International Journal of Sciences & Applied Research

www.ijsar.in

Statistical optimization of medium components by response surface methodology for dextran production by *Weissella confusa*

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Abstract

Exopolysaccharides like dextran produced by different microorganisms have a wide range of applications in the food, pharmaceuticals and other industries. To improve the yield of dextran from *Weissella confusa*, different medium constituents were optimized using response surface methodology (RSM) based on central composite design (CCD). The strain produced 1.3g/100ml of dextran in conventional method by using cortezi medium containing sucrose and yeast extract as a carbon and nitrogen sources. Response surface methodology which was applied to optimize concentrations of sugarcane juice, black gram powder, magnesium chloride, magnesium sulphate and dipotassium hydrogen phosphate improved the productivity to 3.35g/100ml. This value represents 2.25 fold increases in productivity as compared to conventional method. Optimal parameters of maximum dextran yield were determined as sugarcane juice 5%, black gram 2.5%, magnesium chloride 0.25%, magnesium sulphate 0.25% and dipotassium hydrogen phosphate 2.5% in 48 hours. This is significant commercially as not only the production increased but also the optimized medium had cheaper nutrient sources.

Keywords: Central composite design, Dextran, Parameters, Response surface methodology, Sucrose

Introduction

Dextran is a bacterial exopolysaccharide (Tallgren *et al.* 1999) biochemically a branched glucan made up of glucose molecules joined into chains of varying length (Naessens *et al.* 2005). It is produced as low molecular weight and high molecular weight dextrans (From 10 to 150 Kilo Daltons (Shah Ali UL Quader *et al.* 2005). It is produced by certain lactic acid bacteria like *Leuconostoc mesenteroides* (Leathers *et al.* 1995) *Lactobacillus brevis*, *Streptococcus mutants* and *Weissella sps* (Maina *et al.* 2001). Dextran is of particular interest because of its use as blood-plasma volume expander (Anthony and Leonsins, 1952). It finds various other industrial applications in food, pharmaceutical and chemical industries as adjuvant, emulsifier, carrier and stabilizer (Lakshmi Bhavani and Nisha, 2010). Crossed linked dextran known as sephadex (Andrews, 1964) are widely used for separation and purification of various products like proteins in research and industry. In food industry it is being used as thickener for jam and ice cream

(Cortezi *et al.* 2005) as it prevents crystallization of sugar, improves moisture retention, and maintains flavour and appearance of the food stuffs. Intravenously administered iron dextran is used to alleviate iron deficiency anaemia (Blanshard and Mitchell, 2005). Dextran has also been reported to inhibit the multiplication of human immunodeficiency virus (Baba *et al.* 1990). As it has numerous industrial applications, it is being produced by commercially using the strain of *Leuconostoc mesenteroides*. The amount of dextran produced however is practically insufficient to meet the dextran requirements of the various industries. To reach the industrial needs it required to improve the yield of dextran by application of optimization methods. Compared to conventional methods statistical methods like response surface methodology (RSM) are more advantageous as they required less time, labour intensive and simultaneously more than one parameter is optimized at five different levels (Deniz and Ismail, 2007). The present study was focused to apply response surface methodology (RSM) based on central composite design (CCD) for the quantitative optimization of culture medium components in order to enhance dextran production by *Weissella confusa*.

Materials and methods

Isolation of dextran producer *Weissella confusa*

Bacterial culture under study was isolated from idli batter/black gram soaked water, using enrichment culture technique. Sample was inoculated into a cortezi medium (Cortezi *et al.* 2005). Among the diverse dextran producers obtained by primary screening *Weissella confusa* was selected and used for this study due to its highest dextran producing ability. *Weissella confusa* was identified by microscopic, biochemical tests like resistance to vancomycin and confirmed by 16s rRNA gene sequencing analysis.

