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Application of Statistical Experimental Designs for the Optimization of Medium Constituents for the Production of L-Asparaginase by *Serratia Marcescens*

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Abstract

Statistical experimental designs were applied for the optimization of medium constituents for L-Asparaginase production by *Serratia marcescens* NCIM 2919 in solid state fermentation (SSF) using sesame oil cake (SOC) as the sole substrate. Using Plackett–Burman design (PBD), moisture content of substrate, glucose and $NaNO_3$ were identified as significant variables which highly influenced L-Asparaginase production and these variables were subsequently optimized using a Doehlert experimental design (DD). Besides reducing the number of experiments required for optimization, this technique allowed to quantify the amount of L-Asparaginase in any part of the experimental domain. The optimum conditions were found to be moisture content of the substrate 68.64 (%), glucose 3.093 (%w/w) and $NaNO_3$ 1.013 (%w/w). L-Asparaginase activity at these optimum conditions was 110.795 U/g ds (Units/g of dried sesame oil cake waste as substrate).

Keywords: L-Asparaginase; Sesame oil cake; Optimization; Plackett–Burman design; Doehlert experimental design; *Serratia marcescens*

Introduction

The field of cancer research has some good examples of the use of therapeutics. Recent studies show that PEGylated enzyme, Oncaspar (pegasparagase), already in use in the clinic has shown better results for the treatment of children with newly diagonised standard-risk acute lymphoblastic leukemia (Vellard, 2003). L-Asparaginase is an effective antineoplastic agent, used in acute lymphoblastic leukemia chemotherapy (Azmi et al., 2007). The discovery of high L-Asparaginase activity with serum of guinea pig is turned out to be important for the development of L-Asparaginase as a potential antineoplastic agent. Intense interest in asparaginase has resulted since the demonstration that L-Asparaginase from *E.coli* has antitumor activity. Studies on the mechanism of antineoplastic action of this enzyme suggest that a number of tumor cells responding to it lack adequate L-asparagine synthetase activity and require an exogenous supply of L-asparagine. Upon the depletion of this amino acid by asparaginase, *in vitro* or *in vivo*, these tumor cells die.

Although L-Asparaginase has been found in various plant and animal species, due to the difficult extraction procedure of this enzyme, other potential sources like microorganisms were searched. Microorganisms like fungi and bacteria have proved to be very efficient and inexpensive sources of this enzyme. But, tumor inhibitory activity has been demonstrated only with the asparaginases obtained from *E.coli* (Mashburn and Wriston, 1964), *Erwinia aroidea* (Peterson and Ciegler, 1969) and *Serratia marcescens* (Rowley and Wriston, 1967).

Solid state fermentation (SSF) is a very effective technique as the yield of the product is many times higher than in submerged fermentation (Arima, 1964). The use of solid state fermentation process is being reported by several researchers as an alternative to submerged fermentation (Hang and Woodams, 1986; Tran et al., 1998). SSF offers many advantages over submerged fermentation such as lower energy requirements, less risk of bacterial contamination, less waste water generation and less environmental concerns regarding the disposal of solid waste (Doelle et al., 1992). Other advantages include ease of product extraction that does not require complicated methods of treating the fermented residue (Lonsan et al., 1985). In comparison with SmF, SSF offers better opportunity for the biosynthesis of low-volume-high cost products (Balakrishnan and Pandey, 1996). L-Asparaginase production in SSF has been reported earlier on soy bean meal (Abdel-Fattah and Olama, 2002; El-Bessoumy et al., 2004) and wastes from three leguminous crops- bran of *Cajanus cajan, Phaseolus mungo and Glycine max* (Mishra, 2006). Sesame is grown in many parts of the world on over 5 million acres (20,000 km^2). The largest producer of the crop in 2007 was India, followed by China, Myanmar, Sudan, Ethiopia, Uganda and Nigeria. Seventy percent of the world's sesame crop is grown in Asia, with Africa growing 26%. SOC has been exploited for the production of enzymes like lypase, nuetral proteases, L- glutaminase and phytase, and for the production of antibiotics and antioxidants. It is also majorly used as a feed substitute for animal protein hydrolysates, used in the treatment of protein malnutrition (Ramachandran et al., 2007). The use of anbundantly available cheap agro-industrial waste like sesame oil cake which is an ideal sources of proteinaceous nutrients with a crude protein content of 35.6% would be an ideally suited nutrient support in SSF rendering both carbon and nitrogen sources, and is reported to be a good substrate for enzyme production (Kuo, 1967). Hence an attempt has been made in this investigation to utilize the sesame oil cake as a sole substrate for L-Asparaginase production and as well as to carry out the initial process optimization.

