Statistical Optimization of Culture Media and Conditions for Maximize Production of Mannan by Saccharomyces Cerevisiae Using Response Surface Methodology

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Authors' contributions

This work was carried out in collaboration between all authors. Authors MSA and ME designed the study. Author KA managed the statistical analysis. Author MM managed the literature searches. Author VAH performed the statistical analysis, wrote the protocol and the first draft of the manuscript. All authors read and approved the final manuscript.

Original Research Article	

ABSTRACT

Aim: In view of the increase in *Saccharomyces cerevisiae* mannan content, the culture medium and condition for *S. cerevisiae* were optimized in this study.
Study Design: The mathematical model was established by the quadratic rotary combination design via Response Surface Methodology to evaluate the effect of culture condition and media on mannan content in the yeast cell wall.
Methodology: The influence of culture medium ingredients such as carbon source (FOS, in the range of 2 – 9 g/100ml) and nitrogen source (mixture of peptone and yeast extract, in the range of 1-5g/100ml), and enzyme activator (glycerol, in the range of 1-4g/100ml) on mannan production were evaluated using Response surface methodology. Also The influence of original pH (in the range of 4-7), inoculums size (in the range of 2-4%) and temperature (in the range of 26-32ºC), on mannan production was evaluated and confirmed by quadratic design.
Results: The optimized concentrations of culture medium were determined as follows: 8.99

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g/100mL, FOS; 4.25 g/100 ml, mixture of bacto peptone and yeast extract; and 3 g/100mL, glycerol. The order of effect was carbon source› nitrogen source› enzyme activator. Also The order of effect of original pH, inoculums size, and temperature, on mannan production was as follows: temperature > initial pH > inoculation size. The optimized culture condition was pH, 6.62; inoculums size, 4 ml; temperature, 32ºC. The maximum mannan production increased to 94.912 ±9.1 mg/100 ml at the optimum culture condition.
Conclusion: It was evident that the mannan production was affected significantly by culture medium and condition optimization (p < 0.01).

1. INTRODUCTION

Yeast is surrounded by a tough, rigid cell wall that represents 20~25% of the dry weight of the cell [1,2]. Mannan is one of the major components (together with glucan, chitin, and protein) of the yeast cell wall, occupying about 35~45% of it deposited in the outside of the cell wall. The presence of mannan not only provides the cells with rigidity that protects them from osmotic pressure but also helps to maintain their shapes during the cell cycle stage [3].

Mannans are generally linked to proteins through covalent bond and are mainly composed of 80~90% mannose and 5~20% proteins, with the molecular weight ranging from 20,000 to 200,000. The backbone, which is composed of a-1,6-linked mannose residues is 83% branched at O-2 by a single mannose residue as well as oligosaccharide side chains mostly in the form of di-, tri- and tetramers. Longer side chains, penta- to heptamers, are present in lesser amounts. NMR spectra of oligosaccharides showed that they are mostly composed by a-1,2- and -1,3-linked mannose residues [4]. It has been reported that most of the immunological effects observed within the intact yeast cells are reproduced with cell wall components and have been shown to be potent inducers of cellular and humoral immunity. Among them, mannan and mannoprotein are found to be the one with significant activities [5]. Besides, they could also balance the enterobacteria, combine with the extrinsic pathogen, resist tumor and acted as an anti-oxidant agent. The production of mannan is affected significantly by the mannan content in *S. cerevisiae*. Generally, the changes in the physiological state of the cell and its reaction to external influence could be attributed to the dynamic structure of the cell wall [6]. While, the architecture of a cell wall and mechanisms responsible for its synthesis may be controlled by the composition of the culture medium [7] According to Odds and Bernaerts [8] differential culture media should contain indicator substances that can facilitate the development of yeasts without affecting their viability. Reports had shown that culture medium for *S. cerevisiae* included carbon source, nitrogen source, growth gene and other factors. The carbon source used for yeast fermentation include glucose, saccharose, lactose, fructose, maltose and dissolved starch, of which, glucose was been used as an important medium for *S. cerevisiae* fermentation to enhance its yield [9] The organic nitrogen source for *S. cerevisiae* fermentation are peptone and yeast extract, while the inorganic nitrogen source and compound nitrogen source are from sulfuric ammonium, nitre, carbamide and casein. Zeng and co-workers also found that the microorganism could be affected significantly by peptone compounded with yeast extract [10]. Previously it was demonstrated that yeast biomass could be affected significantly by culture medium, however, how the culture medium affects mannan production is unknown. Therefore the culture medium and condition of *S. cerevisiae* were optimized in this study for the production of the high yield of mannan.