Fermentation

Broth studies for dextran production was done in 250 ml Erlenmeyer flasks containing 50ml Cortezi medium with sucrose as main carbon source. The fermentation parameters were studied for their influence on dextran production over a range to identify the most optimum factor. The flasks were incubated for 48 hours broth samples collected from different flasks and tested for dextran production by anthrone method (Morris, 1948) and fructose by resorcinol method (Roe *et al.* 2008). Fructose in broth was tested only to prove that dextran is a polymer of glucose and fructose is left in broth when sucrose is taken in the medium.

Recovery

Dextran was recovered from broth by alcohol precipitation, dried under vacuum over $CaCl_2$ at 30^0C and weighed (Farwa Sarwat *et al.* 2008). Product was assayed and found to contain glucose polymer (Dextran) by using anthrone method. Dextran yield was determined in grams/100ml of fermented broth. Molecular weight of dextran was analysed by HPLC using Agilent Zorbax GF-250, and it indicates presence of low molecular weight dextran (Vijayabaskar *et al.* 2011).

Central composite design (CCD)

An experiment CCD was carried out in order to identify and optimize the nutrients in the production medium (Sugarcane juice, black gram, magnesium chloride, magnesium sulphate and dipotassium hydrogen phosphate) that have a significant effect on dextran production. There are five factors and five levels for each factor. The levels are fixed based on experimentation (Table-1). The CCD model selected for present study contains a total of 54 experiments in two blocks of 42 and 12 experiments. The design of the experiment with the different levels of the factors is given table-2. For each factor under study a range of levels were selected and fermentation experiments done to identify the level giving peak production. This peak level was taken as the central point (0) and the other four levels two higher than this +1, +2 and two lower than this -1, -2 were taken, again taking into consideration the fact that the concentrations of ingredients promoted growth and production and did not inhibit them. The ingredients are added according to the pattern of the design, then inoculated and incubated for 48 hours. Samples were collected and results were subjected to statistical analysis using Indostat software version 9.1.

Once the experiment was performed, results were analysed statistically and the predicted value of dextran produced (Y) was obtained by calculating the coefficient of polynomial model using the equation (1):

$$Y = b_0 - b_{i1}\,x_1 + b_{i2}\,x_2 + b_{i3}\,x_3 + b_{i4}\,x_4 + b_{i5}\,x_5 + b_{j1}\,x_1^2 + b_{j2}\,x_2^2 + b_{j3}\,x_3^2 + b_{j4}\,x_4^2 + b_{j5}\,x_5^2 + b_{k1}\,x_1x_2 + b_{k2}\,x_1x_3 + b_{k3}\,x_1x_4 + b_{k4}x_1x_5 + b_{k5}\,x_2x_3 + b_{k6}\,x_2x_4 + b_{k7}\,x_2x_5 + b_{k8}\,x_3x_4 + b_{k9}x_3x_5 + b_{k10}x_4x_5 + b\,(\text{Block})$$

Where b_0 is the intercept, x_1 to x_5 are the factors b_i series, b_j series and b_k series represent the regression coefficient of linear, quadratic and interactive terms respectively and b is the block.

The significance of each coefficient was determined using the student t-test. Model terms were selected or rejected based on the student t-value or significance as indicated by probability values (Ram Mohan Reddy *et al.* 1999). Three dimensional plots and their respective contour plots of two factors (at five different levels each) versus the amount of dextran produced were drawn keeping the other three factors at their optimum levels. From these three dimensional plots, the interaction of one parameter with other parameter was studied. The optimum concentration of each parameter was identified based on the hump in the three-dimensional plots and central point in the corresponding contour plot.

Results

Five comparatively more important nutrients were selected and a five level, five factor central composite design was adopted (Table-1). The dextran yields were subjected to statistical analysis that gave predicted yields, regression coefficients, t-values and probabilities. Table-2 shows experimental yields and predicted yields of dextran production. R^2adj (0.94793) is reasonably close to 1, and indicates a high degree of correlation between the observed and predicted values. The regression coefficients, corresponding t-values and probabilities of the variables in linear, quadratic and interactive terms were obtained by subjecting the experimental results to statistical analysis and results are indicated in (Table-3).

Table 1: Independent variables and their levels used for central composite design.

