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OPTIMIZATION OF INULINASE PRODUCTION USING COPRA WASTE BY *Kluyveromyces marxianus* var. *marxianus*

Kluyveromyces marxianus var. marxianus was found to secrete a large amount of extracellular inulinase in to the medium. The optimization of inulinase production using copra waste as a carbon source was performed with statistical methodology based on experimental designs. The screening of eighteen nutrients for their influence on inulinase production was achieved using a Plackett-Burman design. Corn steep liquor, $(NH_4)_2SO_4$, $ZnSO_4{\cdot}7H_2O$, K_2HPO_4 and urea were selected based on their positive influence on inulinase production. The selected components were optimized using response surface methodology (RSM). The optimum conditions are: corn steep liquor - 0.0560 (g/gds), $(NH_4)_2SO_4$ - 0.0084 (g/gds), $ZnSO_4{\cdot}7H_2O$ - 0.0254 (g/gds), K_2HPO_4 - 0.0037 (g/gds) and urea - 0.02147 (g/gds). These conditions were validated experimentally which revealed an enhanced inulinase yield of 372 U/gds.

Key words: inulinase; copra waste; K. marxianus var. marxianus; optimization; RSM.

Inulin is a fructan stored in certain higher plants such as *Helianthus tuberosus*, *Chicorium intybus* and *Dahlia pinnata*. It is a polymer of β-D-fructofuranose residues linked by 2 $\rightarrow$ 1 bonds with a terminal glucose unit and can be hydrolyzed into fructose and oligo-fructose by inulinase that can be used as sweeteners and functional food additives [1,2]. It is not only an alternative dietary fiber, but also a cheap and renewable carbon source for some microorganisms. Those microorganisms secrete inulinase, which targets on β-2,1 linkage of inulin and degrades it into fructose and a small amount of glucose [3]. The production of high-fructose syrup from inulin has been widely investigated [4,5]. Recently it has been reported that inulin could be an efficient source for the production of inulo-oligosaccharides [6]. This polymer is a recognized source for the production of either ultra-high fructose syrups, with D-fructose content over 95%, or for production of oligofructose syrups [7]. Fructose is generally accepted as a safe sweetener, sweeter than sucrose, with lower cost, and has functional properties that enhance flavor, color, and product stability, and is thus widely used in many foods and beverages instead of sucrose [8]. Inulin can be hydrolyzed by a chemical approach. However, the chemical approach has many drawbacks [9,10]. Fructose can also be produced from starch by enzymatic methods involving α-amylase, amyloglucosidase and glucose isomerase, resulting in the production of a mixture consisting of oligosaccharides (8%), fructose (42%) and glucose (50%) [1]. However, separation of fructose from this fructose syrup is costly and thus makes this method uneconomical [9]. The best procedure involves the use of microbial inulinases have been described in fungi (*e.g.*, *Penicillium* sp. [11], *Aspergillus niger* [12] and *A. ficuum* [13]), yeast (*e.g.*, *Kluyveromyces marxianus* [14] and *Candida kefyr* [15]) and bacteria (*e.g.*, *Bacillus circulans* [16], *Clostridium acetobutylicum* [17] and *Pseudomonas* sp. [18]).

Among the yeasts, which can produce inulinases, such as strains of *Candida* sp., *Sporotrichum* sp., *Pichia* sp., and *Kluyveromyces* species of *fragilis* and *marxianus* have high potential for producing commercially acceptable yields of the enzyme [19]. The demand of fructose is ever increasing due to its beneficial role for diabetics, increased iron absorption in children, increased ethanol removal from blood of highly intoxicated person [20], apart from being used as a low calorie sweetener [1]. There are some studies on the production of inulinase by solid-state fermentation using *Kluyveromyces marxianus* [19-22]. In those

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studies various carbon sources like wheat brawn, rice bran and sugarcane bagasse were used. Hence the aim of the present work is an exploration of the possibility for an increase of inulinase production using copra waste as a carbon source.

MATERIALS AND METHODS

Yeast strain

Yeast strain used in this work is well preserved in the laboratory. Yeast strain *Kluyveromyces marxianus var. marxianus* MTCC-188 was a stock of the Microbial Type Culture collection Centre (MTCC), Chandigarh, India. The strain was maintained on solid medium at 5 °C. The medium composition (g/l) comprised the following: yeast extract, 3.0; peptone, 10.0; dextrose, 20.0; and agar, 15.0. Cells were harvested from slants and used to inoculate liquid medium.