2. MATERIALS AND METHODS

2.1 Microorganism and Culture Condition

S. cerevisiae PTCC5209(CBS2356) purchased from Persian Type Culture Collection (Tehran, Iran) .It was grown in the Yeast Extract Peptone Dextrose Medium (YPD) composed of 20 g/L, glucose; 20 g/L, peptone; 10 g/L, yeast extract (seed Medium); for the activation. For long term storage of yeast cells after activation time has elapsed, the cells cultured in YPD Agar slants (with the same composition containing 20g of agar per liter),after passing incubation time, samples were kept at 4ºC before use.

2.2 Measurement of Yeast Number

To ensure that the number of yeast was in accordance to the desired level of 10^9 per milliliter in logarithmic phase, the Mcfarland standard solution was used, (The absorbance of Mcfarland NO.7 solution at 600nm is 1.170 that is equal to 10^9 yeast per mililter).different dilutions (v/v) was prepared from the cultured samples and absorption versus the pure culture (as a control) was measured, using spectrophotometer model Unico, seated in Institute of Food Science and Technology,(Mashhad, Iran). It was observed that dilution of 50% (v/v) of the sample is equal to 1.170.

2.3 Create Test and Control Environment

A 3 ml seed medium was inoculated to the liquid fermentation medium containing 100 ml with composition same as the seed medium and cultivated at 28ºC for 72 h on a shaker at 130 rpm in an incubator (HZQ-F160, China). The liquid YPD medium was used as a control, To test the effect of growth conditions on the rate of mannan production by the yeast, took the same approach, except that growth factors including pH in the range from 4-7 the temperature in the range of from 26-32ºC and inoculation size in the range of 2-4% was used as the variables and the impact of each of these factors is evaluated. In order to change the pH of the medium to achieve the desired pH, Hydrochloric acid was used. In order to assess effect of culture medium on mannan production experimental media were prepared by replacing carbon source (Fructo oligo-saccharide), nitrogen source (mixture of yeast extract and bactopeptone) or adding the enzyme activator (glycerol) to the YPD medium and incubation performed at optimized condition [11].

2.4 Preparation of the Yeast Cell Wall

The yeast was cultivated in 100 ml YPD liquid culture media in Erlenmeyer flask for 72 h. A 5 ml yeast suspension was withdrawn into a centrifugal tube and centrifuged at 4,500 g for 10 min. The deposit was washed three times with cold deionized water and then yeast cells were disrupted in 0.5 ml deionized water in the presence of 0.5 g glass beads (Sigma G8772, Sigma Aldrich) with the diameter of 0.4 to 0.6 mm for four cycles of 20 s each using a Mini-Bead Beater-8 (Biospec Mini Bead Beater 8, USA) with intermediate ice cooling (20s) the glass beads were extensively washed with cold deionized water. The supernatant and washing were pooled and centrifuged again at 4,500 g for 10 min. The pellet, containing cell wall was washed several times with cold deionized water until the supernatant became clear and then stored at 4oC before using [12].

2.5 Measurement of Mannan

Wet yeast cell walls were wet with 100 µl 72% H_2SO_4 (w/w) and kept at room temperature for 3h. The slurry was diluted to 1 ml and a final concentration of 2 N H_2SO_4, and then heated in sealed tubes for 4 h at 100ºC. Sulfate ions were neutralized by drop-wise addition of NaOH until neutral pH was reached. The volume was adjusted to 100 ml with phosphate buffer (pH 7.0), and the amount of mannan was measured spectrophotometrically using 0.3 ml of the sample and 0.2 ml of NaCl-H_3BO_3 reagent or distilled water. NaCl-H_3BO_3 reagent consisted of a mixture of 12 g NaCl and 2 g H_3BO_3 in 100 ml distilled water. The absorption of mannose in 90% H_2SO_4 at 70ºC for 30 min was read at 280 nm. The difference between absorption with and without NaCl-H_3BO_3 was directly proportional to mannose concentration [13].