Variables	Levels				
	-2	-1	0	+1	+2
Sugarcane juice (%)	1	2	3	4	5
Black gram (%)	0.5	1	1.5	2.0	2.5
M_gCl_2 (%)	0.05	0.1	0.15	0.2	0.25
M_gSO_4 (%)	0.05	0.1	0.15	0.2	0.25
K_2HPO_4 (%)	0.5	1.0	1.5	2	2.5

Model P value (Prob>F) is very low (<0.0000). This reiterates that the model is significant. The P values are used as a tool to check the significance of each of the coefficients, which in turn are necessary to understand the pattern of the mutual interactions between the test variables. The smaller the magnitude of the P, the more significant is the corresponding coefficient (Muralidhar *et al.* 2001).

Table 2: Central composite design matrix for response surface methodology showing observed and predicted yield of dextran.

RUN	Factor-1	Factor-2	Factor-3	Factor-4	Factor-5	Actual Dextran yield Gm/100ml	Predicted Dextran yield Gm/100ml
1	1	1	1	1	1	3.1	3.0
2	1	1	1	1	-1	2.55	2.59
3	1	1	1	-1	1	2.6	2.53
4	1	1	1	-1	-1	2.2	2.08
5	1	1	-1	1	1	2.65	2.64
6	1	1	-1	1	-1	2.5	2.40
7	1	1	-1	-1	1	2.3	2.22
8	1	1	-1	-1	-1	1.9	1.94
9	0	0	0	0	0	3.3	3.27
10	0	0	0	0	0	3.3	3.27
11	1	-1	1	1	1	2.9	2.88
12	1	-1	1	1	-1	2.55	2.48
13	1	-1	1	-1	1	2.7	2.67
14	1	-1	1	-1	-1	2.3	2.23
15	1	-1	-1	1	1	2.4	2.27
16	1	-1	-1	1	-1	2.15	2.04
17	1	-1	-1	-1	1	2.2	2.12
18	1	-1	-1	-1	-1	1.95	1.86
19	0	0	0	0	0	3.25	3.27
20	0	0	0	0	0	3.3	3.27
21	-1	1	1	1	1	2.85	2.94
22	-1	1	1	1	-1	2.65	2.63
23	-1	1	1	-1	1	2.55	2.68
24	-1	1	1	-1	-1	2.35	2.32
25	-1	1	-1	1	1	2.5	2.45
26	-1	1	-1	1	-1	2.3	2.31
27	-1	1	-1	-1	1	2.3	2.24
28	-1	1	-1	-1	-1	2.0	2.06
29	0	0	0	0	0	3.3	3.27
30	0	0	0	0	0	3.2	3.27
31	-1	-1	1	1	1	2.55	2.38
32	-1	-1	1	1	-1	2.0	2.07
33	-1	-1	1	-1	1	2.4	2.38
34	-1	-1	1	-1	-1	2.0	2.04
35	-1	-1	-1	1	1	1.5	1.64
36	-1	-1	-1	1	-1	1.55	1.51
37	-1	-1	-1	-1	1	1.75	1.71
38	-1	-1	-1	-1	-1	1.6	1.54
39	0	0	0	0	0	3.3	3.27
40	0	0	0	0	0	3.25	3.27

41	2	0	0	0	0	2.55	2.91
42	-2	0	0	0	0	2.7	2.53
43	0	2	0	0	0	2.7	2.69
44	0	-2	0	0	0	1.85	2.04
45	0	0	2	0	0	2.7	2.73
46	0	0	-2	0	0	1.7	1.86
47	0	0	0	2	0	2.8	2.89
48	0	0	0	-2	0	2.35	2.44
49	0	0	0	0	2	2.5	2.61
50	0	0	0	0	-2	1.95	2.03
51	0	0	0	0	0	3.35	3.27
52	0	0	0	0	0	3.3	3.27
53	0	0	0	0	0	3.3	3.27
54	0	0	0	0	0	3.3	3.27

Table 3: Analysis of variance (ANOVA) for response surface quadratic model for the production of dextran.