Solid-state fermentation

Commercial quality copra waste (coconut oil cake) was procured from the local market and used as a substrate for inulinase production. The composition of the copra waste is given in Table 1. Fermentation was carried out in Erlenmeyer flasks (250 ml) with 10 g of copra waste, supplemented with nutrients concentrations defined by the experimental design. Each flask was covered with hydrophobic cotton and autoclaved at 121 °C for 20 min. After cooling, each flask was inoculated with 2 ml of the suspension previously prepared and incubated for 72 h in a chamber with temperature and humidity control.

Table 1. Composition of copra waste

Component	Content, mass%
Dry matter	85.5
Crude protein	15.1
Crude fat	3.5
Crude fiber	12.2
Ash	5.1
Total sugar	4.38
Minerals	0.88

During the preliminary screening process, the experiments were carried out for 5 days and it was found that at the 72 h, the maximum production occurs. Hence experiments are carried out for 72 h.

Extraction of inulinase

After fermentation, the whole sample of the fermented matter was transferred to a 250 mL conical flask. 10 volumes of distilled water were added and the contents were agitated for 30 min at 200 rpm (30 °C). Then the sample was centrifuged at 15000 rpm and the supernatant were analyzed by DNS method [23].

Optimization of inulinase production

RSM consists of a group of empirical techniques used for the evaluation of the relationship between clusters of controlled experimental factors and measured response. A prior knowledge with understanding of the related bioprocesses is necessary for a realistic modeling approach. To determine which variables significantly affect inulinase production by *K. marxianus var. marxianus*, the Plackett-Burman design was used. Eighteen variables (Table 2) were screened in 20 experimental runs (Table 3) and insignificant ones were eliminated in order to obtain a smaller, manageable set of factors. The low level (-1) and high level (+1) of each factor are listed in (Table 3). The statistical software package “Minitab 15”, was used for analyzing the experimental data.

Table 2. Nutrient screening using a Plackett-Burman design

Nutrient code	Nutrient	Level, g/10gds	
		Low (-1)	High (+1)
A	Yeast extract	0.01	0.05
B	Beef extract	0.05	0.15
C	$MnSO_4 \cdot 7H_2O$	0.1	0.5
D	K_2HPO_4	0.02	0.07
E	Soya bean cake	0.4	0.8
F	$MgSO_4 \cdot 7H_2O$	0.002	0.012
G	NH_4Cl	0.01	0.03
H	KCl	0.005	0.015
J	$(NH_4)_2HPO_4$	0.05	0.3
K	NH_4NO_3	0.05	0.1
L	$ZnSO_4 \cdot 7H_2O$	0.1	0.5
M	$(NH_4)_2SO_4$	0.06	0.1
N	Corn steep liquor	0.4	0.8
O	Peptone	0.05	0.15
P	Dextrose	0.1	0.3
Q	$FeSO_4 \cdot 7H_2O$	0.0005	0.002
R	KH_2PO_4	0.1	0.6
S	Urea	0.1	0.3

Once the critical factors were identified through the screening, the central composite design (CCD) was used to obtain a quadratic model, consisting of factorial trials and star points to estimate quadratic effects and central points to estimate the pure process variability with inulinase production as the response. Response surface methodology (RSM) was employed to optimize the five significant factors viz., corn steep liquor, $(NH_4)_2SO_4$, $ZnSO_4 \cdot 7H_2O$, K_2HPO_4 and urea

Table 3. Plackett-Burman experimental design matrix for screening of important variables for inulinase production

Run No.	A	B	C	D	E	F	G	H	J	K	L	M	N	O	P	Q	R	S	Inulinase, U/gds
1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	81.0
2	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	151.0
3	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	61.0
4	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	33.0
5	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	99.0
6	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	42.0
7	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	67.0
8	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	135.0
9	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	185.0
10	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	111.0
11	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	138.0
12	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	71.0
13	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	60.5
14	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	81.8
15	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	59.8
16	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	57.6
17	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	86.5
18	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	273.5
19	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	220.0
20	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	168.5

which enhances the inulinase production. The five independent variables were studied at five different levels (Table 4) and a set of 50 experiments were carried out (Table 5). The statistical software package "Design Expert 7.1.5" was used to analyze the experimental data. All variables were taken at a central coded value of zero. The minimum and maximum ranges of variables investigated are listed in Table 4. Upon the completion of experiments, the average maximum inulinase were taken as the response (Y). A multiple regression analysis of the data was carried out for obtaining an empirical model that relates the response measured to the independent variables. A second order polynomial equation is:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1,i<j}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j$$

where Y is the measured response, β_0 is the intercept term, β_i are linear coefficients, β_{ii} are quadratic coefficients, β_{ij} are interaction coefficients and X_i and X_j are coded independent variables. The optimal concentrations of the critical variables were obtained by analyzing contour plots. The statistical analysis of the model was represented in the form of analysis of variance (ANOVA).

