

Statistical Modeling of β -Galactosidase Production from Novel Yeast Isolate Using Cheese Whey

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Whey, a liquid component remaining after milk fat and casein have been separated from whole milk, is one of the major disposal problems of the dairy industries. To overcome this problem, biotechnologically a better alternative is subjecting the whey to processes through which the value added products can be formed. In the present investigation, Response Surface Methodology (RSM) was applied to optimize the different process parameters for β -galactosidase production from whey based medium using novel yeast isolate. The concentration of yeast extract, urea, pH, incubation time and temperature has shown significant effect on the production of the β -galactosidase. The maximum enzyme activity of 1.73 IU/ mg dry weight was observed under the optimized fermentation conditions. Thus, the processing of whey into useful products not only results in reduction of environmental pollution but also helps in the preparation of valuable products in a very cost effective manner.

Keywords: Yeast; β -Galactosidase, Whey; Optimization, Response Surface Methodology

Introduction

Whey, by-product of dairy industry is the richest source of lactose, protein, and other nutrients. Due to this, the disposal of whey causes major threat to environment. To overcome this problem, a better alternative is subjecting the whey for the production of value added products like β -galactosidase, which can also reduce the cost of production¹. The enzyme β -galactosidase can be produced by different micro-organisms such as bacteria, fungi and yeast². It has large number of applications in food and pharmaceutical sectors. It can be used to avoid the problems of lactose intolerance among the individuals having deficiency of enzyme³. Besides this, it can also be used for the production of prebiotics such as lactulose and galacto-oligosaccharides⁴⁻⁶. The optimization of various parameters by using conventional method does not depict the interactive effect of the various factors on the enzyme activity⁷. In order to overcome this problem, response surface methodology (RSM) can be applied. Keeping the above in view, the present study was performed to formulate whey based medium and to optimize the different process variables for maximum β -galactosidase production using RSM.

Materials and methods

Materials

All the reagents used in the experiments were purchased from HiMedia Laboratories Pvt. Limited, Mumbai, India.

Isolation, screening and identification of β -galactosidase producing yeast

Whey samples were collected from different states of India like Punjab, Haryana, Madhya Pradesh, and Bihar. Isolation of yeast strain has been carried out by following the method of Nahvi and Moeini⁸ with slight modification. The screening of the isolated yeast strains was carried on the basis of their β -galactosidase activity following the method of Miller⁹. Further, the isolate with maximum enzyme activity was identified using 18 S rDNA gene analysis that revealed its phylogenetic relatedness to *Kluyveromyces* sp. from Merck Specialities Private Limited GeNeiTM, Bangalore, India.

Fermentation of yeast cells for β -galactosidase production

The isolated yeast cells were inoculated into the whey based media containing (% w/v) yeast extract (0.3), magnesium sulphate (0.05), urea (0.1) and incubated at pH 5.5, temperature 30 °C for 28 h¹⁰.

Experimental design and statistical analysis

Experiments were conducted according to Central Composite Rotatable Design (CCRD),

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generated by Design Expert, Trial version 6.0 statistical software (Stat-Ease INC., Minneapolis, MN, USA) with five variables at five levels each. The levels of experimental variables and the experimental plan in un-coded form of process variables along with results are presented in Table 1 and Table 2, respectively. The second order polynomial equation (Eq. 1) was fitted to the experimental data of each dependent variable as given below

$$Y_i = \beta_0 + \sum_{i=1}^n \beta_i x_i + \sum_{i=1}^{n-1} \sum_{j=i+1}^n \beta_{ij} x_i x_j + \sum_{i=1}^n \beta_{ii} x_i^2 \quad \dots (1)$$

Where Y_i = Response $\{Y_1$ = Enzyme activity (IU/mg DW) $\}$, x_i = Independent variables (x_1 = yeast extract concentration, x_2 = urea concentration, x_3 = pH, x_4 = Treatment time (min), x_5 = Temperature ($^{\circ}$ C), β_0 is the value of coefficient of fitted response at the central point of design, β_i , β_{ij} , β_{ii} are the linear, quadratic and cross product regression coefficients, respectively.

Estimation of biomass

A known amount of broth containing yeast culture was centrifuged (5000 rpm $\times$ 10 min at 4 $^{\circ}$ C) and washed twice with distilled water. The cell suspension was filtered through the Whatman filter paper. The biomass was dried in oven at 80 $^{\circ}$ C, till a constant weight was obtained.