Variable	**Reg coeff**	**t-value**	**t-prob**	**Levels of significance**
Intercept	3.2709	94.8844	0.0000	***
A- Sugarcane juice	0.0950	4.9629	0.0000	***
B- Black gram	0.1625	8.4892	0.0000	***
C-MgCl2	0.2175	11.3624	0.0000	***
D-$MgSO_4$	0.1125	5.8771	0.0000	***
E- K_2HPO_4	0.1450	7.5749	0.0000	***
A^2	-0.1367	-6.5652	0.0000	***
B^2	-0.2242	-10.7689	0.0000	***
C^2	-0.2429	-11.6697	0.0000	***
D^2	-0.1492	-7.1658	0.0000	***
E^2	-0.2367	-11.3695	0.0000	***
AB	-0.1094	-5.1106	0.0000	***
AC	-0.0313	-1.4602	0.1537	
AD	0.0531	2.4823	0.0183	
AE	0.0250	1.1681	0.2511	
BC	-0.0594	-2.7743	0.0090	
BD	0.0688	3.2124	0.0029	
BE	0.0031	0.1460	0.8848	
CD	0.0156	0.7301	0.4705	
CE	0.0437	2.0443	0.0490	
DE	-0.0094	-0.4381	0.6642	
Block	0.0019	0.0353	0.9719	

Note: * Indicates most significant.**

F ratio for the model was 42.24697. Degree of freedom 20, 33. F probability 0.0000. R^2 0.96758 and R^2 adj 0.94793.

Based on the probability and t- values, significance levels were indicated by three stars for most significant as indicated in table-3. From the table for the factor A (sugarcane juice) ,factor B (black gram powder), factor C (magnesium chloride), factor D (magnesium sulphate) and factor E (dipotassium hydrogen phosphate) linear and quadratic terms are most significant and interactive terms are not significant. It is evident from table that all the factors selected were significant either in linear or quadratic terms while all interactive terms were not significant. With the equation (1) dextran yield can be calculated using the regression coefficients and the factor optimal concentrations.

Dextran yield: $3.2709 + 0.0950x_1 + 0.1625x_2 - 0.2175x_3 + 0.1125x_4 + 0.1450x_5 - 0.1367x_1^2 - 0.2242x_2^2 - 0.2429x_3^2 - 0.1492x_4^2 - 0.2367x_5^2 - 0.1094x_1x_2 - 0.0313x_1x_3 + 0.0531x_1x_4 + 0.0250x_1x_5 - 0.0594x_2x_3 + 0.0688x_2x_4 + 0.0031x_2x_5 + 0.0156x_3x_4 + 0.0437x_3x_5 - 0.0094x_4x_5 +$ Block

Where x_1, x_2, x_3, x_4 and x_5 are the coded values of sugarcane juice, black gram powder, magnesium chloride, magnesium sulphate and dipotassium hydrogen phosphate respectively.

The statistical analysis also gave three dimensional curves and their corresponding contour plots. These were obtained based on the effect of concentrations of two parameter and their interactions on dextran yield keeping the other three parameters at their optimal concentrations (as obtained through analysis of variance). The elliptical shape of the contour indicates good interaction of the two variables and circular shape indicates no interaction between variables (Sen and Swaminathan, 1997). The response surface curves and their corresponding contour plots obtained by application of response surface methodology for dextran medium optimization (Figures 1-10) shows that when sugarcane juice concentration increases from 1- 5% resulted in gradual increase in dextran yield. Similar results were observed for other nutrients also. As concentration of black gram powder increases from 0.5-2.5%, magnesium chloride from 0.05-0.25%, magnesium sulphate from 0.05-0.25% and dipotassium hydrogen phosphate from 0.5- 2.5% increases in dextran production. From central composite design the optimum concentration for sugarcane juice, black gram powder, magnesium chloride, magnesium sulphate and dipotassium hydrogen phosphate were observed to be 5%, 2.5%, 0.25%, 0.25% and 2.5% respectively. The predicted dextran production was 3.27 g/100ml while conducting the experiments at the predicted optimum conditions, the dextran yield obtained was 3.35 g/100ml. The experimental values were found to be very close to the predicted values and hence, the model was successfully validated.