The first step in process optimization is screening of important variables, followed by estimation of optimal levels of these variables. Plackett–Burman design (Plackett and Burman, 1946) is one design which is a well esTablelished and widely used statistical technique for

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screening of medium constituents (Sarat et al., 2010). Following the initial screening, the next step is optimizing the variables selected through Plackett–Burman design. The traditional 'one-factor at a time' technique used for optimizing a multivariable system is not only time consuming but also often easily misses the alternative effects between components. Also, this method requires to carry out a number of experiments to determine the optimum levels when the interactions are significant. These drawbacks of single factor optimization process can be eliminated by optimizing all the affecting parameters collectively by Doehlert experimental design (Doehlert, 1970) using response surface methodology (RSM). Recently, many statistical experimental design methods have been employed in bioprocess optimization. Among them, Doehlert designs (Ferreira et al., 2004) offer the following advantages over the other designs. This method has been successfully applied to optimize different process parameters (Ismail et al., 1999; Carvalheiro et al., 1999; Vanot et al., 2002; Dutra et al., 2006). Basically, this optimization process involves three major steps: performing the statistically designed experiments, estimating the coefficients in a mathematical model, predicting its response and checking the adequacy of the model. Several researchers in biotechnology have applied these techniques for optimization of different parameters (Francis et al., 2003; Bandaru et al., 2006; Singh and Satyanarayana, 2006; Sita and Narsimha, 2010). Hence, the present study reports the application of the Plackett–Burman design and Doehlert experimental design to optimize L-Asparaginase production from sesame oil cake by SSF using *Serratia marcescens* NCIM 2919.

Materials and Methods

Substrate

Sesame (*Sesamum indicum*) oil cake (SOC), procured from a local oil extracting unit (Jeevan Enterprises) of Vizianagaram, Andhra Pradesh, India was used as the substrate. It was dried at 60°C for 72h to reduce the moisture content to around to 5% and ground to a desired size.

Microorganism

S. marcescens NCIM 2919 obtained from National Chemical Laboratory, Pune, India was used throughout the study.

Growth conditions

The culture was maintained on nutrient agar medium slants having the composition (%): beef extract 1.0, NaCl 0.5, peptone 1.0, agar agar 2.0. The pH of the medium was adjusted to 7.0–7.5 and culture was incubated at 30°C for 48h. Sub-culturing was carried out once in every 2 weeks and the culture was stored at 4°C.

Inoculum preparation

The *Serratia* strain was cultivated in a medium containing trypton 15g, soy bean meal 5g and sodium chloride 5 g/l of distilled water. The cells were cultivated in this medium at 30°C on a shaker at 180 rpm for 24h (Abdel-Fattah and Olama, 2002).

Media preparation

Ten grams of substrate was weighed into a 250 ml Erlenmeyer flask and to this a supplemental salt solution was added to the desired moisture level. The composition of the salt solution was as follows (% w/w): $MgSO_4$. 7 H_2O: 0.05; $FeSO_4$. 7 H_2O: 0.00 1; KCl: 0.05; and K_2HPO_4: 0.1. $NaNO_3$ and glucose were taken as nitrogen and carbon sources as per the design respectively. The contents were thoroughly mixed and autoclaved at 121°C (15 psi) for 20 min (Mukherjee et al., 2000).

Solid state fermentation

The sterilized substrate including media as shown in the above Section 2.5 was inoculated with 2 ml of inoculum. The contents were mixed thoroughly and incubated in a slanting position at 37°C. All the experiments were carried out in duplicate and samples were collected after 4 days of incubation.