Assay of enzyme activities

Enzymes were assayed by measuring the concentration of reducing sugars released from inulin or sucrose. The reaction mixture containing 1 ml of diluted crude enzyme and 4 ml of 2% inulin or 2% sucrose (dissolved in 0.1 M acetate buffer, pH 5.0) was incubated at 50 °C. After incubating for 30 min, aliquots of 0.5 ml were withdrawn and increase in reducing sugar was estimated by a 3,5-dinitrosalicylic acid method [23] using calibration curve obtained with

Table 4. Ranges of the independent variables used in RSM

Component	Code	Level, g/10gds				
		-2.38	-1	0	+1	+2.38
Corn steep liquor	X_1	0.4	0.5	0.6	0.7	0.8
$(NH_4)_2SO_4$	X_2	0.06	0.07	0.08	0.09	0.1
$ZnSO_4 \cdot 7H_2O$	X_3	0.1	0.2	0.3	0.4	0.5
K_2HPO_4	X_4	0.02	0.03	0.04	0.05	0.06
Urea	X_5	0.1	0.15	0.2	0.25	0.3

Table 5. Central composite design (CCD) of factors in coded levels with enzyme activity as response

Run No.	X_1	X_2	X_3	X_4	X_5	Inulinase, U/gds	
						Experimental	Predicted
1	-1	-1	1	1	-1	179.4	202.8
2	1	1	-1	1	-1	196.4	193.3
3	-1	1	1	1	-1	315.4	314.7
4	-1	1	1	-1	-1	326.8	304.7
5	0	0	0	0	0	341.8	341.8
6	-1	1	-1	-1	1	325.4	315.9
7	-1	-1	-1	1	1	330.5	343.5
8	-1	-1	-1	1	-1	326.8	320.8
9	1	1	1	-1	-1	345.7	312.8
10	0	0	0	0	0	341.8	341.8
11	0	0	0	0	0	341.7	341.8
12	0	0	0	0	0	341.6	341.8
13	1	-1	-1	1	-1	255.0	273.3
14	-1	-1	1	-1	1	209.7	204.4
15	-1	-1	1	1	1	208.0	212.9
16	0	0	2.38	0	0	294.7	284.0
17	1	1	-1	-1	-1	268.2	244.6
18	-2.38	0	0	0	0	298.4	290.1
19	1	1	-1	-1	1	281.4	273.3
20	1	1	-1	1	1	200.2	201.4
21	0	0	0	0	0	341.6	341.8
22	1	-1	1	-1	-1	222.9	211.7
23	0	2.38	0	0	0	266.3	290.2
24	-1	1	1	1	1	326.8	325.1
25	-1	1	1	-1	1	320.0	335.8
26	0	0	0	0	-2.38	177.0	172.9
27	-1	-1	-1	-1	-1	323.0	303.5
28	0	0	0	0	0	341.8	341.9
29	-1	-1	1	-1	-1	166.2	173.7
30	0	0	0	0	0	341.6	341.8
31	1	1	1	1	1	296.5	268.9
32	0	0	-2.38	0	0	345.6	358.3
33	0	-2.38	0	0	0	251.3	229.2
34	0	0	0	0	0	341.6	341.8
35	1	-1	-1	-1	-1	314.0	305.6
36	-1	1	-1	1	1	319.2	293.6
37	0	0	0	-2.38	0	270.1	294.6
38	1	-1	-1	-1	1	323.0	334.0
39	1	1	1	-1	1	302.6	328.9
40	0	0	0	2.38	0	266.3	243.6
41	2.38	0	0	0	0	215.3	225.5
42	-1	1	-1	-1	-1	270.0	272.4
43	1	-1	-1	1	1	296.5	281.1
44	1	-1	1	-1	1	222.9	227.5
45	1	1	1	1	-1	239.9	273.2
46	1	-1	1	1	-1	171.9	191.2
47	1	-1	1	1	1	198.3	186.4
48	-1	1	-1	1	-1	241.8	270.6
49	-1	-1	-1	-1	1	336.2	346.8
50	0	0	0	0	2.38	213.4	219.2

a standard solution of fructose [24]. Absorbance was read at 575 nm. A higher absorbance indicated a high level of reducing sugar produced and consequently, a high enzyme activity. One inulinase unit is the amount of enzyme which forms 1 μmol fructose per min.