Contour plot and 3D Response surface plot showing interactive effect among variables

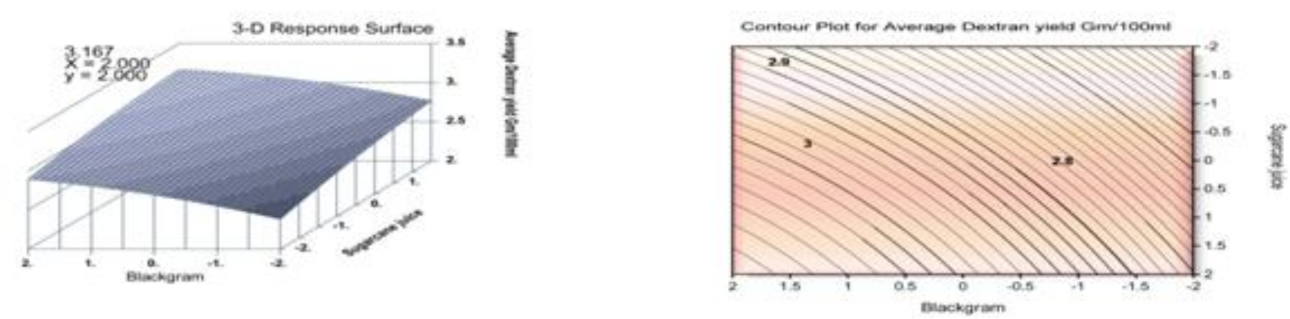


Fig. 1: Contour plot and 3D Response surface plot showing interactive effect of black gram powder and sugarcane juice.

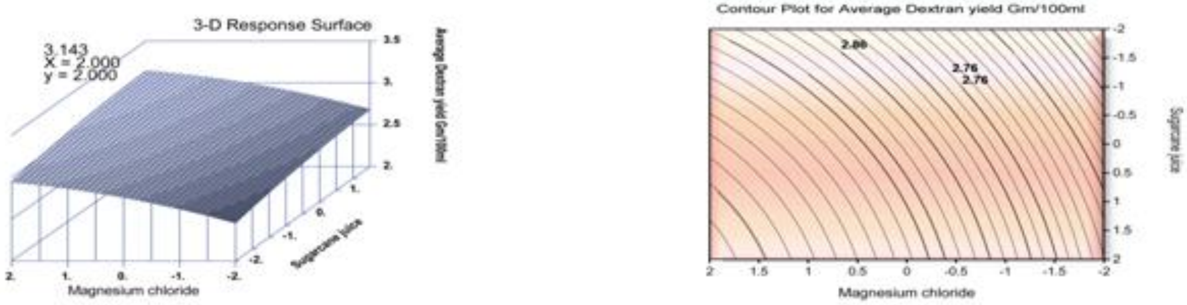


Fig. 2: Contour plot and 3D Response surface plot showing interactive effect of magnesium chloride and sugarcane juice.

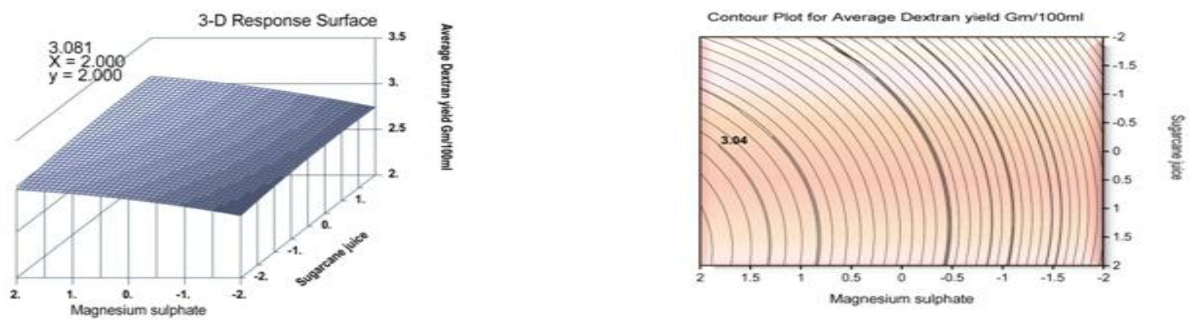


Fig. 3: Contour plot and 3D Response surface plot showing interactive effect of magnesium sulphate and sugarcane juice.