Extraction of enzyme and assay method

The crude enzyme from the fermented material was recovered by simple extraction method. For this, the fermented substrate was mixed thoroughly with 100 ml of 0.01 M sodium phosphate buffer (pH 7.0) and the contents were agitated for 1h at room temperature in a rotary shaker at 150 rpm. At the end of extraction, the liquid was filtered off through Whatman No.1 filter paper and the resulting clear filtrate was used for enzyme assay. L-Asparaginase activity was assayed by the method Nesslerization (Geckil and Gencer, 2004). A standard curve was prepared with ammonium sulphate. One L-Asparaginase unit of activity (IU) was defined as the amount of enzyme that liberated 1µ mole of ammonia per gram of dry substrate per min under optimal assay conditions. (Mishra, 2006).

Experimental design and optimization

Plackett–Burman design: The purpose of the first optimization step was to identify which ingredients of the medium have a significant effect on L-Asparaginase production. The Plackett–Burman (Plackett and Burman, 1946) statistical experimental design is very useful for screening the most important variables and does not consider the interaction effects between the variables. The total number of experiments to be carried out is $K+1$, where K is the number of variables. Each variable is represented by two levels, high and low denoted by (+) and (–), respectively. The statistical software package (Stat-Ease Inc., Minneapolis, MN, USA) was used for analyzing the experimental data.

The effect of each variable on L-Asparaginase production was calculated by using the following equation:

$$E_{(Xi)} = \frac{\Sigma Y_{(+)i} - \Sigma Y_{(-)i}}{L/2} \quad (1)$$

where, $E_{(Xi)}$ is the effect of levels of the tested variables, $\Sigma Y(+)_i$ and $\Sigma Y(-)_i$ are the L-Asparaginase production from the experimental runs in which the variables being tested are added to the medium at their maximum and minimum levels respectively and L is the number of experiments carried out. When the value of concentration effect ($E_{(Xi)}$) of the tested variable is positive, the influence of the variable is greater at high concentration, and when it is negative, the influence of the variable is greater at low concentration.

Doehlert experimental design: Doehlert experimental design (Doehlert, 1970) was used in the optimization of L-Asparaginase production. Moisture content of substrate ($X1$, %), glucose (X_2, (%w/w)) and NaNO3 (X_3, (%w/w)) of fermentation were chosen as the independent input variables, amount of L-Asparaginase (Y, U/gds) was used as the dependent output variable and were shown in Table 3 and Table 4 . For statistical calculations the variables Xi were coded as xi according to Eq. (2)

$$x_i = \left(\frac{X_i - X_{oi}}{\Delta X_i} \right) \alpha_i \quad (2)$$

where x_i is the coded value of the i_{th} factor, X_i the natural value, X_{oi} the value at the center point, ΔX_i the step change value, and α_i is the maximum value of the coded factor (i.e. 1.0, 0.866 and 0.816 for five levels, seven levels and three levels, respectively).

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A three variable Doehlert experimental design involving a total number of 15 experiments including three replicates at the center point (Table 4) was employed for the optimization of the parameters. The second degree polynomials (Eq. (3)) were calculated with the statistical package (Stat-Ease Inc., Minneapolis, MN, USA) to estimate the response of the dependent variable.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 + b_{12}X_1X_2 + b_{23}X_2X_3 + b_{13}X_1X_3 \quad (3)$$

where Y is predicted response, X_1, X_2, X_3 are independent variables, b_0 is offset term, b_1, b_2, b_3 are linear effects, b_{11}, b_{22}, b_{33} are squared effects and b_{12}, b_{23}, b_{13} are interaction terms.

Results and Discussion

Identification of important medium constituents using plackett–burman design

A total of seven medium components were screened in eight experimental runs and the corresponding Plackett–Burman experimental design matrix for screening of important variables for L-Asparaginase production were shown in Table 1. The resulting effects ($E_{(Xi)}$) of the variables on the responses, the associated *t*-values and significant levels were shown in Table 2. A *p*-value less than 0.05 for the three variables viz., moisture content of substrate (X_1), glucose (X_2) and $NaNO_3$ (X_3) indicates that they are significant. The same was confirmed from the pareto graph (Figure 1) which was also used to show the effect of all variables on L-Asparaginase production. These variables had confidence level above 95% in comparison to other variables and thus were considered to be significant for L-Asparaginase production. These significant variables were further optimized by DD involving RSM.

Optimization of the selected medium constituents using doehlert experimental design

Based on the results of the Plackett–Burman design, moisture content of substrate, glucose and $NaNO_3$ were chosen as the independent input variables and L-Asparaginase activity was used as the dependent output variable. A DD was employed to analyze the interactive effect of these parameters and to arrive at an optimum. The three variables which influence the fermentative production of L-Asparaginase highly were moisture content of substrate, glucose and $NaNO_3$. Out of them glucose had shown stronger effect on L-Asparaginase production and hence it was assigned seven levels (Ferreira et al., 2004).

