextransucrase production by various *Leuconostoc* strains under flask cultures and batch fermentation using the 'one-variable at a time' experiments [11–15]. The 'one-variable at a time' approach is time consuming and does not account for the interactions among the medium components. The statistical approach for medium optimization is believed to be a better alternative to the classical approach because of the utilization of fundamental principles of statistics, randomization, replication, and duplication [16–19]. A combination of factorial design and response surface optimization are used to identify the factors and their levels for obtaining the optimum response. The response surface method (RSM) is one of the popularly used optimization procedures, mainly developed based on full factorial central composite design (CCD). *Leuconostoc mesenteroides* NRRL B-512F, which is used for industrial production of dextran elaborates a contaminating levansucrase in lower amounts along with dextransucrase [20]. Whereas *L. mesenteroides* NRRL B-640 elaborates only dextransucrase, there is no contamination of levansucrase [21]. So, there is a requirement of the optimization of higher dextransucrase production from homogeneous producers. In the present study, the production of dextransucrase from *L. mesenteroides* NRRL B-640 was studied by a sequential study of factorial Plackett–Burman design followed by CCD. The factorial design of Plackett–Burman was used to screen the most significant factors affecting enzyme production. A CCD was used to identify the optimum levels of the significant variables to generate optimal response.

Materials and Methods

Microorganism and Cultivation Conditions

L. mesenteroides NRRL B-640 was procured from Agricultural Research Service (ARS-Culture collection), USDA, Peoria, USA. The ingredients required for maintenance and enzyme production media were from Hi-Media, India. The culture was maintained in modified MRS [22] with sucrose replaced by glucose as stub at 4 °C and subcultured every 2 weeks. A loop full of culture from the stub was transferred to 5 ml of medium as described by Tsuchiya et al. [11]. The cultures were grown at 25 °C with shaking at 200 rpm. One percent of the culture inoculum reading an optical density of 0.6 was used to inoculate 100 ml enzyme production medium. The culture broth was centrifuged at 9,200×*g* for 10 min at 4 °C to separate the cells. The cell-free extract was analyzed for enzyme activity.

Dextransucrase Activity Assay

The assay of dextransucrase was carried out in 1 ml of a reaction mixture in 20 mM sodium acetate buffer, pH 5.4, containing 146 mM (5%) sucrose and using the cell-free extract (10–20 μl) as the enzyme source. The reaction mixture was incubated at 30 °C for 15 min. The enzyme activity was measured by estimating the liberated reducing sugar by the Nelson–Somogyi procedure [23, 24]. One unit (U) of dextransucrase activity is defined as the amount of enzyme that liberates 1 μmol of reducing sugar per minute at 30 °C in 20 mM sodium acetate buffer, pH 5.4.

Protein Determination

The total protein content of the cell-free extract was estimated by the Lowry method [25]. Bovine serum albumin with the concentration range of 25–500 μg/ml was used as a reference to plot a standard curve.

Optimization Procedure and Experimental Design

Screening of Factors Affecting Dextransucrase Production

The Plackett–Burman factorial design was employed for screening the important nutrients for dextransucrase production resulting in higher specific activity. Six nutrients, viz. sucrose, yeast extract, K_2HPO_4, peptone, beef extract, and Tween 80, were used to determine the key ingredients significantly affecting dextransucrase production. Based on the Plackett–Burman factorial design, each factor was examined at two levels: −1 for low level and +1 for high level, and a center point was run to evaluate the linear and curvature effects of the variables [26]. Table 1 shows the Plackett–Burman experimental design with six factors under investigation as well as the levels of each factor used in the experimental

Table 1 Plackett–Burman design showing six variables with coded values (+ or −) and the real values along with the observed results for dextransucrase production.

