JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2014. Vol. **8**(3), p. 2359-2367

Production of Dextran using *Leuconostoc mesenteroides* NCIM-2198 and its Media Optimization by Response Surface Methodology

Shivendu Ranjan[1], Amod Kumar[1,2], Nandita Dasgupta[1], Sathiavelu Arunachalam[2]* and Ramalingam Chidambaram[1]

[1]Instrumentation and Food Analysis Laboratory, Industrial Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore - 632014, India.
[2]Environmental Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore - 632014, India.

(Received: 20 November 2013; accepted: 02 February 2014)

For the production of dextran a high enzyme active strain of L. mesenteroides NCIM-2198 was selected. To increase the yield of dextran, media was optimized using Resonse Surface Methodology (RSM). A Central Composite Design (CCD) with four variables was used to determine the optimum combination of the variables. A total 31 runs were generated by varying the concentrations of four media components sucrose, peptone, yeast extract and K_2HPO_4 and yield as a response. The temperature for the production medium was kept at 30 °C and the pH of the production medium was 7.0 before sterilization. The optimal conditions were obtained; condition 1: sucrose 10.157 g/100ml, yeast 1.611 g/100ml, peptone 0.573 g/100ml, K_2HPO_4 1.845 g/100ml and condition 2 as: sucrose 10.313 g/100ml, yeast 1.000 g/100ml, peptone 0.718 g/100ml, K_2HPO_4 1.000 g/100ml. Under these conditions, the model predicted a dextran production of 3.493 g/100mL and 3.466 g/100ml respectively. Validation of the optimization showed that dextran production of 3.186 g/100ml and 3.157 g/100ml was observed under optimal conditions 1 and 2 respectively.

Key words: Dextran, production, Response surface methodology, Media optimization, central composite design, *L. mesenteroides* NCIM-2198.

Dextran is an extracellular bacterial polymer of D-glucopyranose with predominantly α- (1→6) linkage in the main chain and a variable amount of α-(1→2), α- (1→3), α-(1→4) branched linkages[1-3]. It is a group of high molecular mass polysaccharides that are synthesized from sucrose and composed of chains of D-glucose units[4]. Dextran is produced by species of leuconostoc, streptococcus and acetobacter. Hucker and Pederson[5] was the first who reported the production of dextran from sucrose by strains of Leuconostoc species. It has been reported the formation of dextran from different strains of bacteria that were primarily Leuconostoc strains[6]. Species of bacteria from other genera have been also found to produce dextran. In 1941 dextran production from sucrose by Streptococcus species was reported and compared the dextran produced by acetobacter species and found them similar to that of leuconostoc species[7]. Among many dextran producing species the dextran produced by *L .mesenteroides* NRRL B512F[8] and *L. mesenteroides*

* To whom all correspondence should be addressed.
Mob.: +91-9443457024;
E-mail: asathiavelu@vit.ac.in

NRRL B1299 (*9*) have been well characterized and classified. Dextran from *L. mesenteroides* B512F contains 95% of α- (1→6) linkages and 5% of α-(1→3) branch linkages; whereas insoluble dextran from *L. mesenteroides* 1299 (*10*) contains 63% α-(1→6), 27% of α-(1→2) and 8% of α-(1→3) linkages.

Dextran has found industrial applications in food, pharmaceutical and chemical industries as adjuvant, emulsifier, carrier and stabilizer (*11-14*). Cross-linked dextran is known as sephadex, which is widely used for the separation and purification of protein. In food industry dextran is currently used as thickener for jam and ice cream. It prevents crystallization of sugar, improves moisture retention, and maintains flavor and appearance of various food items.

As an important subject in the statistical design of experiments, the *Response Surface Methodology* (*RSM*) is a collection of mathematical and statistical techniques useful for the modeling and analysis of problems in which a response of interest is influenced by several variables and the objective is to optimize this response. RSM is useful for developing, improving, and optimizing the response variable. This can be explained by equation 1 in which the variables *x1* and *x2* are independent variables where the response *y* depends on them. The dependent variable *y* is a function of *x1*, *x2*, and the experimental error term, denoted as *e*.

$$y = f(x1, x2) + e \quad \text{...(1)}$$

In this study a CCD with four variables was used to study the response pattern and to determine the optimum combination of the variables for maximum yield of dextran.