Table 1 — Levels of different process variables for β -galactosidase production

Factor	Process parameter	Levels				
		-2.378	-1	0	+1	+2.378
X_1	Yeast extract (% w/v)	0.06	0.2	0.3	0.4	0.54
X_2	Urea (% w/v)	0.03	0.07	0.1	0.13	0.17
X_3	pH	4.3	5.0	5.5	6.0	6.69
X_4	Temperature ($^{\circ}$ C)	18.11	25	30	35	41.89
X_5	Incubation time (h)	18.49	24	28	32	37.51

Table 2 — Experimental design of process variables and values of experimental data for optimization of enzyme activity

Yeast extract Conc. (% w/v)	Urea Conc. (% w/v)	pH	Incubation temp. ($^{\circ}$ C)	Incubation Time (h)	Enzyme activity (IU/ mg DW*)
0.2	0.07	5	25	24	1.301
0.4	0.07	5	25	24	1.461
0.2	0.13	5	25	24	1.332
0.4	0.13	5	25	24	1.521
0.2	0.07	6	25	24	1.265
0.4	0.07	6	25	24	1.386
0.2	0.13	6	25	24	1.314
0.4	0.13	6	25	24	1.498
0.2	0.07	5	35	24	1.252
0.4	0.07	5	35	24	1.453
0.2	0.13	5	35	24	1.301
0.4	0.13	5	35	24	1.444
0.2	0.07	6	35	24	1.201
0.4	0.07	6	35	24	1.348
0.2	0.13	6	35	24	1.321
0.4	0.13	6	35	24	1.439
0.2	0.07	5	25	32	1.321
0.4	0.07	5	25	32	1.526
0.2	0.13	5	25	32	1.342
0.4	0.13	5	25	32	1.586
0.2	0.07	6	25	32	1.281
0.4	0.07	6	25	32	1.481
0.2	0.13	6	25	32	1.321
0.4	0.13	6	25	32	1.54
0.2	0.07	5	35	32	1.287
0.4	0.07	5	35	32	1.489
0.2	0.13	5	35	32	1.321

(Contd.)

Table 2 — Experimental design of process variables and values of experimental data for optimization of enzyme activity (*Contd.*)

Yeast extract Conc. (% w/v)	Urea Conc. (%, w/v)	pH	Incubation temp. (°C)	Incubation Time (h)	Enzyme activity (IU/ mg DW*)
0.4	0.13	5	35	32	1.516
0.2	0.07	6	35	32	1.254
0.4	0.07	6	35	32	1.421
0.2	0.13	6	35	32	1.246
0.4	0.13	6	35	32	1.501
0.062	0.1	5.5	30	28	1.229
0.537	0.1	5.5	30	28	1.701
0.3	0.028	5.5	30	28	1.324
0.3	0.171	5.5	30	28	1.472
0.3	0.1	4.31	30	28	1.543
0.3	0.1	6.69	30	28	1.524
0.3	0.1	5.5	18.10	28	1.218
0.3	0.1	5.5	41.89	28	1.084
0.3	0.1	5.5	30	18.48	1.475
0.3	0.1	5.5	30	37.51	1.608
0.3	0.1	5.5	30	28	1.701
0.3	0.1	5.5	30	28	1.712
0.3	0.1	5.5	30	28	1.695
0.3	0.1	5.5	30	28	1.687
0.3	0.1	5.5	30	28	1.698
0.3	0.1	5.5	30	28	1.708
0.3	0.1	5.5	30	28	1.635
0.3	0.1	5.5	30	28	1.727

*DW (Dry weight)

Results and Discussion

The different process parameters were optimized to achieve maximum β -galactosidase production using CCRD.