Run order	Sucrose (g/l)	Yeast extract (g/l)	K_2HPO_4 (g/l)	Peptone (g/l)	Beef extract (g/l)	Tween 80 (g/l)	Specific activity (U/mg)
1	30 (+)	10 (−)	30 (+)	1 (−)	5 (−)	1 (−)	0.40
2	30 (+)	30 (+)	10 (−)	5 (+)	5 (−)	1 (−)	0.34
3	10 (−)	30 (+)	30 (+)	1 (−)	15 (+)	1 (−)	0.21
4	30 (+)	10 (−)	30 (+)	5 (+)	5 (−)	2 (+)	0.41
5	30 (+)	30 (+)	10 (−)	5 (+)	15 (+)	1 (−)	0.30
6	30 (+)	30 (+)	30 (+)	1 (−)	15 (+)	2 (+)	0.38
7	10 (−)	30 (+)	30 (+)	5 (+)	5 (−)	2 (+)	0.05
8	10 (−)	10 (−)	30 (+)	5 (+)	15 (+)	1 (−)	0.19
9	10 (−)	10 (−)	10 (−)	5 (+)	15 (+)	2 (+)	0.15
10	30 (+)	10 (−)	10 (−)	1 (−)	15 (+)	2 (+)	0.38
11	10 (−)	30 (+)	10 (−)	1 (−)	5 (−)	2 (+)	0.02
12	10 (−)	10 (−)	10 (−)	1 (−)	5 (−)	1 (−)	0.10
13	20 (0)	20 (0)	20 (0)	3 (0)	10 (0)	1.5 (0)	0.34
14	20 (0)	20 (0)	20 (0)	3 (0)	10 (0)	1.5 (0)	0.33
15	20 (0)	20 (0)	20 (0)	3 (0)	10 (0)	1.5 (0)	0.35

design and the response. The Plackett–Burman experimental design is based on the first order polynomial model:

$$Y = \beta_o + \sum \beta_i x_i \tag{1}$$

where Y is the response (productivity and specific activity), β_o is the model intercept and β_i is the linear coefficient, and x_i is the level of the independent variable. This model does not describe the interaction among factors and it is used to screen and evaluate the important factors that influence the response. In the present work, 6 assigned variables were screened in 12 experimental runs in addition to 3 runs at their center points. Enzyme activity assays and protein estimations were carried out in duplicate and the averages of the specific activities was taken as response Y_1 (Table 1). From the regression analysis of the variables, the factors significant at the 90% level ($P<0.1$) were considered to have significant effect on dextransucrase production and were further optimized by RSM.

Central Composite Design and Statistical Analysis

The effects of sucrose, yeast extract, K_2HPO_4, and beef extract on dextransucrase production were studied by RSM. A CCD with five settings for each of the four factor levels was used to evaluate the quadratic effects and two-way interactions among these variables. A full factorial CCD with 8 star points ($\alpha=2$) and 7 replicates at the center point resulting in a total of 31 experiments, which covered the entire range of combinations of variables, were used to optimize the chosen key variables for dextransucrase productivity in shake flask culture.

For predicting the optimal point, a second-order polynomial equation was fitted to correlate the relationship between the variables and response (specific activity). The equation is:

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_i \sum_j \beta_{ij} X_i X_j \tag{2}$$

where Y is the predicted response, k is the number of factor variables, β_o is the model constant, β_i is the linear coefficient, β_{ii} is the quadratic coefficient, and β_{ij} is the interaction coefficient.

The following equation was used for coding the variables:

$$xi = \frac{Xi - Xo}{\Delta Xi}, i = 1, 2, 3, \ldots, k \tag{3}$$

where x_i is the dimensionless value of an independent variable, X_i is the real value of an independent variable, X_o is the value of X_i at the center point, and ΔX_i is the step change.

Statistical analysis of the data was performed using the Statistical software MINITAB 15 to evaluate the analysis of variance (ANOVA) to determine the significance of each term in the equations fitted and to estimate the goodness of fit in each case. Response surfaces were drawn for the experimental results obtained from the effect of different variables on dextransucrase production to determine the individual and cumulative effects of these variables and the mutual interactions between them.

Experimental Validation of the Optimized Conditions by Flask Culture and Bioreactor

To experimentally confirm and validate the optimized conditions by flask culture and bioreactor, the statistically optimized medium composition was used for dextransucrase

production and triplicate fermentation runs in 250 ml culture flask (containing 100 ml medium) optimized at 25 °C and shaking at 200 rpm and duplicate runs in a 3-l bench-top bioreactor (Applikon, model Bio Console ADI 1025) in batch culture experiments (with 1.4 l working volume) optimized at 25 °C, agitation speed of 200 rpm, and aeration rate of 1.5 vvm were carried out.

Results and Discussion

Evaluation of Factors Affecting Dextransucrase Production Using the Placket–Burnman Design

Dextransucrase specific activity was estimated in the cell-free extract of *L. mesenteroides* NRRL B-640 culture broth at 12 h, as dextransucrase production was maximum at 12 h. All the assays were carried out in duplicates and the average values are reported. The data in Table 1 indicated that there was a wide variation of dextransucrase specific activity from 0.02 to 0.41 U/mg in the 15 trials. This variation reflected the significance of factors on the enzyme specific activity. The analysis of regression coefficients and *t* value of six ingredients for specific activity are shown in Table 2.