MATERIALS AND METHOD

L. mesenteroides NCIM-2198 was obtained by NCIM (National Center for Industrial Microbiology, Pune, India). The microbial strain is then subculture in MRS broth (HiMedia specialist Ltd., India) and incubated for 24 h at 26 °C. After that streak plating is done for future preservation purpose.

Cultivation media

5 ml of sterilized MRS broth is then inoculated for 24 h subculture and incubated for 24 h at 26 °C. After 24 h the inoculums were transferred in 45.0 ml culture medium and incubated again at same above conditions.

Production and precipitation of dextran

The inoculums were transferred in 450 ml Sucrose broth (Table 1) and incubated at 26 °C for 24 h. After the incubation the culture medium was centrifuge at 5000 rpm for 5 min to remove the cells. The supernatant was decanted. In the second step, chilled ethanol (Merck, India) was added with constant stirring and centrifuged, precipitates of dextran appeared, supernatant was again decanted. After 10 min chilled ethanol was added again and dextran was precipitated in very fine form. The precipitated dextran was dried over calcium chloride at 30 °C.

Purification of dextran

The supernatant was removed and the precipitate was dissolved in a minimal volume of water. Dextran was again precipitated with cold ethanol as described above, this cycle of redissolving, precipitation and washing was repeated three times and the precipitate was dried at 30 °C.

Estimation of total protein

Total protein of the cell-free filtrate was determined by the method of Bradford. Bovine serum Albumin was used as a standard curve ranging from 20 µg/ml to 100 µg/ml[15].

Estimation of total sugar and reducing sugar

The produced dextran was used as unknown for the estimation of total and reducing sugar. Anthrone method was used for total sugar and DNSA method was used for reducing sugar estimation. For Anthrone method glucose was used as a standard curve ranging from 80 µg/ml to 400 µg/ml and for DNSA 100 µg/ml to 500 µg/ml.

Media optimization using RSM

CCD with four variables was used to study the response pattern and to determine the optimum combination of the variables. CCD combines vertices of the hypercube whose co-ordinates are given by a 2^n factorial design and two star points (outsider points) to provide for the estimation of curvature of the model. Table 3 shows the (-α), (+α), maximum and minimum values of variables.

A complete CCD coded and un-coded value with respective responses is shown in Table

4. An *Analysis of Variance (ANOVA)* was conducted to determine the significant effects of process variables on the response. Optimum conditions for production of dextran were determined to obtain maximum conversion of sucrose into dextran. Quadratic model equations obtained in this study (coefficients from) were utilized for each response in order to determine optimum conditions.

Optimization using overlaid contour plots, a graphic optimization technique was adopted to determine optimized conditions for maximum yield response. It also provides comprehensive and informative insight into the system, which leads to faster process optimization and may replace other complex statistical analyses[16]. Optimization was also done by drawing optimization plot in response optimizer of Minitab15 software by taking conditions shown in Table 7, 8.

RESULTS AND DISCUSSION

Production of dextran

Dextran was produced by a bacterium *L. mesenteroides* NCIM-2198. Results have suggested that the enzyme activity and dextran production are depending on media composition. The conversion of sucrose into of dextran was varied according to media content, medium 2 has more salts than medium hence it increases the conversion of sucrose into dextran that also suggest the high enzyme activity in salt rich medium (Table 2).

Media for the production of dextran was optimized by using CCD and RSM approach. Response for the same i.e. product (g/100ml) was measured. A complete CCD (uncoded value) with respective response is shown in Table 4.