Validation of the experimental model

The statistical model was validated with respect to inulinase production under the conditions predicted by the model in shake-flasks level. Samples were

drawn at desired intervals and inulinase activity was determined as described above.

Plate count of *K. marxianus* var. *marxianus*

Fermented matter was adequately mixed with 10 volumes of sterilized water (w/v, based on initial weight of the substrate). The mixture was aseptically diluted to suitable concentration by tenfold dilution. A 0.1 ml volume of diluted mixtures in different concentrations was spread on malt extract medium in quintuplicate. After incubation for 48 h at 30 °C, the number of CFU/g (1CFU = 1 colony-forming unit) was calculated according to the dilution factor and the number of colonies on the plates with 15-250 colonies.

RESULTS AND DISCUSSION

Plackett-Burman experiments (Table 3) showed a wide variation in inulinase activity. This variation reflected the importance of optimization to attain higher productivity. From the Pareto chart (Fig. 1) the variables viz., corn steep liquor, $(NH_4)_2SO_4$, $ZnSO_4{\cdot}7H_2O$, K_2HPO_4 and urea were selected for further optimization to attain a maximum response.

The levels of factors (corn steep liquor, $(NH_4)_2SO_4$, $ZnSO_4{\cdot}7H_2O$, K_2HPO_4 and urea) and the effect of their interactions on inulinase production were determined by central composite design of RSM. Fifty experiments were performed at different combinations of the factors shown in Table 4. The predicted and observed responses along with design matrix are presented in Table 5 and the results were analyzed by ANOVA. The second-order regression equation provided the levels of inulinase activity as the function of corn steep liquor, $(NH_4)_2SO_4$, $ZnSO_4{\cdot}7H_2O$, K_2HPO_4 and urea, which can be presented in terms of coded factors as in the following equation:

$$Y = 341.86 - 13.56X_1 + 12.18X_2 - 15.60X_3 - 10.72X_4 + 9.71X_5 - 14.84X_1^2 - 14.50X_2^2 - 3.65X_3^2 - 12.84X_4^2 - 25.74X_5^2 - 7.47X_1X_2 + 8.96X_1X_3 - 12.39X_1X_4 - 3.72X_1X_5 + 40.53X_2X_3 - 4.76X_2X_4 + 0.063X_2X_5 + 2.94X_3X_4 - 3.14X_3X_5 - 5.16X_4X_5$$

where Y is the inulinase activity (U/gds), X_1, X_2, X_3, X_4 and X_5 are corn steep liquor, $(NH_4)_2SO_4$, $ZnSO_4{\cdot}7H_2O$, K_2HPO_4 and urea, respectively. ANOVA for the response surface is shown in Table 6. The model F-value of 25.43 implies the model is significant. There is only a 0.01% chance that a "model F-value" this large could occur due to noise. Values of "Prob > F" less than 0.05 indicate model terms are significant. Values greater than 0.1 indicate the model terms are not significant. In the present work, all the linear, interactive effects of X_1X_2, X_1X_3, X_1X_4 and X_2X_3 and square effects of X_1, X_2, X_4 and X_5 were significant for inulinase production. The coefficient of determination (R^2) for inulinase activity was calculated as 0.9461, which is very close to 1 and can explain up to 94.61% variability of the response. The predicted R^2 value of 0.7919 was in reasonable agreement with the adjusted R^2 value of 0.9089. An adequate precision value greater than 4 is desirable. The adequate precision value of 16.55 indicates an adequate signal and suggests that the model can be used to navigate the design space.

The above model can be used to predict the inulinase production within the limits of the experimental factors. Figure 2 shows that the actual response values agree well with the predicted response values.