Both the *t* value and *P* value ($P<0.10$) of the statistical parameters were used to confirm the significance of the parameters studied [19]. Sucrose was significant at 99.99% confidence level for dextransucrase production. Yeast extract, K_2HPO_4, and beef extract were found significant with 96%, 98%, and 95% confidence levels, respectively, for dextransucrase specific activity. Peptone and Tween 80 were found insignificant with higher *P* values for enzyme specific activity. Neglecting the variables which were insignificant, the model equation for dextransucrase production can be written as:

$$Y_{\text{specific activity}} = 0.243 + 0.124\,X_1 - 0.027\,X_2 + 0.031\,X_3 + 0.025\,X_5 \qquad (4)$$

where X_1=sucrose, X_2=yeast extract, X_3=K_2HPO_4, and X_5=beef extract.

On the basis of calculated *P* values (Table 2), sucrose, yeast extract, K_2HPO_4, and beef extract were chosen for further optimization, as these factors significantly affected the

Table 2 Statistical analysis of the Plackett–Burman design showing coefficient values, *t* value, and *P* value for each variable for specific activity after 12 h.

Variable	Dextransucrase activity (U/mg)			
	Coefficient	*t* stat	*P* value	Confidence level (%)
Intercept	0.243	22.69	0.0001	99.99
Sucrose (X_1)	0.124	11.57	0.0001[a]	99.99
Yeast extract (X_2)	−0.027	−2.54	0.039[a]	96.1
K_2HPO_4 (X_3)	0.031	2.91	0.023[a]	97.7
Peptone (X_4)	−0.004	−0.44	0.674	32.6
Beef extract (X_5)	0.025	2.35	0.051[a]	94.9
Tween 80 (X_6)	−0.012	−1.18	0.276	72.4

[a] Indicates the factors with 95% confidence.

dextransucrase specific activity. Peptone and Tween 80 were added at their median values for subsequent experiments.

Optimization of Medium Composition for Higher Specific Activity of Dextransucrase by RSM

At the end of the screening experiments, four nutritional factors were believed to play a significant role in dextransucrase production. A CCD with 31 experiments was carried out. The respective low and high levels of each variable with the coded levels in parenthesis and the CCD with dextransucrase specific activities are given in Table 3. The fit of the model was checked by the coefficient of determination R^2 [27, 28]. The nearer the values of R^2 to 1, the model would explain better for the variability of experimental values to the predicted values. The second-order response surface model presented a high determination coefficient (R^2=0.9397) explaining 94% of the variability in the response, i.e., specific activity. The

Table 3 Full factorial CCD matrix of four variables in real and coded units (in parenthesis) and the response of dextransucrase activity.

Run no.	Sucrose (X_1)	Yeast extract (X_2)	K_2HPO_4 (X_3)	Beef extract (X_4)	Specific activity (U/mg)
1	10 (−1)	10 (−1)	10 (−1)	5 (−1)	0.40
2	30 (1)	10 (−1)	10 (−1)	5 (−1)	0.58
3	10 (−1)	30 (1)	10 (−1)	5 (−1)	0.10
4	30 (1)	30 (1)	10 (−1)	5 (−1)	0.63
5	10 (−1)	10 (−1)	30 (1)	5 (−1)	0.25
6	30 (1)	10 (−1)	30 (1)	5 (−1)	0.39
7	10 (−1)	30 (1)	30 (1)	5 (−1)	0.16
8	30 (1)	30 (1)	30 (1)	5 (−1)	0.27
9	10 (−1)	10 (−1)	10 (−1)	15 (1)	0.15
10	30 (1)	10 (−1)	10 (−1)	15 (1)	0.45
11	10 (−1)	30 (1)	10 (−1)	15 (1)	0.01
12	30 (1)	30 (1)	10 (−1)	15 (1)	0.37
13	10 (−1)	10 (−1)	30 (1)	15 (1)	0.20
14	30 (1)	10 (−1)	30 (1)	15 (1)	0.54
15	10 (−1)	30 (1)	30 (1)	15 (1)	0.02
16	30 (1)	30 (1)	30 (1)	15 (1)	0.30
17	0 (−2)	20 (0)	20 (0)	10 (0)	0.06
18	40 (+2)	20 (0)	20 (0)	10 (0)	0.66
19	20 (0)	0 (−2)	20 (0)	10 (0)	0.31
20	20 (0)	40 (+2)	20 (0)	10 (0)	0.18
21	20 (0)	20 (0)	0 (−2)	10 (0)	0.09
22	20 (0)	20 (0)	40 (+2)	10 (0)	0.15
23	20 (0)	20 (0)	20 (0)	0 (−2)	0.48
24	20 (0)	20 (0)	20 (0)	20 (2)	0.41
25	20 (0)	20 (0)	20 (0)	10 (0)	0.53
26	20 (0)	20 (0)	20 (0)	10 (0)	0.51
27	20 (0)	20 (0)	20 (0)	10 (0)	0.53
28	20 (0)	20 (0)	20 (0)	10 (0)	0.50
29	20 (0)	20 (0)	20 (0)	10 (0)	0.51
30	20 (0)	20 (0)	20 (0)	10 (0)	0.52
31	20 (0)	20 (0)	20 (0)	10 (0)	0.55