An *analysis of variance* (ANOVA) was conducted to determine the significant effects of process variables on response. Based on the regression coefficient (Table 5), coefficients of quadratic equation (Eq. 2) were obtained using Minitab 15.0 (trial version) software. F-values and t-values were compared with standard tabular values to check the significance of the model and individual term respectively. Coefficient of determination (R^2) was also calculated to check the adequacy of the model fit. The R^2 is the

Table 1. Media composition (Sucrose Broth) for dextran production from *L. mesenteroides* NCIM-2198

Ingredients	Medium	
(g/100ml)	Medium 1	Medium 2
Sucrose	10.000	10.000
Yeast extract	0.500	2.000
Peptone	0.500	0.700
K_2HPO_4	0.500	1.500
NaCl	0.001	0.001
$MgSO_4.7H_2O$	0.001	0.001
$MnCl_2.H_2O$	0.001	0.001
$FeSO_4.7H_2O$	0.001	0.001
$CaCl_2$	0.005	0.005

Table 2. Amount of dextran produced in different medium

	Sucrose (g/100ml)	Dextran produced (g/100ml)	% conversion of sucrose
Medium 1	10	2.95	29.5
Medium 2	10	3.30	33.0

Table 3. Variables - α, + α, maximum and minimum values of variables

	-α	-1	0	1	+α
Sucrose (g/100ml)	1.0	4.0	7.0	10.0	13.0
Yeast extract (g/100ml)	0.0	0.5	1.0	1.5	2.0
Peptone (g/100ml)	0.4	0.5	0.6	0.7	0.8
K_2HPO_4 (g/100ml)	0.0	0.5	1.0	1.5	2.0

α = 2.000

Table 4. Variables with coded and un-coded values for central composite design with respective response (dextran production) and % conversion of sucrose

Run	Coded Value				Un-coded value				Response	
	Sucrose (g/100ml)	Yeast (g/100ml)	Peptone (g/100ml)	K_2HPO_4 (g/100ml)	Sucrose (g/100ml)	Yeast (g/100ml)	Peptone (g/100ml)	K_2HPO_4 (g/100ml)	Dextran (g/100ml)	Percent conversion of sucrose
1	-1	-1	-1	-1	4	0.5	0.5	0.5	1.200	30.00
2	1	-1	-1	-1	10	0.5	0.5	0.5	3.200	32.00
3	-1	1	-1	-1	4	1.5	0.5	0.5	1.220	30.50
4	1	1	-1	-1	10	1.5	0.5	0.5	3.310	33.10
5	-1	-1	1	-1	4	0.5	0.7	0.5	1.210	30.25
6	1	-1	1	-1	10	0.5	0.7	0.5	3.290	32.90
7	-1	1	1	-1	4	1.5	0.7	0.5	1.260	31.50
8	1	1	1	-1	10	1.5	0.7	0.5	3.360	33.60
9	-1	-1	-1	1	4	0.5	0.5	1.5	1.200	30.00
10	1	-1	-1	1	10	0.5	0.5	1.5	3.300	33.00
11	-1	1	-1	1	4	1.5	0.5	1.5	1.340	33.50
12	1	1	-1	1	10	1.5	0.5	1.5	3.400	34.00
13	-1	-1	1	1	4	0.5	0.7	1.5	1.260	31.50
14	1	-1	1	1	10	0.5	0.7	1.5	3.310	33.10
15	-1	1	1	1	4	1.5	0.7	1.5	1.400	35.00
16	1	1	1	1	10	1.5	0.7	1.5	3.390	33.90
17	-2	0	0	0	1	1.0	0.6	1.0	0.340	34.00
18	2	0	0	0	13	1.0	0.6	1.0	4.500	34.60
19	0	-2	0	0	7	0.0	0.6	1.0	2.100	30.00
20	0	2	0	0	7	2.0	0.6	1.0	2.330	33.20
21	0	0	-2	0	7	1.0	0.4	1.0	2.210	32.60
22	0	0	2	0	7	1.0	0.8	1.0	2.240	32.00
23	0	0	0	-2	7	1.0	0.6	0.0	2.230	31.80
24	0	0	0	2	7	1.0	0.6	2.0	2.290	32.70
25	0	0	0	0	7	1.0	0.6	1.0	2.280	32.50
26	0	0	0	0	7	1.0	0.6	1.0	2.290	32.70
27	0	0	0	0	7	1.0	0.6	1.0	2.280	32.50
28	0	0	0	0	7	1.0	0.6	1.0	2.270	32.40
29	0	0	0	0	7	1.0	0.6	1.0	2.290	32.70
30	0	0	0	0	7	1.0	0.6	1.0	2.280	32.50
31	0	0	0	0	7	1.0	0.6	1.0	2.300	32.80

proportion of variability in the response values explained or accounted for by the model[16].