Experiments were carried out as per the design, and the L-Asparaginase obtained after 4 days fermentation with 15 experiments of different combinations of moisture content, glucose and $NaNO_3$ was estimated (Table 3 and Table 4). By applying multiple regression analysis on the experimental data, the following second

Run no.	Levels	Variables							L-Asparaginase activity (U/gds)
		Glucose (%w/w)	Maltose (%w/w)	$(NH_4)_2SO_4$ (%w/w)	Urea (%w/w)	$NaNO_3$ (%w/w)	Casein (%w/w)	Moisture content (%)	
	+	5	5	2	2	2	2	80	
	–	1	1	0.5	0.5	0.5	0.5	30	
1		–	–	–	+	+	+	–	71.29
2		+	–	–	–	–	+	+	84.56
3		–	+	–	–	+	–	+	76.32
4		+	+	–	+	–	–	–	80.19
5		–	–	+	+	–	–	+	78.92
6		+	–	+	–	+	–	–	82.19
7		–	+	+	–	–	+	–	76.56
8		+	+	+	+	+	+	+	82.16

Table 1: Plackett–Burman experimental design matrix for screening of important variables for L-Asparaginase production.

Variables	Effect (*E*)	*t*(1)	*P*–value
Glucose	6.50250	41.727	0.015254[a]
Maltose	–0.43250	–2.775	0.220161
$(NH_4)_2SO_4$	1.86750	11.984	0.053000
Urea	–1.76750	–11.342	0.055983
$NaNO_3$	–2.06750	–13.267	0.047893[a]
Casein	–0.76250	–4.893	0.128340
Moisture content	2.93250	18.818	0.033798[a]

[a]Significant at $p \leq 0.05$, Standard error=0.155833

Table 2: Effects for L-Asparaginase production from the results of Plackett–Burman design.

Factors	Range and levels						
Coded variable, x_1	–1	–0.5	0	0.5	1		
Moisture content, X_1 (%)	50	60	70	80	90		
Coded variable, x_2	– 0.866	– 0.577	– 0.288	0	0.288	0.577	0.866
Glucose, X_2 (%w/w)	1	1.67	2.33	3	3.66	4.33	5
Coded variable, x_3	– 0.816	0	0.816				
$NaNO_3$, X_3 (%w/w)	0.5	1.0	1.5				

Table 3: Experimental range and levels of the factors.

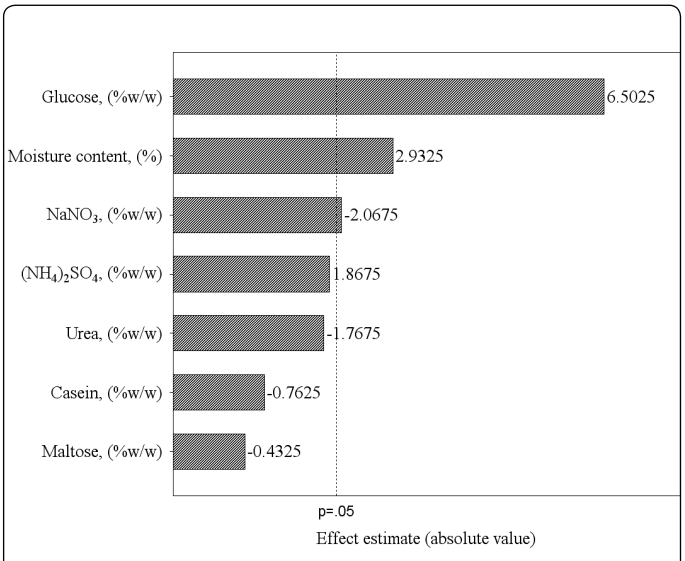

Figure 1: Pareto graph showing effect of various variables on L-Asparaginase production based on the observations of Plackett Burman design.