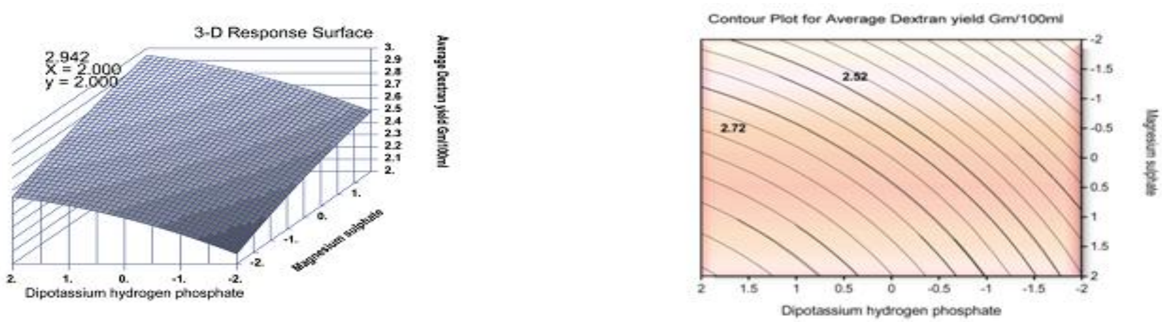


Fig. 4: Contour plot and 3D Response surface plot showing interactive effect of dipotassium hydrogen phosphate and magnesium sulphate.

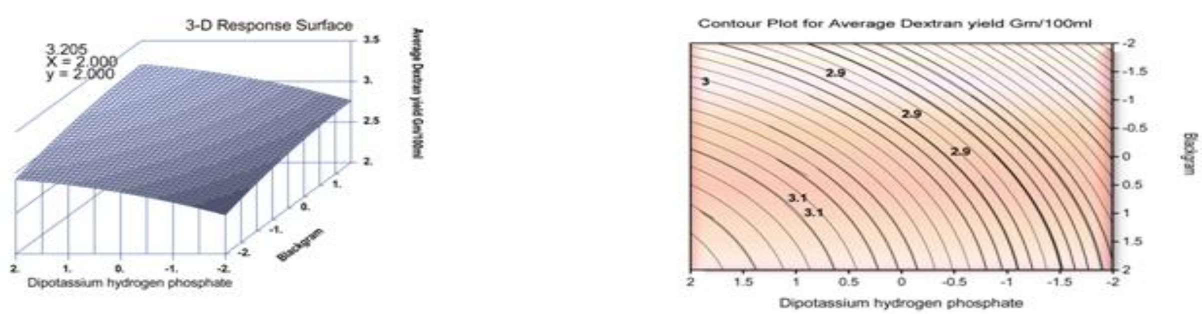


Fig. 5: Contour plot and 3D Response surface plot showing interactive effect of dipotassium hydrogen phosphate and black gram.