Diagnostic checking of fitted model and surface plots

The result of analysis of variance (ANOVA) of quadratic model for β -galactosidase activity is indicated in Table 3. The model *F*-value (Fisher variation ratio) of 32.98 implies that the model is significant. There is only a 0.01% chance that this large model *F*-value could occur by chance and only 4.21% of the total variation was not explained by the model ($R^2 = 95.79\%$). The relative magnitude of β -coefficients revealed that the linear term of yeast extract had maximum positive effect followed by urea concentration and incubation time. The interactive terms 'yeast extract concentration and incubation time' has the maximum positive effect followed by 'urea concentration and pH', 'yeast extract and urea concentration' on β -galactosidase production (Figure 1-2). The effect of yeast extract concentration and incubation time on β -galactosidase production depicts that at low level of incubation time, enzyme activity increased with the increase in yeast extract

concentration, it might be due to increase in biomass (Figure 1). However, β -galactosidase activity decreased with an increase in incubation time, since the cells may have reached the stationary phase. The maximum β -galactosidase activity were observed at 0.36-0.38 (% w/v) and 26-28 h, respectively. The optimal production of β -galactosidase enzyme from *K. marxianus* reached after 30-40 h¹¹. The effect of urea concentration and pH on β -galactosidase production (Figure. 2) indicates an increase in enzyme activity with increase of urea concentration upto a range of 0.09-0.12 (% w/v), after which the production decreased, which might be due to increase in pH that affect the functioning of enzyme or transport of nutrients into the cell. The maximum enzyme activity was observed at pH range 5.3-5.5 and incubation temperature of 29-31 °C. An optimal pH of 5 has been observed for β -galactosidase production using *Kluyveromyces marxianus* DIV13-247¹². However, a pH range from 5.2 to 5.5 was found to be best for β -galactosidase production using *Kluyveromyces lactis*¹³. The equation (Eq. 2) of the model fitted for β -galactosidase production in the actual form of process variables after eliminating the non significant terms is:

Table 3 — Regression summary and ANOVA table for enzyme activity for uncoded value of process variable

Source	β -value	Sum of Squares	DF	Mean Square	F- value	Prob. > F
Model	1.687	1.272	1	0.064	32.976	< 0.0001
* Yeast extract	0.094	0.383	1	0.383	198.51	< 0.0001
* Urea	0.027	0.031	1	0.0315	16.327	0.0004
* pH	-0.016	0.010	1	0.0107	5.553	0.0254
* Temp	-0.023	0.023	1	0.0231	11.98	0.0017
* Time	0.021	0.019	1	0.0192	9.962	0.0037
* Yeast extract ²	-0.048	0.131	1	0.131	67.915	< 0.0001
* Urea ²	-0.060	0.203	1	0.203	105.09	< 0.0001
* pH ²	-0.036	0.074	1	0.074	38.26	< 0.0001
*Temp ²	-0.104	0.602	1	0.602	311.95	< 0.0001
* Time ²	-0.035	0.068	1	0.068	35.352	< 0.0001
* Yeast extract * urea	0.0045	0.0006	1	0.0006	0.336	0.5667
* Yeast extract * pH	-0.004	0.0005	1	0.0005	0.265	0.6103
Yeast extract temp	-0.003	0.0003	1	0.0003	0.143	0.7079
* Yeast extract * time	0.013	0.0056	1	0.0056	2.912	0.0986
* Urea * pH	0.008	0.002	1	0.0023	1.181	0.2861
* Urea * temp	-0.001	7.2 x10 ⁻⁵	1	7.2 x10 ⁻⁵	0.037	0.8482
* Urea * time	-0.006	0.00113	1	0.00113	0.585	0.4506
* pH * temp	-0.0008	2.45 x10 ⁻⁵	1	2.45 x10 ⁻⁵	0.0127	0.911
* pH * time	-0.0015	7.81 x10 ⁻⁵	1	7.81 x10 ⁻⁵	0.0405	0.8419
* Temp * time	-0.0014	6.05 x10 ⁻⁵	1	6.05 x10 ⁻⁵	0.031	0.8607
Residual		0.0559	29	0.0019	32.98	< 0.0001
Lack of Fit		0.0507	22	0.0023	198.51	< 0.0001
Pure Error		0.0052	7	0.0007	16.327	0.0004
Cor Total		1.328	49	0.0636	5.553	0.0254
R-Squared		0.958				
Adj R-Squared		0.929				

* Non-significant at 5% level.