Experiment number	x_1	x_2	x_3	X_1	X_2	X_3	L-Asparaginase activity (U/gds)	
							Experimental	Predicted
1	1	0	0	90	3	1.0	91.78	93.3013
2	–1	0	0	50	3	1.0	98.35	96.8293
3	0.5	0.866	0	80	5	1.0	92.13	91.8324
4	–0.5	–0.866	0	60	1	1.0	91.24	91.5315
5	0.5	–0.866	0	80	1	1.0	92.67	92.1380
6	–0.5	0.866	0	60	5	1.0	95.43	95.9669
7	0.5	0.288	0.816	80	3.66	1.5	93.24	92.0129
8	–0.5	–0.288	–0.816	60	2.33	0.5	90.28	91.5065
9	0.5	–0.288	–0.816	80	2.33	0.5	90.43	89.4434
10	0	0.577	–0.816	70	4.33	0.5	91.59	91.3501
11	–0.5	0.288	0.816	60	3.66	1.5	92.48	93.4660
12	0	–0.577	0.816	70	1.67	1.5	91.27	91.5111
13	0	0	0	70	3	1.0	108.68	108.6069
14	0	0	0	70	3	1.0	107.91	108.6069
15	0	0	0	70	3	1.0	109.23	108.6069

Table 4: Doehlert three factor experimental design along with experimental and predicted values.

Term	Coefficient	Value	Standard error of coefficient	*t*-value	*p*-value
Constant	b_0	–136.651	21.60265	–6.3257	0.001455[b]
Moisture content	b_1	4.720	0.50908	9.2713	0.000245[b]
Glucose	b_2	23.730	3.48820	6.8030	0.001045[b]
$NaNO_3$	b_3	92.030	13.78706	6.6751	0.001140[b]
Moisture content × moisture content	b_{11}	–0.034	0.00343	–9.8578	0.000183[b]
Glucose × glucose	b_{22}	–3.089	0.25757	–11.9913	0.000071[b]
$NaNO_3$ × $NaNO_3$	b_{33}	–48.273	3.47665	–13.8848	0.000035[b]
Moisture content × glucose	b_{12}	–0.059	0.03762	–1.5753	0.176003
Glucose × $NaNO_3$	b_{23}	–0.534	1.37679	–0.3880	0.713974
$NaNO_3$ × moisture content	b_{31}	0.109	0.15858	0.6894	0.521273

b Significant at $p \leq 0.05$

Table 5: Model coefficients estimated by multiple linear regression (significance of regression coefficients).

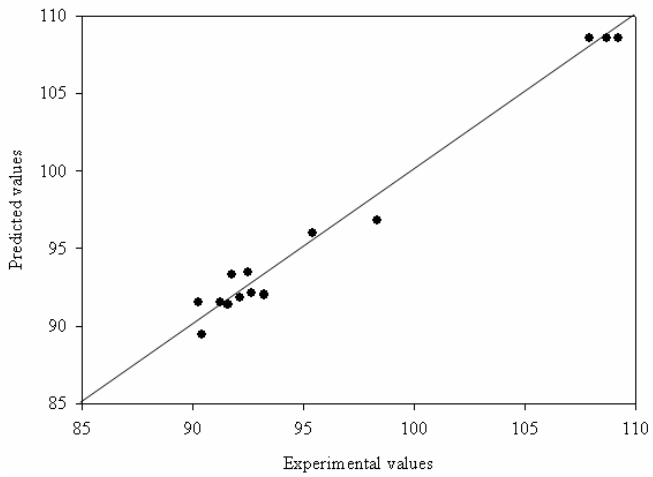

Figure 2: Parity plot showing the distribution of experimental vs. predicted values of L-Asparaginase activity.

order polynomial equation was found to represent the L-Asparaginase production adequately.

$$Y=-136.651+4.720X_1+23.730X_2+92.030X_3-0.034X_1^2-3.089X_2^2-48.273X_3^2-0.059X_1X_2-0.534X_2X_3+0.109X_3X_1 \quad (4)$$

The coefficients of the regression model (Eq. (4)) calculated are listed in Table 5, which contain three linear, three quadratic and three interaction terms and one block term. The significance of each coefficient was determined by Student's *t*-test and *p*-values, which are listed in Table 5. The larger the magnitude of the *t*-value and smaller the *p*-value, the more significant is the corresponding coefficient (Akhnazarova and Kafarov, 1982; Khuri and Cornell, 1987). This implies that the first order and second order main effects of moisture