X_1, X_2, X_3, and X_4 are given in g/l.

coefficients of regression were calculated and the following regression equation was obtained:

$$Y_{\text{specific activity}} = 0.52 + 0.14\,X_1 - 0.057\,X_2 - 0.018\,X_3 - 0.037\,X_4 + 0.020\,X_1X_2 - 0.031\,X_1X_3 + 0.020\,X_1X_4 - 0.01\,X_2X_3 - 0.011\,X_2X_4 + 0.045\,X_3X_4 - 0.039\,X_1^2 - 0.068\,X_2^2 - 0.099\,X_3^2 - 0.018\,X_4^2 \quad (5)$$

The statistical significance of Eq. 5 was checked by F test [18], the results of ANOVA for dextransucrase specific activity demonstrated that the model is highly significant, and is evident from Fischer's test with an F value of 17.82 and a very low probability value$[(P_{\text{model}} > F = 0.0001)$.

Model coefficients estimated by regression analysis for each variable representing for their effect on specific activity is shown in Table 4. The model terms X_1, X_2, X_4,X_1^2, X_2^2, X_3^2, and X_3X_4 exhibited confidence levels above 95% ($P<0.05$). This indicated that the linear effects of sucrose, yeast extract, and beef extract concentrations; the quadratic effects of yeast extract and K_2HPO_4 concentrations; and the interaction effect of K_2HPO_4 and beef extract were significant model terms (Table 4). The interaction effect of sucrose and K_2HPO_4 (X_1X_3) was significant by 92% ($P<0.07$). Higher observed P values for X_3, X_4^2, X_1X_2, X_1X_4, X_2X_3, and X_2X_4 indicated that they were less significant dextransucrase specific activity. The negative coefficient indicated that the decrease in the variable concentration can increase the dextransucrase production. The positive coefficients of the variables indicated an increase in dextransucrase specific activity with the increase in the variable concentration in the medium.

The 3-D response surface plots and the 2-D contour plots are the graphical representation of the regression equation. The response surface plots can be used to visualize the effect of the concentration of the variables on required response and can also measure the approximate required concentrations of the variables for obtaining the maximum response [18, 19].

Table 4 Model coefficient estimated by multiple linear regression for specific activity of dextransucrase.

Model term	Parameter estimate	Standard error	Computed t value	P value
Intercept	0.52	0.025	21.15	0.0001
X_1	0.14	0.013	10.70	0.0001[a]
X_2	−0.057	0.013	−4.25	0.001[a]
X_3	−0.018	0.013	−1.29	0.213
X_4	−0.037	0.013	−2.76	0.014[a]
X_1^2	−0.039	0.012	−3.20	0.006[a]
X_2^2	−0.068	0.012	−5.47	0.0001[a]
X_3^2	−0.099	0.012	−8.16	0.0001[a]
X_4^2	−0.018	0.012	−1.48	0.156
X_1X_2	0.020	0.016	1.22	0.238
X_1X_3	−0.031	0.016	−1.90	0.076
X_1X_4	0.020	0.016	1.19	0.251
X_2X_3	−0.01	0.016	−0.64	0.531
X_2X_4	−0.011	0.016	−0.69	0.500
X_3X_4	0.045	0.016	2.74	0.014[a]

[a] Indicates model terms with above 95% significance.