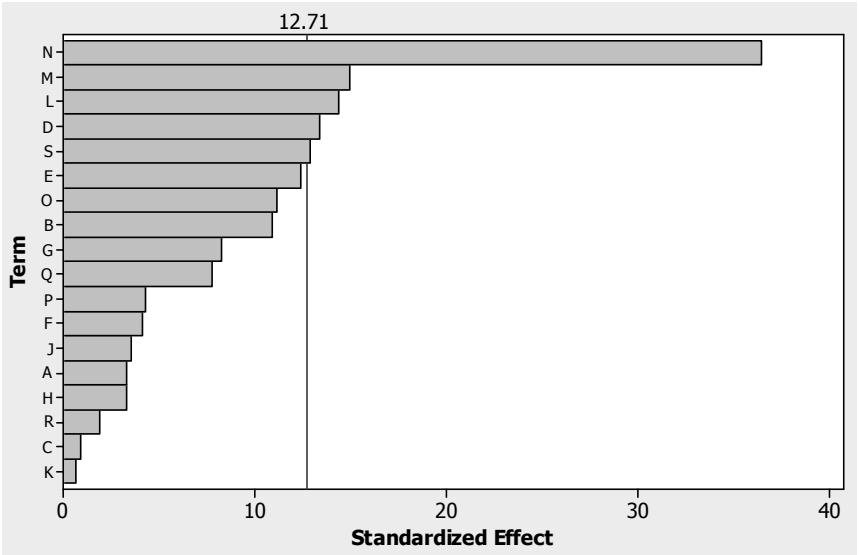

Figure 1. Pareto chart showing the effect of media components on inulinase activity.

Table 6. Analysis of variance (ANOVA) for response surface quadratic model for the production of inulinase

Source	Coefficient factor	Sum of squares	DF	F	P > F
Model	341.86	515E+05	20	25.43	< 0.0001
X_1	-13.56	7967	1	26.75	< 0.0001
X_2	12.18	6729	1	21.58	< 0.0001
X_3	-15.60	10540	1	35.39	< 0.0001
X_4	-10.72	4980	1	16.72	0.0003
X_5	9.71	4080	1	13.70	0.0009
X_1*X_2	-7.47	1788	1	6	0.0206
X_1*X_3	8.96	2567	1	8.62	0.0065
X_1*X_4	-12.39	4915	1	16.50	0.0003
X_1*X_5	-3.72	443	1	1.49	0.2327
X_2*X_3	40.53	52553	1	176.44	< 0.0001
X_2*X_4	-4.76	726	1	2.44	0.1294
X_2*X_5	0.063	0.13	1	4.2E-04	0.9838
X_3*X_4	2.94	277	1	0.93	0.3426
X_3*X_5	-3.14	316	1	1.06	0.3113
X_4*X_5	-5.16	851	1	2.86	0.1017
X_1*X_1	-14.84	12245	1	41.11	< 0.0001
X_2*X_2	-14.50	11683	1	39.22	< 0.0001
X_3*X_3	-3.65	742	1	2.49	0.1253
X_4*X_4	-12.84	9159	1	30.75	<0.0001
X_5*X_5	-25.74	36825	1	123.63	< 0.0001
Residual	-	8638	29	-	-
Lack of fit	-	8638	22	39976.53	<0.0001
Pure Error	-	0.069	7	-	-
Cor Total	-	1.602E+05	49	-	-