Source of Variation	Sum of squares (SS)	Degree of freedom (d.f.)	Mean squares (MS)	*F*-value	Probe >*F*
Regression	663.5711	9	73.73012	32.55999	0.000651
Residual	11.3222	5	2.26444		
Total	674.8933	14			

R = 0.99157638; R^2 = 0.98322372; Adjusted R^2 = 0.95302643

Table 6: ANOVA for the entire quadratic model.

content of substrate, glucose and $NaNO_3$ are highly significant as is evident from their respective *p*-values. They are more significant at the second order. This indicates that they can act as limiting nutrients and small variations in their concentration will alter either growth rate or product formation rate or both to a considerable extent. All the interaction effects were found to be insignificant ($p > 0.05$). The parity plot (Figure 2) showed a satisfactory correlation between the experimental and predicted values (obtained from Eq. (4)) of L-Asparaginase, wherein, the points cluster around the diagonal line which indicates the good fit of the model, since the deviation between the experimental and predicted values was minimal.

The results of the second order response surface model fitting in the form of ANOVA were given in Table 6. It is required to test the significance and adequacy of the model. The Fisher variance ratio, the *F*-value ($= S^2_r/S^2_e$), is a statistically valid measure of how well the factors describe the variation in the data about its mean. The greater the *F*-value is from unity, the more certain it is that the factors explain adequately the variation in the data about its mean, and the estimated factor effects are real. The analysis of variance of the regression model demonstrates that the model is highly significant, as is evident from the Fisher's *F*-test ($F_{model}=32.55999$) and a very low probability value ($P_{model} > F=0.000651$). Moreover, the computed *F*-value ($F_{0.01(9,5)} = S^2_r/S^2_e = 32.55999$) is greater than the Table *F*-value at the 1% level, indicating that the treatment differences are significant.

The goodness of the fit of the model was checked by the determination coefficient (R^2). The R^2 value provides a measure of how

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much variability in the observed response values can be explained by the experimental factors and their interactions. The R^2 value is always between 0 and 1. The closer the R^2 value is to 1, the stronger the model is and the better it predicts the response (Cochran and Cox, 1957). In this case, the value of the determination coefficient (R^2= 0.98322) indicates that 98.32% of the variability in the response could be explained by the model. The R^2 value also indicates that only 1.678% of the variation is not explained by the model. In addition, the value of the adjusted determination coefficient (Adj R^2=0.95302) is also very high to advocate for a high significance of the model. Also a higher value of the correlation coefficient (R=0.9915) justifies an excellent correlation between the independent variables (Box and Hunter, 1978). The predicted optimum levels of moisture content of substrate, glucose and $NaNO_3$ were obtained by applying the regression analysis to the Eq. (4).

Figures 3–5 represent the response surface and contour plots for the optimization of medium constituents of L-Asparaginase production. The effect of the moisture content and glucose on the L-Asparaginase production was shown in Figure 3. An increase in moisture content with glucose up to the optimum point, increased the L-Asparaginase production to a maximum level, but with a further increase in the moisture content with glucose, the trend is reversed. The interaction effect of moisture content and NaNO3 on L-Asparaginase production in Figure 4 clearly indicates a proper combination for production of L-Asparaginase. An increase in the $NaNO_3$ with moisture content increased the L-Asparaginase production gradually, but at a higher $NaNO_3$ and moisture content, the trend is reversed. The optimum for maximum L-Asparaginase production lies near the center point of the $NaNO_3$ and moisture content. A similar effect on the response was observed for glucose at any level of $NaNO_3$. An increase in glucose with $NaNO_3$ up to the optimum point increased the L-Asparaginase production to maximum level, but a further increase in the glucose with $NaNO_3$ decreased the L-Asparaginase production as shown in Figure 5. Therefore, an optimum was observed near the central value of glucose, moisture content and $NaNO_3$. The optimum medium constituents for higher L-Asparaginase production can be attained at the concentration of 68.64% of moisture content, 3.093 (%w/w) of glucose and 1.013 (%w/w) of NaNO3. At these optimum medium concentrations, maximum L-Asparaginase activity 110.795 U/gds was obtained.