2.6 Experimental Design and Statistical Analysis

Since the main objective of the study was to evaluate the main effects and interaction factors of temperature, pH and inoculation size as well as the main and interactive effects of carbon source, nitrogen source and enzymeactivator, on the mannan content of the cell wall, two separated statistic design of Response Surface Methodology were employed to estimate effect of independent variables on the mannan content of cell wall and designing the experimental data

X_1 (FOS), X_2 (mixture of bactopeptone and yeast extract), X_3 (glycerol);

X_1(pH), X_2 (temperature), X_3 (inoculation size); Tables 1 and 2

Table 1. Independent variable and their values in yeast model to evaluate the effect of culture condition

Variable	Coded factor	+1	0	-1
pH	X_1	7	5.5	4
Temperature	X_2	32	29	26
IS[1]	X_3	4	3	2

Table 2. Independent variable and their values in yeast model to evaluate the effect of culture media

variable	Coded factor	+1	0	-1
Carbon source	X_1	9	5.5	2
Nitrogen source	X_2	5	3	1
EA[2]	X_3	4	2.5	1

The RSM[3] was applied to the experimental data using a commercial statistical package Design-Expert version 8.01 (Statease Inc. Minneapolis, USA) Experiments Tables 3 and 4 were randomized in order to minimize the effects of unexplained variability in the observed responses due to extraneous factors. The experimental design included star points and six centre points to calculate the repeatability of the method [14]. The response functions (Y)

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[1] *Inoculation size*

[2] *Enzyme activator*

[3] *Response Surface Methodology*

was the mannan content of the cell wall that was related to the coded variables by a second order polynominal using equation below:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3$$

The coefficients of the polynomial model were represented by, β_0 (constant factor), β_1 β_2 β_3 (linear effects), β_{11} β_{22} β_{33} (effects of squares), β_{12} β_{13} β_{23} (effects of interactions). Statistical significance of the terms in the regression equations was examined. The significant terms in the model were found by analysis of variance (ANOVA) for each response. The adequacy of model was checked accounting for R^2 and adjusted-R2. Numerical and graphical optimization technique of the Design Expert software was used for optimization of the response. The desired goals for each variables and response were chosen. All the independents variables were kept within range while the response was maximized.

Table 3. Treatment applied in evaluating culture condition

Run	pH	Temperature	Inoculation size
1	5.5	32	3
2	7	32	4
3	5.5	29	3
4	4	32	4
5	4	29	3
6	5.5	29	3
7	7	32	2
8	5.5	29	3
9	7	26	2
10	5.5	29	3
11	7	29	3
12	7	26	4
13	5.5	26	3
14	5.5	29	3
15	4	26	2
16	4	26	4
17	5.5	29	3
18	5.5	29	2
19	5.5	29	4
20	4	32	2

Table 4. Treatment applied in evaluating culture media

Run	Carbon source	Nitrogen source	Enzyme source
1	2	3	2.5
2	9	1	4
3	5.5	3	1
4	9	3	2.5
5	9	1	1
6	5.5	3	2.5
7	5.5	3	2.5
8	2	1	1
9	2	5	4

Table 4 Continued…

10	5.5	5	2.5
11	5.5	3	2.5
12	2	5	1
13	5.5	1	2.5
14	5.5	3	2.5
15	9	5	4
16	5.5	3	2.5
17	2	1	4
18	9	5	1
19	5.5	3	2.5
20	5.5	3	4

3. RESULTS AND DISCUSSION

3.1 Model Fitting

The second order polynomial response surface model was fitted to the response variables (Y) for the corresponding fitting of the explanatory models and the variation of the mannan content the sum of squares of the sequential model was analyzed. These analyses indicated that adding terms up to quadratic significantly improved the model Table 5. Therefore, could be the most appropriate model for the response variable.