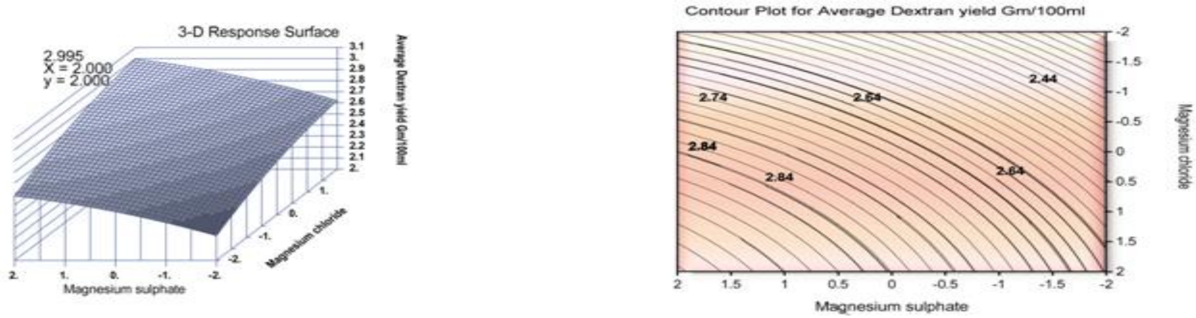


Fig. 6: Contour plot and 3D Response surface plot showing interactive effect of magnesium sulphate and magnesium chloride.

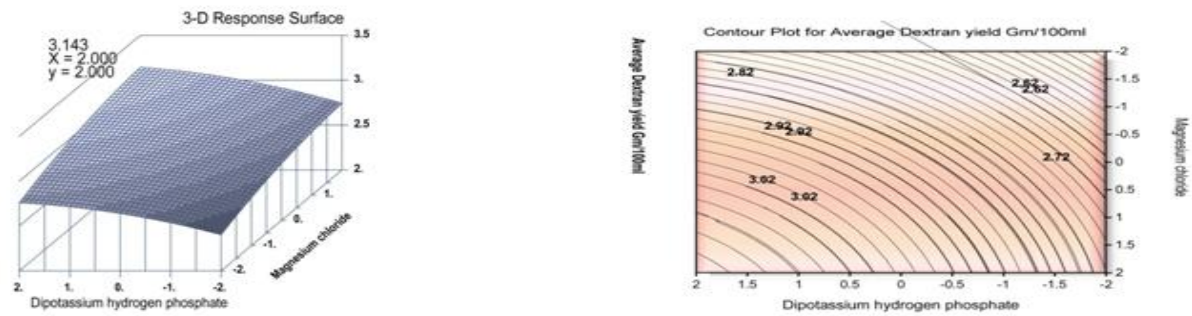


Fig. 7: Contour plot and 3D Response surface plot showing interactive effect of dipotassium hydrogen phosphate and magnesium chloride.

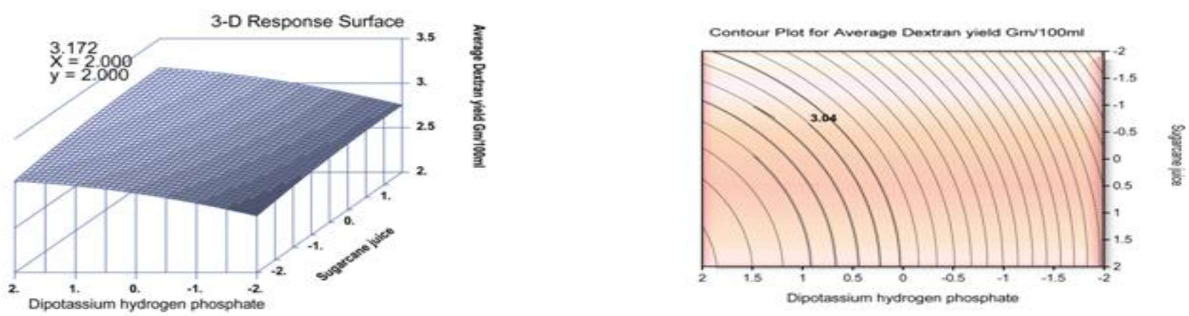


Fig. 8: 3D Response surface plot showing interactive effect of dipotassium hydrogen phosphate and sugarcane juice.