Figure 1a shows the response for the interactive factors, sucrose and yeast extract, where the K_2HPO_4 and the beef extract were kept at the zero level. Maximum specific activity in this condition was predicted to be 0.64 U/mg, corresponding to the high amount of sucrose (4 g/l) and low amount of yeast extract (2 g/l). It indicates the negative effect of the high concentration of yeast extract on dextransucrase specific activity. The shape of the response surface indicates no interaction between these variables. Figure 1b shows the moderate interaction of sucrose and K_2HPO_4 for dextransucrase specific activity. The increase in sucrose concentration from 1 up to 4 g/l along with the increase in K_2HPO_4 concentration up to 2 g/l results in higher specific activity. Figure 1c with elliptical contours shows good interaction between sucrose and beef extract for dextransucrase specific activity. The increase in the sucrose concentration up to 4 g/l by keeping the beef extract concentration below 1.5 g/l results in a dextransucrase specific activity of 0.64 U/mg. Figure 1d with elliptical contours shows good interaction of K_2HPO_4 with beef extract for dextransucrase specific activity. The lower beef extract concentrations (below 1 g/l) with both yeast extract

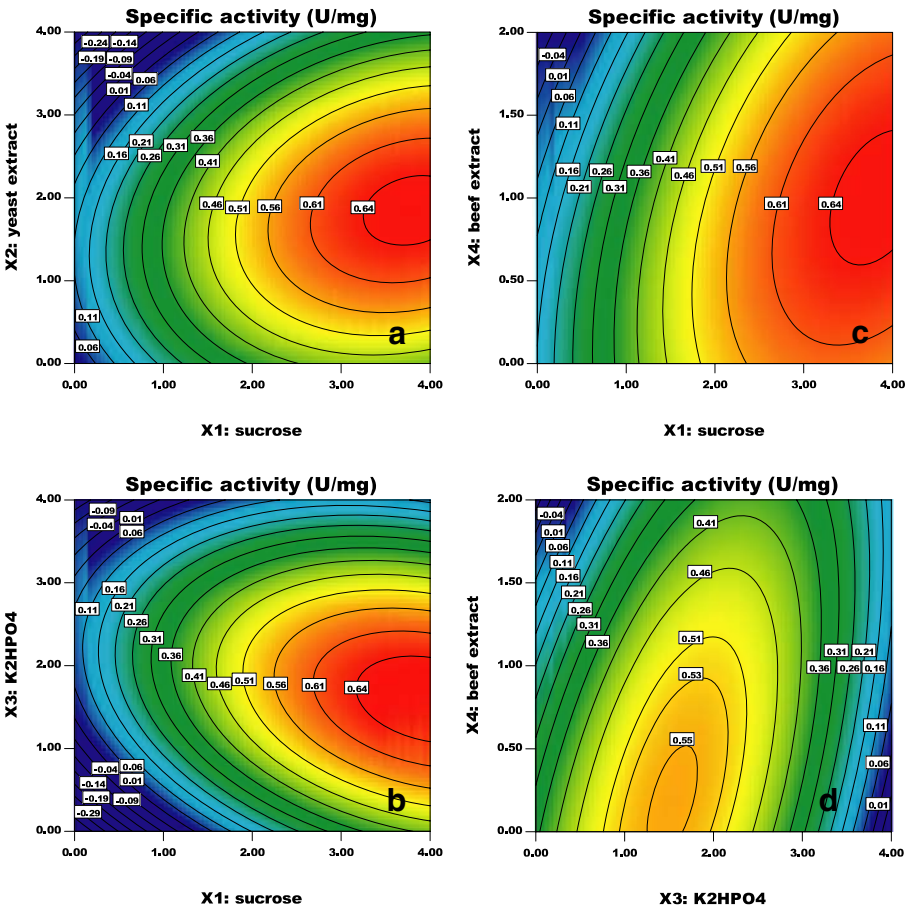

Fig. 1 Contour plots of the combined effects of **a** sucrose and yeast extract, **b** sucrose and K_2HPO_4, **c** sucrose and beef extract, and **d** $K_2HPO_4$and beef extract on dextransucrase production by *L. mesenteroides* NRRL B-640 (where the other two components are at their fixed levels; fixed levels are given in g/100 ml medium: sucrose=2, yeast extract=2, K_2HPO_4=2, and beef extract=1)

and K_2HPO_4 in below 2 g/l results in a dextransucrase specific activity of 0.54 U/mg. Further increase in yeast extract and K_2HPO_4 resulted in a gradual decrease in the specific activity.