The ANOVA (Table 5) for the data obtained indicates the high value of R^2 for the response (99.95) which suggests that the model is a good fit. The F-values of the response at 95% confidence level (2360.76) are much higher than that of the table value ($F_{crit\ (0.05,14,16)}$ =2.373) also confirms the adequacy of the model. ANOVA table also indicates the significant Lack of fit (12.27). The lack of fit measures the failure of the model to represent data in the experimental domain at points which are not included in the regression (*16*). However, based on high R^2 values and F-values, the model can be considered as a good fit. From the ANOVA table, it is evident that the regression terms is also significant ($P \leq 0.05$) for the response. For dextran production all four variables as well as

Table 5. The analysis of variance (ANOVA) table for the full quadratic model

Source	DF	Dextran (g/100ml)			
		Seq SS	Adj MS	F	P
Regression	14	25.3100	1.80786	2360.76	0.000
Linear	4	25.2145	6.30363	8231.51	0.000
Square	4	0.0920	0.02299	30.02	0.000
Interaction	6	0.0035	0.00058	0.76	0.612
Residual Error	16	0.0123	0.00077		
Lack-of-Fit	10	0.0117	0.00117	12.27	0.003
Pure Error	6	0.0006	0.00010		
Total	30	25.3222			
R square	99.95%				
R square (pred)	99.73%				
Model F value	2360.76				
Lack of Fit F value	12.27				

Fcrit (0.05,14,16) = 2.373

Table 6. Regression coefficients and respective t-values for dextran (g/100ml)

Term	Dextran (g/100ml)		
	Coef	T	P
Constant	2.28429	218.396	0.000
Sucrose	1.02333	181.162	0.000
yeast	0.04875	8.630	0.000
peptone	0.01542	2.729	0.015
K_2HPO_4	0.02792	4.942	0.000
sucrose*sucrose	0.04768	9.213	0.000
yeast*yeast	-0.01795	-3.468	0.003
peptone*peptone	-0.01545	-2.985	0.009
K_2HPO_4*K_2HPO_4	-0.00670	-1.294	0.214
sucrose*yeast	0.00063	0.090	0.929
sucrose*peptone	-0.00187	-0.271	0.790
sucrose*K_2HPO_4	-0.00437	-0.632	0.536
yeast*peptone	-0.00187	-0.271	0.790
yeast*K_2HPO_4	0.01313	1.897	0.076
peptone*K_2HPO_4	-0.00437	-0.632	0.536

Tcrit (0.05,10) = 2.228

Table 7. Global solutions for after optimization by response optimizer and predicted response with their desirability on response

Parameter	Global solution	Predicted response	Composite desirability
Sucrose (g/100ml)	10.157	3.493	0.98641
Yeast (g/100ml)	1.611		
Peptone (g/100ml)	0.574		
K_2HPO_4 (g/100ml)	1.845		

Table 8. Optimized conditions obtained from overlaid contour plot with respective predicted response

Run	Optimized Condition				Predicted Response
	Sucrose (g/100ml)	Yeast (g/100ml)	Peptone (g/100ml)	K_2HPO_4 (g/100ml)	Dextran (g/100ml)
1	10.157	1.611	0.573	1.845	3.493
2	10.222	1.000	0.600	1.669	3.457
3	10.179	1.568	0.600	1.000	3.455
4	10.313	1.000	0.718	1.000	3.466

Table 9. Validation of optimized conditions with respective predicted and experimental values

Run	Optimized Condition				Predicted Response Dextran (g/100ml)	Experimental Response Dextran (g/100ml)
	Sucrose (g/100ml)	Yeast (g/100ml)	Peptone (g/100ml)	K_2HPO_4 (g/100ml)		
1	10.157	1.611	0.573	1.845	3.493	3.186
4	10.313	1.000	0.718	1.000	3.466	3.157

interactive terms of sucrose*sucrose, yeast*yeast, peptone*peptone are significant since their T value is greater than T_{crit} (Table 6). Response surface plots were generated for these terms to study the interactive effect among variables on production of dextran (Fig. 1).