Table 5. Sequential model sum of squares for different culture condition and media

Model	Culture Condition			Culture Media		
	DF[4]	Sum of squares	PR›F	DF	Sum of squares	PR›F
Mean	1	1.219E+005		1	1.551E+005	
Linear	3	1359.76	‹0.0001	3	1056.11	‹0.0001
Interaction	3	19.66	0.4890	3	111.02	0.1775
Quadratic	3	94.51	‹0.0001	3	203.13	0.0006
Cubic	4	4.26	0.0219	4	27.71	0.1918
Residential	6	0.97		6	19.31	
Total	20	1.234E+005		20	1.566E+005	

Regression analysis and ANOVA were used for fitting the model and to examine the statistical significance of the terms. The estimated regression coefficient of the quadratic polynomial model for the response variable, along with the corresponding coefficient of determination (R^2) are given in Table 6, In addition, adj-R^2 and coefficient of variation (CV) were calculated to check the model adequacy. The lack of fit is an indication of the failure for a model representing the experimental data at which points were not included in the regression or variations in the models cannot be accounted for random error [14]. If there is a significant lack of fit which could be indicated by a low probability value, the response predictor is discarded. The lack of fit illustrated in Table 6, did not result in a significant p-value for selected variable, meaning that the model were sufficiently accurate for predicting the relevant response. Coefficient of determination, R^2 is the proportion of variation in the response attributed to the model rather than to random error and was suggested that for a good fitted model, R^2 should not be less than 80%. When R^2 approaches to the unity,

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[4] *Degree of freedom*

signifies the suitability of fitting empirical model to the actual data. The lower value of R^2 shows the inappropriateness of the model to explain the relation between variables [15,16]. Our results showed that the R^2 value for these response variables were higher than 0.80, indicating the regression models were suitable to explain the behavior. The R^2 value for mannan content in evaluating culture media and conditions were found to be 0.96 and 0.99 respectively. It should be noted that adding a variable to the model will always increase R^2, regardless of whether the additional variable is statistically significant or not. Thus, a large value of R^2 does not always imply the adequacy of the model. For this reason, it is more appropriate to use an adj-R^2 of over 90% to evaluate the model adequacy.   The adj-R^2 values were found to be higher than 0.93 for the responses. Higher adj-R2 indicated that non-significant terms have not been included in the model. Moreover, coefficient of variation (CV) describes the extent to which the data were dispersed. As a general rule, the coefficient of variation (CV) should not be greater than 10% [17]. Reported that a high CV indicates that variation in the mean value is high and does not satisfactorily develop an adequate response model. Our results showed that the coefficients of variation (CV) were less than 10% for the responses Table 6. Representing a better precision and reliability of the conducted experiments.

Fig. 1. (A) and (B) show that the polynomial regression model was in good agreement with the experimental results. In this figure, each of the observed values is compared to the predicted value calculated from the model. The result suggests that the models used in this research were able to identify operating conditions for increasing mannan content in the cell wall of *Saccharomyces cerevisiae*.

Table 6. ANOVA and regression coefficients of the second-order polynomial model for the response variable (actual values)