Conclusion

The present study involved the use of statistical experimental designs to optimize medium constituents of the fermentation medium for the production of L-Asparaginase from sesame oil cake by SSF using *S. marcescens* NCIM 2919. Three variables: moisture content of substrate, glucose and $NaNO_3$ were identified as significant by Plackett–Burman design for L-Asparaginase production. These variables were further optimized using Doehlert experimental design involving RSM. Among the three variables tested for the correlation between their concentrations and the production of L-Asparaginase,

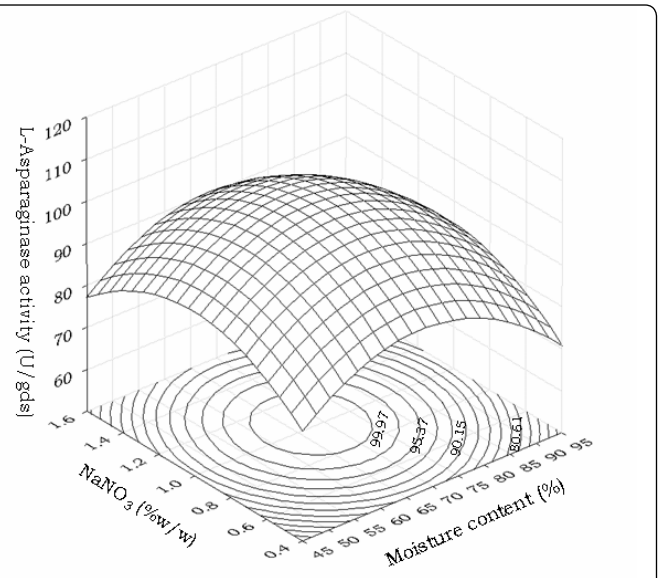

Figure 4: Response surface and contour plot of $NaNO_3$ vs. moisture content on L-Asparaginase activity (glucose was kept constant at 3 (%w/w)).

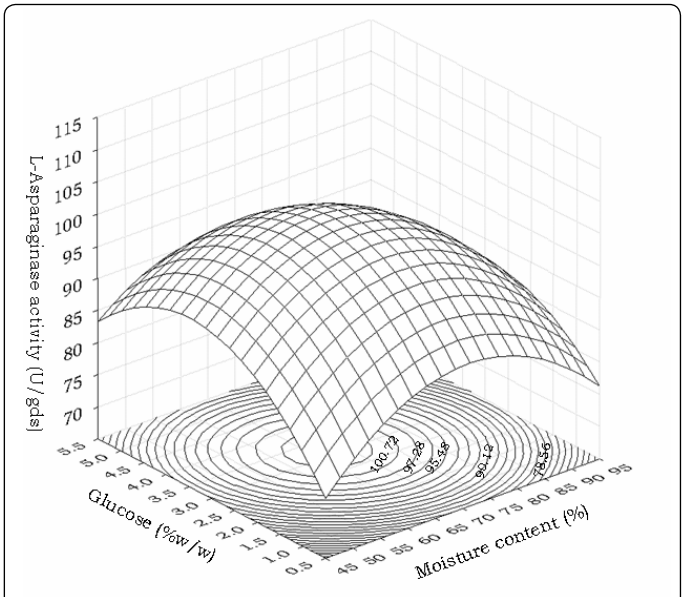

Figure 3: Response surface and contour plot of glucose vs. moisture content on L-Asparaginase activity ($NaNO_3$was kept constant at 1.0 (%w/w)).

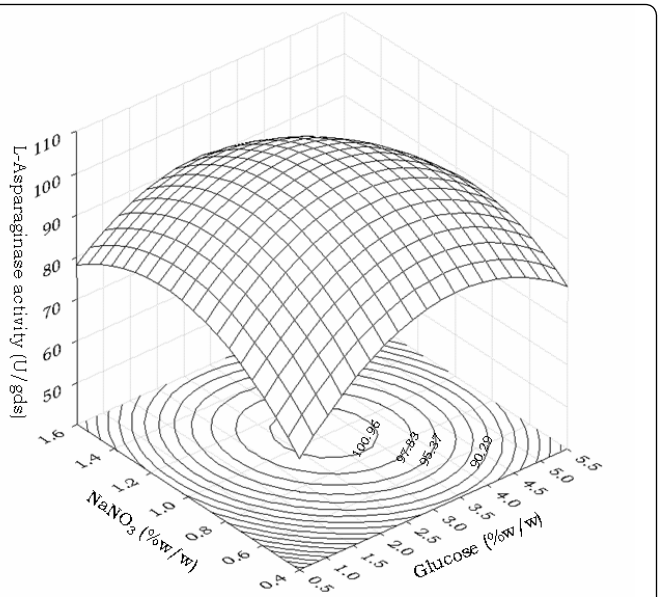

Figure 5: Response surface and contour plot of glucose vs. $NaNO_3$ on L-Asparaginase activity (moisture content was kept constant at 70%).