An optimum condition for dextran production was determined to obtain maximum conversion of sucrose into dextran to increase the yield. Quadratic model equations obtained in this study (coefficients from Table 6) was utilized for the response in order to determine optimum conditions.

By applying the method of desirability function i.e. response optimizer (Fig. 2) and graphic optimization i.e. overlaid contour plot (Fig. 3) the optimized condition was determined as tabulated in Table 7 which also shows the predicted value for dextran production and composite desirability at the optimized point.

The response optimization condition for drawing response optimizer plot was tabulated in Table 8 and the global solution for the parameters and predicted responses are shown. Fig. 2 shows the global solution and predicted response, and composite desirability for the factor on response and graphical representation i.e. of the effect change in parameters on response with effect of composite desirability i.e. if we increase sucrose, yeast, peptone and K_2HPO_4 there will be sharp increase in the composite desirability. So, from this graph also we can conclude that all the variables i.e. sucrose, yeast, peptone and K_2HPO_4 have equal contribution.

From response optimizer (Fig. 2) and overlaid contour plot (Fig. 3) we following conditions can be optimized (Table 8). Since run 1, 3 and run 2, 4 are similar so we can consider run 1 and 4 (from Table 8) as optimized conditions.

The adequacy of the model for predicting

the optimum response, and predicting the response for any random values other than the CCD model values was checked using the recommended optimum (run 1 and 4 from Table 8) condition. The predicted and experimental results at the optimized conditions are tabulated (Table 9).

By applying multiple regression analysis on the experimental data (Table 6), the following second order polynomial equation was found to explain the dextran production regardless of the significance of coefficients:

$$Y = 2.28429 + 1.02333 * X1 + 0.04875 * X2 + 0.01542 * X3 + 0.02792 * X4 + 0.04768 * X1 * X1 - 0.01795 * X2 * X2 - 0.01545 X3 * X3 \quad ...(2)$$

Where, Y is the predicted response i.e. Dextran production, and X1, X2, X3 and X4 are coded values of sucrose, yeast, peptone, K_2HPO_4, respectively.