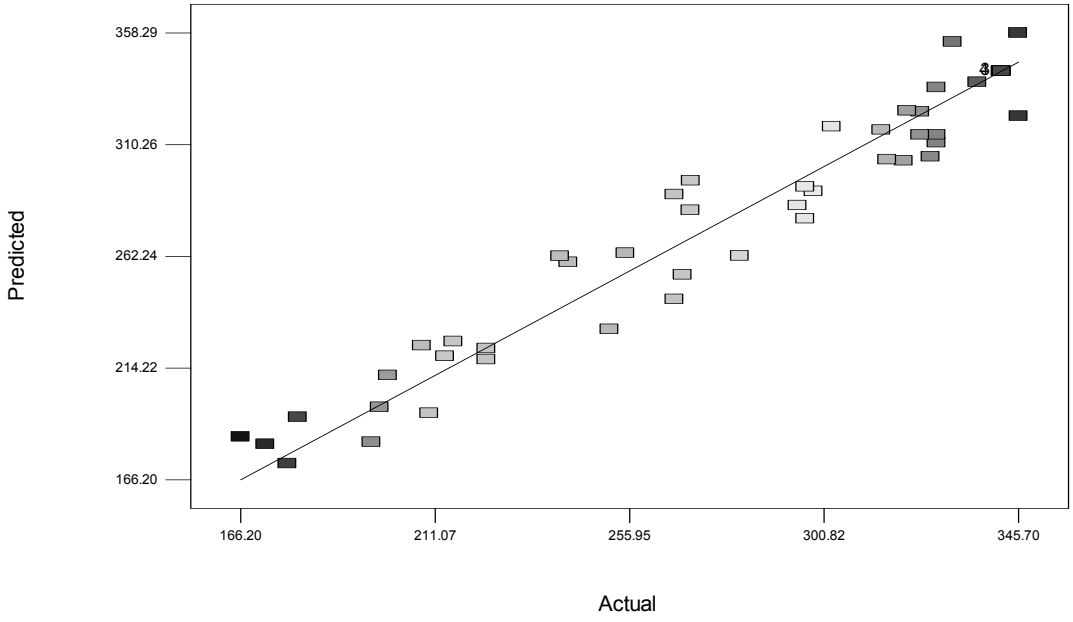

Figure 2. Predicted response versus actual value (Std. Dev.: -17.26; R^2 = 0.9461; Mean: 279.85; adj R^2: -0.9089; C.V.: -6.17%; pred. R^2: -0.7919; adeq. precision: -16.55.

The interaction effects of variables on inulinase production were studied by plotting 3D surface curves against any two independent variables, while keeping another variable at its central (0) level. The 3D curves of the calculated response (inulinase production) and contour plots from the interactions between the variables are shown in Figs. 3-12. Figure 3 shows the dependency of inulinase on corn steep liquor, $(NH_4)_2SO_4$.

The inulinase activity increased with increase in corn steep liquor to about 0.56 g/10gds and thereafter inulinase activity decreased with further increase in corn steep liquor. The same trend was observed in Figs. 4-6. Increase in $(NH_4)_2SO_4$ resulted increase in inulinase activity up to 0.085 g/10gds. This is evident from Figs. 3 and 7-9. Figures 4, 7, 10 and 11 show the dependency of inulinase activity on $ZnSO_4 \cdot 7H_2O$. Low levels of $ZnSO_4 \cdot 7H_2O$ show higher inulinase activity. The effect of K_2HPO_4 on inulinase observed was similar to corn steep liquor (Figs. 5, 8, 10 and 12). Inulinase activity increased with the increase in urea in the region from -0.5 to 0, and then decreased with further increase in urea. The optimal operation conditions of corn steep liquor, $(NH_4)_2SO_4$, $ZnSO_4 \cdot 7H_2O$, K_2HPO_4 and urea for maximum inulinase activity were determined by response surface analysis and also estimated by regression equation. The predicted results are shown in Table 5. The predicted values from the regression equation closely agreed with that obtained from experimental values.

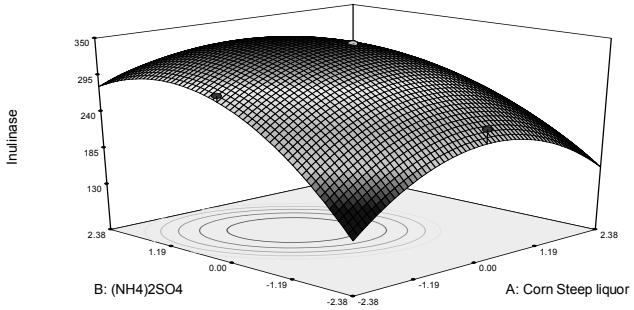

Figure 3. 3D Plot showing the effect of corn steep liquor and $(NH_4)_2SO_4$ on inulinase activity.

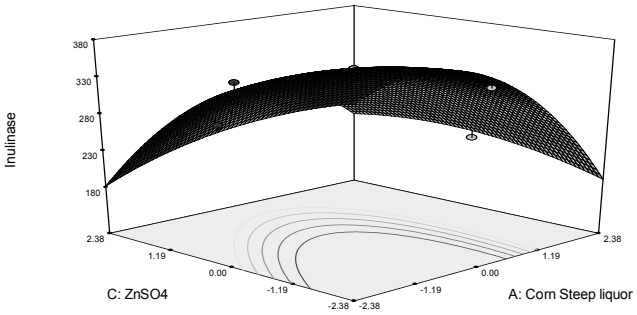

Figure 4. 3D Plot showing the effect of corn steep liquor and $ZnSO_4 \cdot 7H_2O$ on inulinase activity.

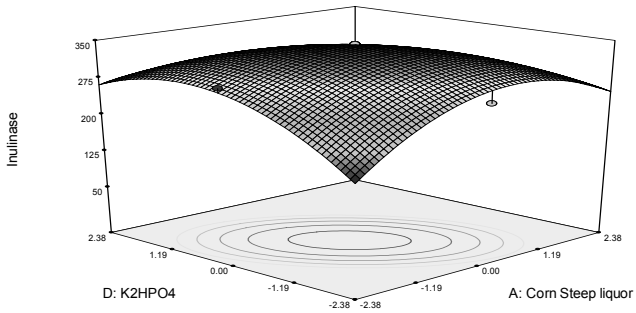

Figure 5. 3D Plot showing the effect of corn steep liquor and $K_2 HPO_4$ on inulinase activity.