[Abbreviation - β -value: β -coefficient value; DF: degrees of freedom; F- value: Fisher variation ratio; R-Squared: correlation coefficient]

$$\text{Enzyme activity (IU/ mgDW)} = -9.45866 + 3.39233X_1 + 12.46264 X_2 + 1.57230 X_3 + 0.25174 X_4 + 0.12925X_5 - 4.85554X_1^2 - 67.11050X_2^2 - 0.14578X_3^2 - 4.16253x10^{-3} X_4^2 - 2.18950x10^{-3}X_5^2 \dots 2$$

Optimization of β -galactosidase production

In order to optimize the process conditions for β -galactosidase production, numerical optimization technique has been used. The optimum operating conditions for β -galactosidase production process to achieve maximum enzyme activity were yeast extract concentration 0.37 (% w/v), urea concentration 0.11 (% w/v), pH 5.36, temperature 30 °C, incubation time 27 h. At these conditions, the predicted value of enzyme activity was found to be 1.73 IU/ mg dry weight, which was confirmed by conducting the experiments in triplicate and $\pm 0.2\%$ deviation in the enzyme activity values was obtained.

Effect of Incubation Time on Biomass Density and β -Galactosidase Production

The incubation time for the production of β -galactosidase and the biomass density under the

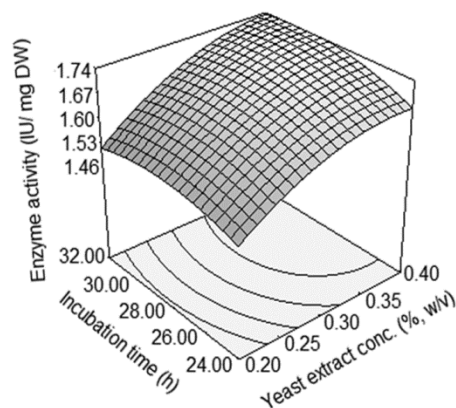


Fig.1 — Effect of yeast extract concentration and incubation time on enzyme activity

above optimized process conditions were examined for the period of 35 h (Figure. 3). The maximum biomass density (4.21 ± 0.23 g dry weight/ L) and β -galactosidase activity (1.73 ± 0.021 IU/ mg dry weight) was achieved after 27 h of incubation, after that no significant increase was observed, which may be due

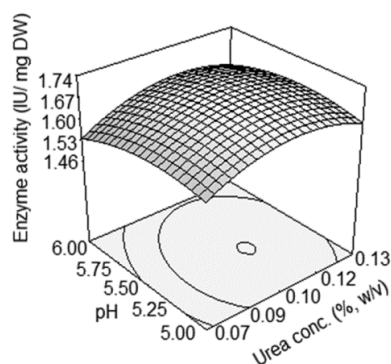


Fig. 2 — Effect of urea concentration and pH on enzyme activity

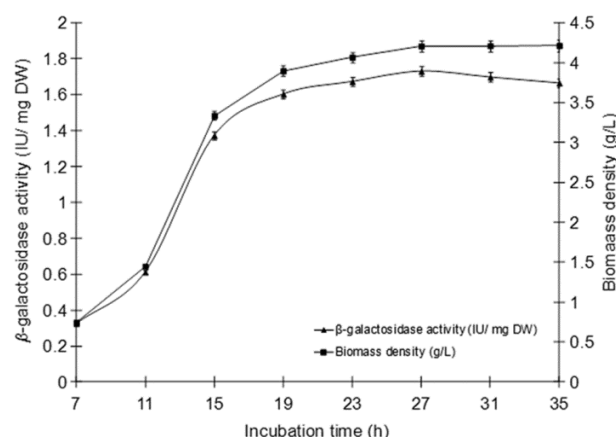


Fig. 3 — Effect of incubation time on β -galactosidase production and biomass density

to the depletion in the nutrients or alteration in the pH of the media¹⁴. From the result, it was found that β -galactosidase produced by *Kluyveromyces* sp. is an example of growth-associated product¹⁵.

Conclusions

Response surface methodology proved to be useful tool in developing optimum conditions for β -galactosidase production from whey. The optimum process parameters for the maximum β -galactosidase production (1.73 IU/ mg dry weight) were yeast extract concentration 0.37 % (w/v), magnesium sulphate 0.05 % (w/v), urea concentration 0.11 % (w/v), pH 5.36, temperature 30 °C, incubation time 27 h using whey. The use of whey for β -galactosidase production can not only improve the economics of the process, but also overcome the problems associated with its disposal.

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