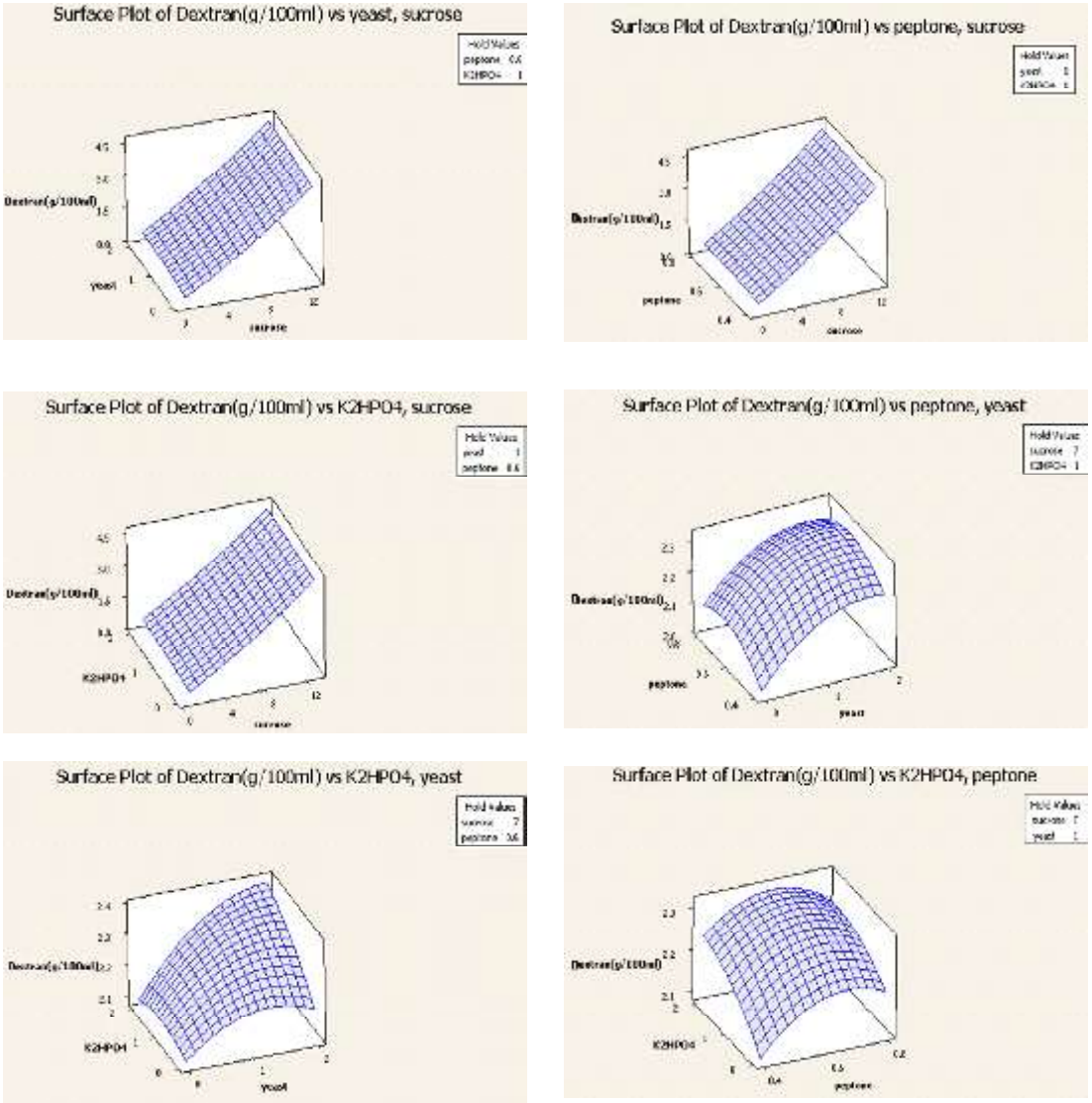

Fig. 1. Interactive effect among variables on production of dextran (a) Surface plots of Dextran g/100ml vs sucrose, yeast (b) Surface plots of Dextran g/100ml vs sucrose, peptone (c) Surface plots of Dextran g/100ml vs sucrose, K2HPO4 (d) Surface plots of Dextran g/100ml vs peptone, yeast (e) Surface plots of Dextran g/100ml vs K2HPO4, yeast (f) Surface plots of Dextran g/100ml vs K2HPO4, peptone

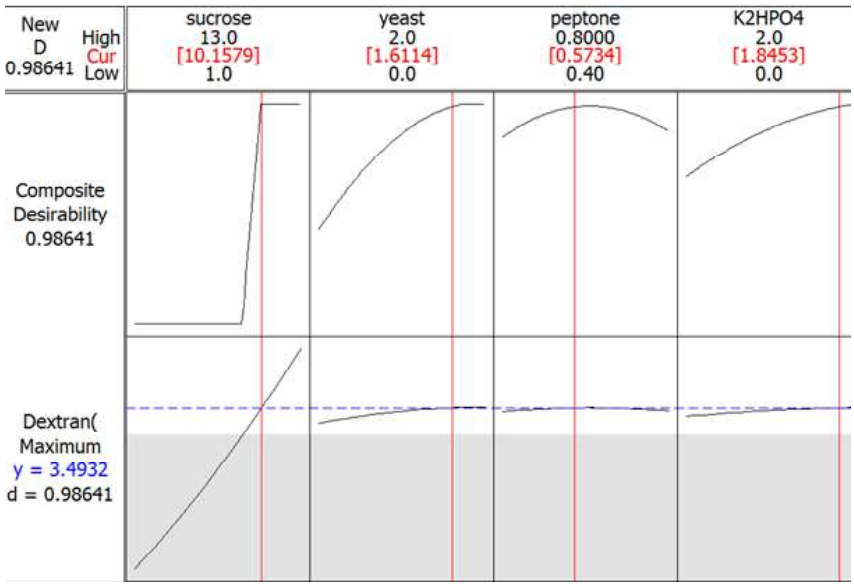

Fig. 2. Response Optimization Plot for factors and response in dextran production