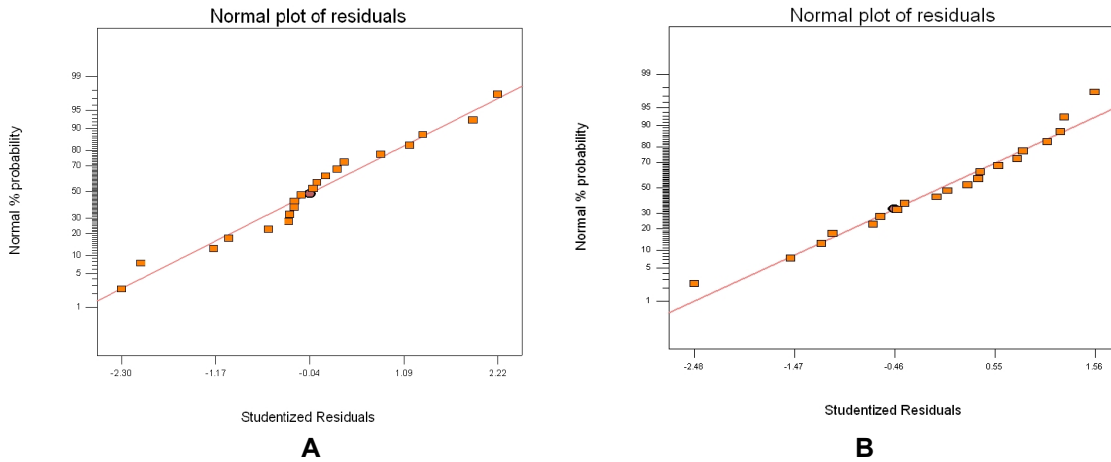| Source	Culture Condition				Culture Media			
	DF	Coefficient	Sum of squares	P-Value	DF	Coefficient	Sum of squares	P-Value
Model	9	76.36	1473.94	<0.0001	9	91.76	1370.25	<0.0001
Linear								
B_1	1	3.83	147	<0.0001	1	9.04	816.44	<0.0001
B_2	1	10.89	1185.96	<0.0001	1	4.25	180.63	0.0001
B_3	1	1.64	26.80	<0.0001	1	2.43	59.04	0.0053
Quadratic								
B_{11}	1	-2.70	20.12	0.0001	1	-1.27	4.47	0.3526
B_{22}	1	4.37	52.49	<0.0001	1	-2.92	23.41	0.0497
B_{33}	1	1.77	8.64	0.0023	1	-3.17	27.67	0.0357
Interaction								
B_{12}	1	0.090	0.064	0.7331	1	-2.84	64.55	0.0041
B_{13}	1	0.031	7.750E-003	0.9055	1	-2.41	46.33	0.0105
B_{23}	1	-1.56	19.59	0.0001	1	-0/13	0.13	0.8721
Residual	10		5.22		10		47.03	
Lack of fit	5		4.35	0.0519	5		36	0.1099
Pure error	5		0.88		5		11.02	
Total	19		1479.16		19		1417.28	
R^2		0.9965				0.9668		
Adj-R^2		0.9933				0.9370		
CV		0.93				2.46		

Fig. 1. Comparison between predicted and actual values of different culture condition and different culture media

3.2 The Effect of Independent Variables on the Response

3.2.1 Effect of culture conditions on the response variable

The variation of mannan content with temperature and pH at constant inoculation size [3] is presented in Fig. 2(A). The microorganism metabolism, individual growth and breeding could be affected by medium temperature. If the temperature is retained comparably to the growth of S. cerevisiae, then the fermentation process could remain stabilized and the fermentation periods could be shortened effectively. It can be seen that the mannan production was affected significantly by temperature ($p< 0.05$); As the temperature increases the mannan content linearly increases, the maximum mannan production (95.447±8.8 mg/100 ml) was obtained at 32°C. These results are in accordance with [11] that mannan production could be affected by medium temperature. Increasing in mannan content of yeast cell during increasing pH is less severe, given the significant quadratic effect of temperature and pH, curvature on the surface and contour can be expected. Fig. 2(B) shows the interaction between temperature and inoculation size. From the plot it can be seen the specific effect of temperature on yeast growth followed by greater mannan content in the cell wall, while No significant changes in the mannan production were observed between the different inoculums size and Fig2(C) shows the simultaneous effect of PH and inoculation size it is clear that inoculation size had much less effect on mannan content than PH and Temperature due to inappreciable slope. Hong Zhi et al. [11] reported similar trends while growing *S. cerevisiae* for mannan extraction

The order for the effect of culture condition factors is:

temperature> initial pH > inoculation size

3.2.2 effects of culture medium on the response variable

The variation of mannan content with carbon source & nitrogen source at constant enzyme activator (2.50) is illustrated in Fig. 3.(A), Carbon sources are important to tally multiplication, and the growth of microorganisms could be influenced greatly by the kind and quantity of carbon sources. Past results have shown that the cell wall and cytoplasmic membrane could

be changed significantly depending on the carbon sources [18,19]. The results showed that the yield of mannan could be affected significantly by the concentration of carbon sources (p<0.05), It was found that the highest mannan produced was 96.262 with Fos at 9 mg/100mlit, besides, cultivation on the different carbon source (glucose and Fos) shows that the maximum mannan production of 98.123 mgr/100mlit was during usage of Fos, that represent easier and faster breakdown of it by S.crevisiae, which is in accordane with Hong Zhi et al. [11], that reported a cell-associated activity is capable of hydrolysing froctoligosaccharide and it can be hydrolyzed fructose from the onset of the cultivation, thereby making it to be more easily utilized by S. cerevisiae. By comparing the highest mannan production to the nitrogen sources at different concentrations, it was observed increasing concentrations of nitrogen sources used (mixture of peptone and yeast extract with 2:1 ratio) has also a significant effect on the growth of the yeast cell wall followed by better Mannan content.