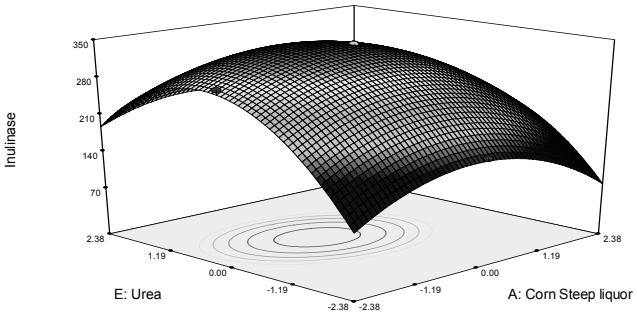

Figure 6. 3D Plot showing the effect of corn steep liquor and urea on inulinase activity.

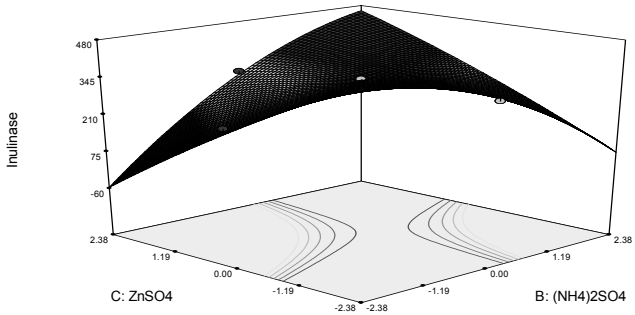

Figure 7. 3D Plot showing the effect of $(NH_4)_2SO_4$ and $ZnSO_4 \cdot 7H_2O$ on inulinase activity.

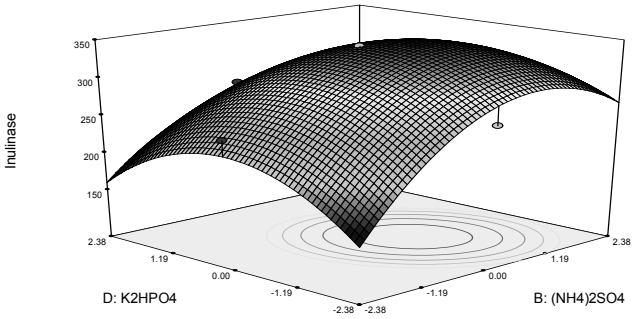

Figure 8. 3D Plot showing the effect of $(NH_4)_2SO_4$ and K_2HPO_4 on inulinase activity.

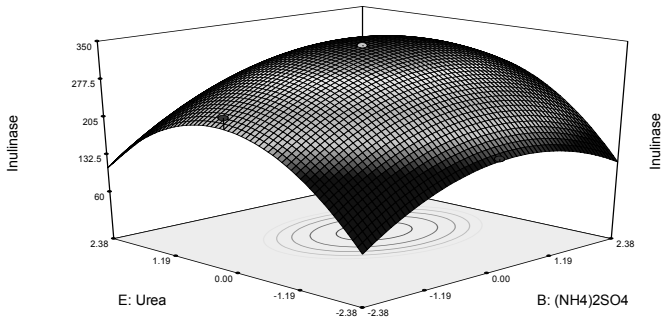

Figure 9. 3D Plot showing the effect of $(NH_4)_2SO_4$ and urea on inulinase activity.

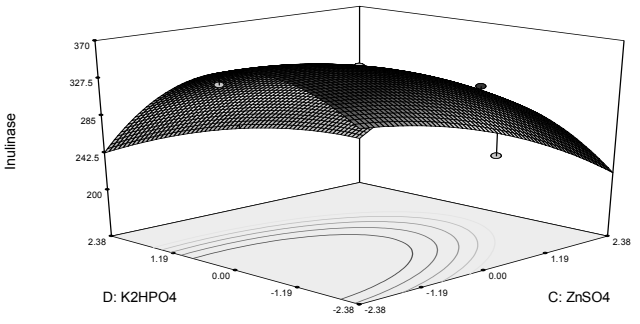

Figure 10. 3D Plot showing the effect of $ZnSO_4{\cdot}7H_2O$ and K_2HPO_4 on inulinase activity.