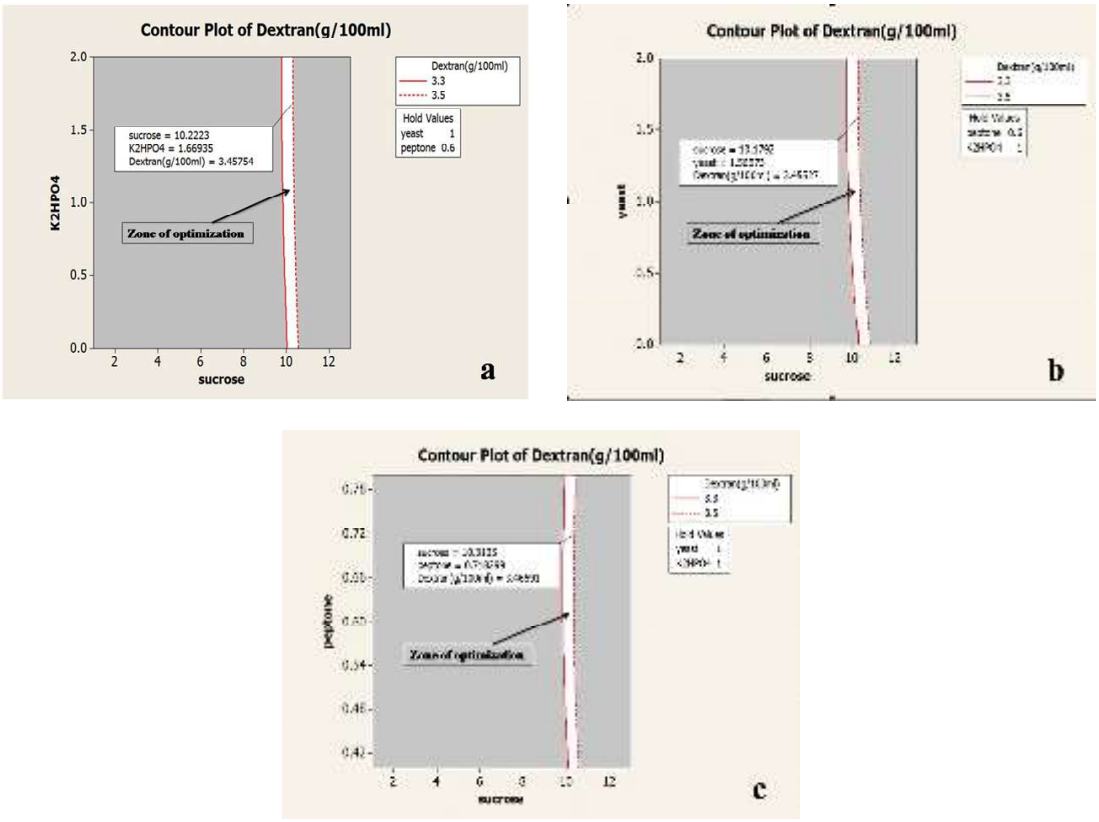

Fig. 3. Overlaid contour plot showing the zone of optimization (white zone) of the response as a function of (a) Sucrose and K_2HPO_4 , (b) Sucrose and Yeast and (c) Sucrose and Peptone