Experimental Validation of the Optimized Medium Composition by Flask Culture and Bioreactor

The optimum levels of the variables were obtained by solving the regression equation and by analyzing the response surface contour plots. The model predicted a maximum dextransucrase specific activity of 0.65 U/mg appearing with sucrose, 30 g/l; yeast extract, 18.5 g/l; K_2HPO_4, 15.3 g/l; and beef extract, 5 g/l and by keeping the other components at their middle levels. To validate the predicted model, three runs were conducted using this optimized medium composition at flask cultures with 100 ml medium. The *L. mesenteroides* NRRL B-640 grown under flask culture with 100 ml optimized medium at 25 °C and shaking at 200 rpm gave dextransucrase with specific activity of 0.68 U/mg, which was perfectly matched with the model predicted value of 0.65 U/mg dextransucrase specific activity. The scale-up of batch cultivation from the flask culture containing 100 ml medium to a bioreactor containing 1.4 l of the same optimized medium under optimized experimental conditions at 25 °C, agitation speed of 200 rpm, and aeration rate of 1.5 vvm resulted in a dextransucrase specific activity of 0.70 U/mg (Fig. 2). The maximum enzyme activity in the bioreactor run was observed at 12 h of the fermentation at a pH of 4.8 and at 38% dissolved oxygen (DO) and dry cell weight of 8.9 mg/ml (optical density of 8.0 at 600 nm) was observed (Fig. 2). The most studied *L. mesenteroides* NRRL B-512F for its large scale production of dextran was reported for maximum dextransucrase specific activity by 0.22 U/mg [20]. The present results showed that the specific activities for dextransucrase obtained with flask culture (0.68 U/mg) and bioreactor (0.70 U/mg) were in perfect agreement with the predicted value (0.65 U/mg) by the statistical model. The flask culture specific activity obtained using optimized medium was three times higher than the activity of dextransucrase produced by the strain *L. mesenteroides* NRRL B-512F. The excellent correlation of enzyme activity between predicted and measured values of these experiments justifies the validity of the response model and the existence of an optimum point. The statistical methods proved to be a powerful tool for maximizing dextransucrase production from *L. mesenteroides* NRRL B-640.

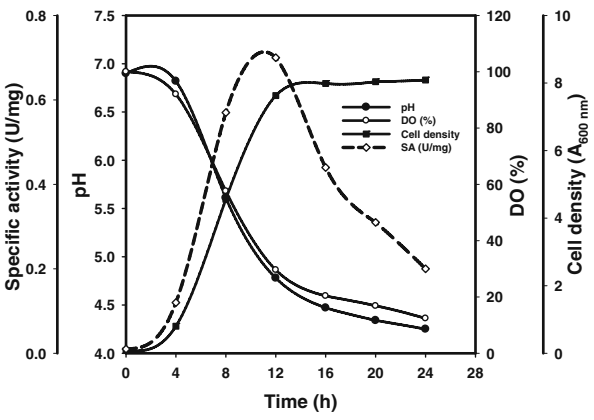

Fig. 2 Dextransucrase production in a bioreactor run with optimized media

Conclusions

To improve dextransucrase production from *L. mesenteroides* NRRL B-640, the culture medium was screened and optimized using the statistical design techniques of Plackett–Burman and RSM. Plackett–Burman design with six variables, viz. sucrose, yeast extract, K_2HPO_4, peptone, beef extract, and Tween 80, was performed to screen the nutrients that were significantly affecting dextransucrase production. The proposed model equation illustrated the quantitative effect of the variables and the interactions among the variables upon dextransucrase production. The variables sucrose, yeast extract, K_2HPO_4, and beef extract showed above 90% confidence levels for dextransucrase production and were considered as significant factors for optimization using RSM. 2^4-CCD was used for RSM optimization. The experimental results were fitted to a second-order polynomial model which gave a coefficient of determination $R^2=0.939$ for dextransucrase specific activity. The model predicted a maximum dextransucrase specific activity of 0.65 U/mg appearing at sucrose, 30 g/l; yeast extract, 18.5 g/l; K_2HPO_4, 15.3 g/l; and beef extract, 5 g/l and by keeping the other components at their middle levels. The dextransucrase specific activity of 0.68 U/mg obtained with the above medium composition in a flask culture perfectly matched with the predicted value of dextransucrase specific activity (0.65 U/mg). The same optimized medium when used in a bioreactor gave an experimental value of dextransucrase specific activity of 0.7 U/mg, which again corresponded well with the predicted values by the statistical model.

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