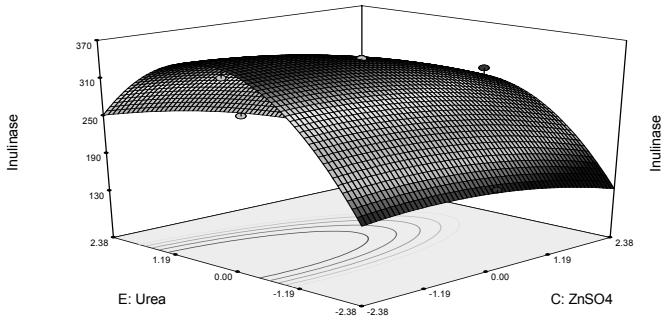

Figure 11. 3D Plot showing the effect of $ZnSO_4{\cdot}7H_2O$ and urea on inulinase activity.

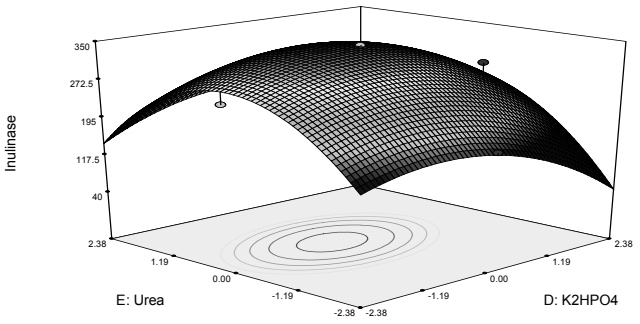

Figure 12. 3D Plot showing the effect of K_2HPO_4 and urea on inulinase activity.

Validation of the experimental model

Validation of the experimental model was tested by carrying out the batch experiment under optimal operation conditions (corn steep liquor – 0.0560 g/gds, $(NH_4)_2SO_4$ - 0.0084 (g/gds), $ZnSO_4.7H_2O$ - 0.0254 g/gds, K_2HPO_4 - 0.0037 g/gds and urea - 0.02147 g/gds) established by the regression model. Three repeated experiments were performed and the results were compared. The inulinase activity (372 U/gds) obtained from experiments was very close to the actual response (368 U/gds) predicted by the regression model, which proved the validity of the model.

CONCLUSION

In this work, Plackett Burman design was used to test the relative importance of medium components on inulinase production. Among the variables, corn steep liquor, $(NH_4)_2SO_4$, $ZnSO_4{\cdot}7H_2O$, K_2HPO_4 and urea were found to be the most significant variables. From further optimization studies the optimized values of the variables for inulinase production were as follows: corn steep liquor – 0.0560 g/gds, $(NH_4)_2SO_4$ - 0.0084 (g/gds), $ZnSO_4.7H_2O$ – 0.0254 g/gds, K_2HPO_4 - 0.0037 g/gds and urea - 0.02147 g/gds. This study showed that the copra waste constitutes a good carbon source for the production of inulinase. Using the optimized conditions, the produced activity reaches 372 U/gds. The results show a close concordance between the expected and obtained activity level.

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NAUČNI RAD

OPTIMIZACIJA PRODUKCIJE INSULINAZE KVASCEM *Kluyveromyces marxianus* var. *marxianus* KORIŠĆENJEM OTPADAKA SUVOG JEZGRA KOKOSOVOG ORAHA

Utvrđeno je da Kluyveromyces marxianus var. marxianus izlučuje veliku količinu ekstraćelijske inulinaze u fermentacionu tečnost. Produkcija inulinaze pomoću otpada suvog jezgra kokosovog oraha kao izvora ugljenika je optimizovana statističkom metodologijom zasnovanoj na eksperimentalnom dizajnu. Trijaža 18 nutrienata na njihov uticaj na produkciju inulinaze je izvršena Plackett-Burman-ovim dizajnom. Zbog pozitivnog uticaja na produkciju inulinaze odabrani su kukuruzna močevina, $(NH_4)_2SO_4$, $ZnSO_4{\cdot}7H_2O$, K_2HPO_4 i urea. Odabrane komponente su optimizovane primenom metodologije površine odziva. Optimalni uslovi su: kukuruzna močevina, 0.0560 g/g, $(NH_4)_2SO_4$, 0.0084 g/g, $ZnSO_4{\cdot}7H_2O$, 0.0254 g/g, K_2HPO_4, 0.0037 g/g i urea, 0.02147 g/g. Ovi uslovi su eksperimentalno potvrđeni, a prinos inulinaze je bio 372 U/g.

Ključne reči: peroksidni broj; ruzmarin; žalfija; timijan; izop; natkritična ekstrakcija.