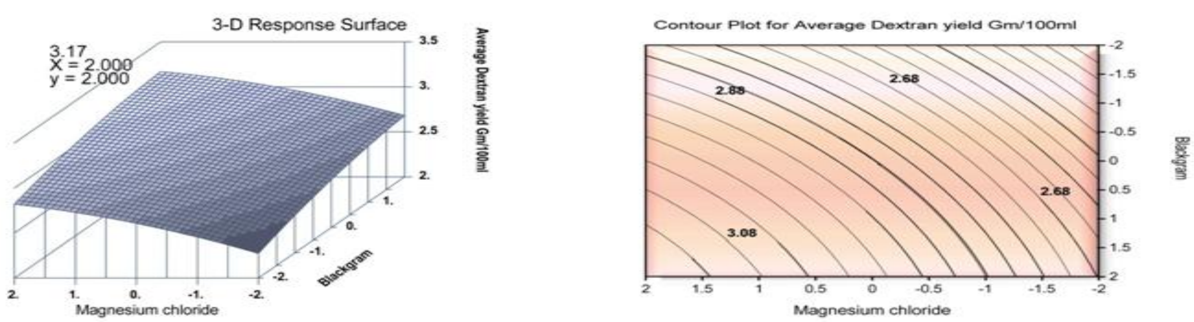
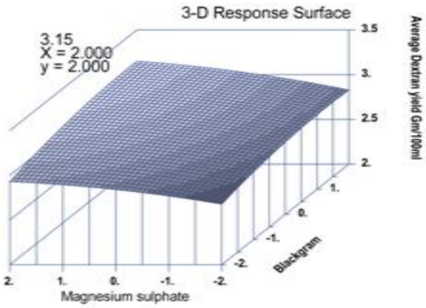


Fig. 9: Contour plot and 3D Response surface plot showing interactive effect magnesium chloride and black gram powder.



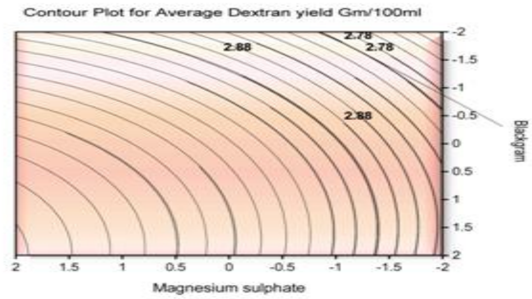


Fig. 10: Contour plot and 3D Response surface plot showing interactive effect of magnesium sulphate and black gram powder.

Discussion

An optimized culture medium significantly influences commercial production as it ensures that the required nutrients are present in appropriate forms and at non inhibitory concentrations. Carbon is an important constituent of the cellular components and it plays a central role in energy production in living cells (Onilude *et al.* 2013). Sucrose being one of the most suitable carbon source for dextran production by lactic acid bacteria (Smitinont *et al.* 1999). In this study sugarcane juice (carbon source) at 5% concentration induced highest dextran production. This is of particular importance when considering the cost of dextran production which is mostly based on sucrose containing medium. Microbes require nitrogen to support the biosynthesis of proteins like enzymes and structural proteins. Dextran through an exopolysaccharide requires dextran sucrase enzyme for production (Naessens *et al.* 2005). In the present study natural nitrogen source like black gram powder at 2.5% shows good response. Different concentrations of micronutrients and macronutrients also influences the activity of enzyme dextran sucrase required for dextran production (Onilude *et al.* 2013). In this study micronutrients like magnesium chloride and magnesium sulphate at 0.25% and macronutrient like dipotassium hydrogen phosphate at 2.5% shows positive effect.

Conclusion

A potential dextran producer was isolated and identified by microscopic, cultural, biochemical and by 16s-rRNA sequencing as *Weissella confusa*. Response surface methodology was used successfully to find the optimum values of the significant factors to achieve maximum dextran production. The predicted yield was 3.27g/100ml. On experimentation, 3.35g/100ml dextran yield was obtained. The experimental values were found to be very close to the predicted values and hence, the model was successfully validated. The optimized medium gave 2.5 fold higher dextran productions than in the unoptimized medium by the isolate *Weissella confusa.* This is significant commercially as not only the production increased but also the optimized medium had cheaper nutrient sources.

Acknowledgment

The authors (Srinivas and Naga Padma) are grateful to the management of BVB Bhavan's Vivekananda College for encouraging to carry out this work.

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