Yeast mannan synthetase is a multi enzyme complex, probably constituted by a minimum of 10 different mannosyl transferases. The *S. cerevisiae* mating pheromone α factor induces the activation of yeast mannan synthetase both in vivo and in vitro. Mannan synthetase is usually obtained by lysis of yeast protoplasts; it is closely associated with the plasmalemma. Those located inside cytoplasm, as well as associated with plasmalemma are the principal sites for mannan biosynthesis [20].

Enzyme can be activated by inorganic ions, small molecular, and macromolecules [21]. The enzyme activation on mannan production was studied by adding and glycerol to the medium Fig.3 (B) and the results showed that the highest mannan production was achieved up to 95.675mg/100 mL at a glycerol concentration of 2.5%, which was. Many microorganisms were able to utilize glycerol as sole energy during aerobic growth. In S. cerevisiae, glycerol degradation occured via a two stepa glycerol phosphorylative pathway, in the first step, glycerol is converted to glycerol-3-phosphate by cytosolic glycerol kinase (Gut 1p). The Glycerol-3-phosphate then passed the outer mitochondrial membrane and was oxidized to dihydroxyacetone phosphate by the inner mitochondrial membrane enzyme, FAD-dependent glycerol-3-phosphate dehydrogenase (Gut 2p). Finally dihydroxyacetone phosphate entered the cytosol, where it is used either in the glycolytic or in the gluconeogenic pathway [22], it can be seen that increasing glycerol is less effective in increasing cell wall mannan, and the significant quadratic effect of enzyme activator leads to curvature of the surface and contour graphs, increasing glycerol to higher amount (up to 3%) associated with a small reduction in tha amount of mannan obtained that could be due to decomposition of side braches of mannan in glycerol reactions. Fig.3 (C) shows the simultaneous effect of carbon source and enzyme activator on mannan production that indicate significant effect of carbon source and partial effects of enzyme activator source. The order for the effects of culture medium can be categorized as follow:

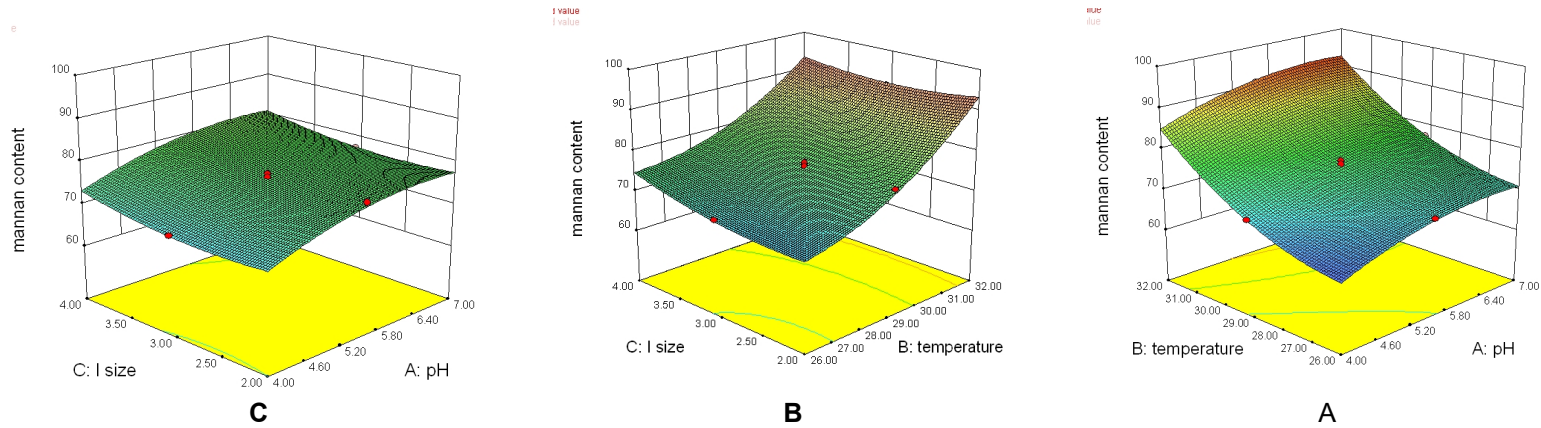Carbon source› nitrogen source› enzyme activator