CONCLUSION

Statistical optimization method for fermentation process could overcome the limitations of classic empirical methods and has been proved to be a powerful tool for the optimization of dextran production by *Leuconostoc Mesenteroides* NCIM2198. Under optimal conditions (condition 1: sucrose 10.157g/100ml, yeast 1.611g/100ml, peptone 0.573g/100ml, K_2HPO_4 1.845g/100ml and condition 2: sucrose 10.313g/100ml, yeast 1.000g/100ml, peptone 0.718g/100ml, K_2HPO_4 1.000g/100ml), the predicted dextran production was 3.493g/100mL and 3.466g/100ml respectively. Validation experiments were also carried out to verify the availability and the accuracy of the model, and the results showed that the predicted value agreed with the experimental value (3.186g/100ml and 3.157g/100ml for optimal conditions 1 and 2 respectively) well. Production of dextran using sucrose is greatly depends on its concentration. As the concentration of sucrose increases in the medium yield of dextran also increases. Other salts also affect the yield of dextran but after increasing their concentration yield decreases. Use of RSM method for media optimization is very helpful as it generates a series of runs that gives an optimised condition by using the response of the runs and is more precise.

REFERENCES

1. *Encyclopedia of polymer science and technology.* John Wiley and Sons Inc., New York, NY, USA. 1966; pp 27-39.
2. Sidebotham R.L. Dextrans. *Adv. Carbohydr. Chem. Biochem.* 1974; 30: 371–444.
3. Monsan P., Bozonnet S., Albenne C., Joucla G., Willemot R.M., Remaund-Simeon M. Homopolysaccharides from lactic acid bacteria. *Int. Dairy J.* 2001; **11***:* 675–685.
4. Baily R.W., Oxford A.E. A quantitative study of the production of dextran from sucrose by rumen strains of *Streptococcus bovis. J. Gen. Microbiol.* 1958; **19**: 130–145.
5. Whittenbury R. A study of the genus leuconostoc. *Archives of Microbiology*, 1966; **53**: 317-327.
6. Jeanes A., Haynes W.C., William C.A., Rankin J.C., Melvin E.H., Austin M.J., *et al.,* Characterization and classification of dextran from ninety-six strains of Bacteria. *J. Am. Chem. Soc.* 1954; **76**: 5041–5052.
7. Hehre E.J., Hamilton D.M. Bacterial conversion of dextrin into polysaccharide with the serological properties of dextran. *Proc. Soc. Expt. Biol. Med.* 1949; **71**: 336–339.
8. Kim D., Robyt J.F. Production, selection, and characteristics of mutants of *Leuconostoc mesenteroides* B-742 constitutive for dextransucrases. *Enzyme Microb. Technol.* 1995; **17**: 689–695.
9. Kobayashi M., Matsuda K. The dextransucrase isoenzyme from *L. mesenteroides* NRRL B-512F. *Biochim. Biophys. Acta.* 1974; **370**: 441–449.
10. Shah A.U., Qader L., Iqbal L., Aman A., Shireen E., Azhar A. Production of Dextran by newly isolated strains of *Leuconostoc mesenteroides* PCSIR-4 and PCSIR-9. *Turk. J. Biochem.* 2006; **26**: 21-26.
11. Leathers T.D., Hayman G.T., Cote G.L. Rapid Screening of Dextran. *Current Microbiol.* 1995; **31**: 19-22.
12. Sutherland I.W. Microbial biopolymers from agricultural products: production and potential. *Int. Biodeter. Biodegr.* 1996; **38**: 249-261.
13. Sarwat F., Qader S.A.U., Aman A., Ahmed N. Production & Characterization of a Unique Dextran from an Indigenous *Leuconostoc mesenteroides* CMG713. *Int. J. Biol. Sci.* 2008; 4: 379-386.
14. Kim D., Day D.F. A New process for the production of clinical dextran by mixed culture fermentation of *Lipomyces starkeyi* and *Leuconostoc mesenteroides*. *Enzyme Micro.Technol.* 1994; 16: 844- 848.
15. Lowry O.H., Rosebrough N.J., Farr A.L., Randall R.J. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 1951; 193: 265–275.
16. Rastogi N.K., Rashmi K.R. *Optimisation of enzymatic liquefaction of mango pulp by response surface methodology. Eur. Food Res. Technol.* 1999; 209: 57-62.