Fig. 2. Simultaneous effects of A: PH and temperature, B: inoculation size and temperature, C: inoculation size and PH
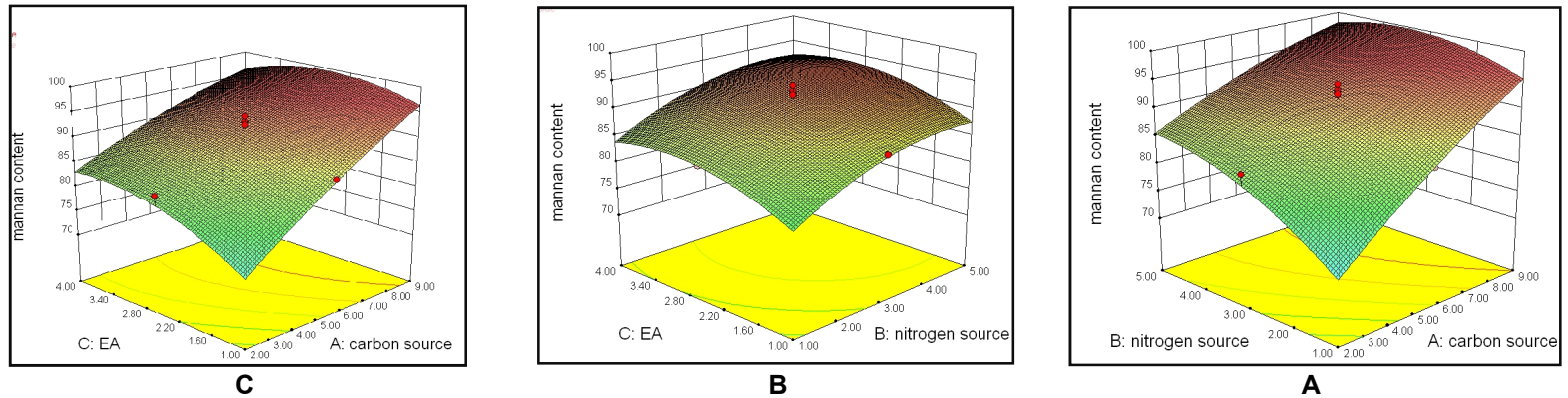

Fig. 3. Simultaneous effects of A: carbon and nitrogen sources, B: nitrogen source and enzyme activator, C: carbon source and enzyme activator

3.3 Optimization

The optimum condition for maximum mannan content of yeast cell wall is tabulated in Table 7, that provides the highest value of 94.912 & 96.4117 mgr/100mlit mannan in optimizing culture condition & culture medium respectively. The range of optimum cultivation condition was determined by superimposing the contour plots of the response. Fig. 4(A) & (B) presents the overlaying contour plots for the response which was evaluated as a fumction of different culture condition & different culture conditions respectively. These plots illustrate the determination of the best combination factors for maximum mannan content of S.cerevisaie cell wall. After the influence factors were determined, response surface method was used to optimize the culture conditions & medium for enhancing the mannan production using the quadratic rotary combination design [23.24]. The independent and dependent variables were analyzed to get regression equation, which is an empirical relationship between the yield of mannan and the test variable in coded units, which could predict the response under the given range. The regression equations obtained for the mannan production are as follows:

Table 7. Predicted optimum condition for culturing *S. cerevisiae*

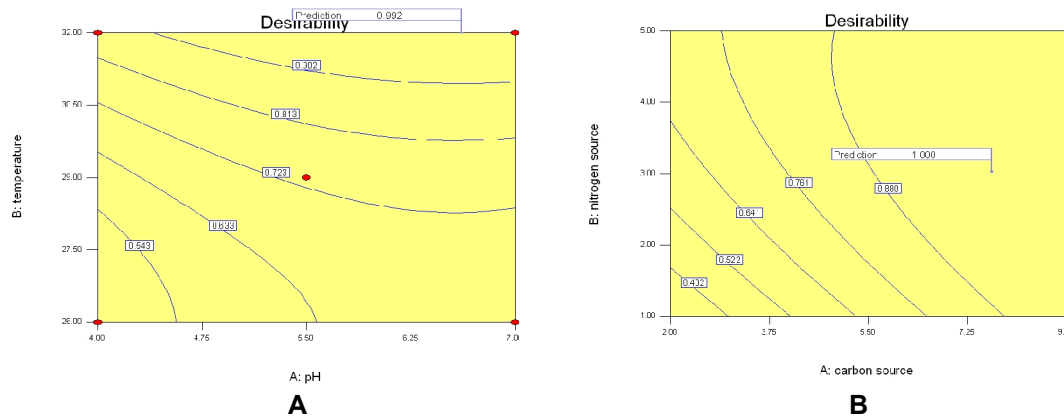| Factor	Low	High	Optimum
PH	4	7	6.62
Temperature	26	32	32
Inoculation size	2	4	4
Carbon source	2	9	8.99
Nitrogen source	1	5	4.25
Enzyme activator	1	4	3

Fig. 4. The optimum region by overlaying contour plots of the response evaluated as a function of A: different culture condition, B:different culture media

Regression equation for culture condition:

$$y = 76.36 + 3.83x_1 + 10.89x_2 + 1.64x_3 - 2.70x_{11} + 4.37x_{22} + 1.77x_{33} + 0.09x_{12} + 0.031x_{13} - 1.56x_{23}$$

Regression equation for culture medium:

$$y = 91.76 + 9.04x_1 + 4.25x_2 + 2.43x_3 - 1.27x_{11} - 2.92x_{22} - 3.17x_{33} - 2.84x_{12} - 2.41x_{13} - 0.13x_{23}$$

Each of the observed value was compared with the predicted value, which was calculated from the model, as depicted in Table 8.

To validate the practicability and veracity of the equation, the experiment was run at optimum conditions within the experimental range obtained from the above study. Mannan productions obtained at the optimum level were 92.128 and 95.234 mg/100mL for optimum culture condition and culture media respectively. This is in agreement with the calculated ones.

Table 8. Comparison of actual values and predicted values

Run	Evaluating Culture Condition		Evaluating Culture Media	
	Actual value	**Predicted value**	**Actual value**	**Predicted Value**
1	61.31	61.99	62.23	63.30
2	69.35	69.42	92.27	91.87
3	86.82	86.72	77.14	77.74
4	94.18	94.51	95.77	94.94
5	68.72	68.34	72.88	73.23
6	75.84	75.89	93.24	92.17
7	86.93	86.81	87.23	87.16
8	95.45	94.72	96.28	94.73
9	69.90	69.82	83.39	81.45
10	77.22	77.49	95.67	99.52
11	70.26	69.84	84.54	84.5
12	91.01	91.62	91.24	93.09
13	77.49	76.50	86.59	86.15
14	77.58	79.77	88.68	91.01
15	77.26	76.36	93.35	91.76
16	76.40	76.36	92.55	91.76
17	76.36	76.36	90.88	91.76
18	76.21	76.36	94.24	91.76
19	76.17	76.36	92.96	91.76
20	76.18	76.36	90.38	91.76

4. CONCLUSION

The optimization of cultural medium and culture condition are critical processes to improve the mannan production of *Saccharomycess cerevisiae ptcc5209*. On the basis of the single factor experiment, the carbon and nitrogen sources and enzyme activator were determined, and then the mathematical model was established by the quadratic rotary combination design, through response surface analysis. Mannan production was influenced significantly by culture medium optimization; Fos, bactopeptone, yeast extract, and glycerol had high significance on correlation of coefficients with the low p-values of < 0.05. The order of the effect was carbon source > nitrogen source > enzyme activator. The influence of original pH, inoculums size, and temperature on mannan production was evaluated and the influence

factors were confirmed by quadratic rotary design, temperature > initial pH > inoculums size. It is evident that the production of mannan was affected significantly by medium optimization and culture condition optimization ($p < 0.01$).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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