all the three variables showed significant influence on the enzyme production. The significant interactions between the three variables were also observed from the contour plots. The maximum amount of L-Asparaginase produced from sesame oil cake was predicted to be 110.795 U/gds (Units/g of dried sesame oil cake) when the optimized medium constituents of the fermentation medium was set as follows: moisture content of substrate 68.64 (%), glucose 3.093 (% w/w) and $NaNO_3$ 1.013 (% w/w). Applying statistical experimental designs to optimize the selected factors for maximal production is an efficient method that tests the effect of factors interaction with minimum number of experiments. The methodology as a whole proved to be adequate for the design and optimization of the bioprocess for obtaining a therapeutically valuable product like L-Asparaginase from an abundantly available, low grade agro-industrial waste like sesame oil cake. Thus, it is useful to advise the microbial industry sponsors to apply such experimental designs to maintain high efficiency and economic bioprocess.

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References

1. Abdel-Fattah YR, Olama ZA (2002) L-Asparaginase production by Pseudomonas aeruginosa in solid-state culture: evaluation and optimization of culture conditions using factorial designs. Process Biochem 38: 115-122.
2. Akhnazarova S, Kafarov V (1982) Experiment optimization in chemistry and chemical engineering. Moscow: MIR.
3. Arima K (1964) Microbial enzyme production. In: Starr MP, editor Global Impacts of Appl Microbiol 279-294.
4. Azmi W, Narta UK, Kanwar SS (2007) Pharmacological and clinical evaluation of L-Asparaginase in the treatment of leukemia. Critical Rev in Oncol/Hematol 61: 208-221.
5. Balakrishnan K, Pandey A (1996) Production of biologically active secondary meTableolites in solid state fermentation. J Sci Ind Res 55: 365-372.
6. Bandaru VVR, Somalanka SR, Mendu DR, Madicherla NR, Chityala A (2006) Optimization of fermentation conditions for the production of ethanol from sago starch by co-immobilized amyloglucosidase and cells of Zymomonas mobilis using response surface methodology. Enzyme Microb Technol 38: 209-214.
7. Box GEP, Hunter JS, Hunter WG (1978) Statistics for experiments. New York Wiley.
8. Carvalheiro F, Roseiro JC, G´ırio FM (1999) Interactive effects of sodium chloride and heat shock on trehalose accumulation and glycerol production by Saccharomyces cerevisiae. Food Microbiol 16: 543-550.
9. Cochran WG, Cox GM (1957) Experimental Designs, second ed. New York Wiley 346-354.
10. Doehlert DH (1970) Uniform shell designs. Appl Stat 19: 231-239.
11. Doelle HW, Mitchell DR, Rolz CE (1992) Solid Substrate Cultivation. Elsevier Applied Science London 7-16.
12. Dutra RL, Maltez HF, Carasek E (2006) Development of an on-line preconcentration system for zinc determination in biological samples. Talanta 69: 488-493.
13. El-Bessoumy AA, Sarhan M, Mansour J (2004) Production, isolation and purification of L-Asparaginase from Pseudomonas aeruginosa 50071 using solid-state fermentation. J Biochem Mol Biol 37: 387-393.
14. Ferreira SLC, dos Santos WNL, Quintella CM, Neto BB, Bosque-Sendra JM (2004) Doehlert matrix: A chemometric tool for analytical chemistry—review. Talanta 63: 1061-1067.
15. Francis F, Sabu A, Nampoothiri KM, Ramachandran S, Ghosh S, Szakacs G, Pandey A (2003) Use of response surface methodology for optimizing process parameters for the production of a-amylase by Aspergillus oryzae. Biochem Eng J 15: 107-115.
16. Geckil H, Gencer S (2004) Production of L-Asparaginase in Enterobacter aerogenes expressing Vitreoscilla hemoglobin for efficient oxygen uptake. Appl Microbiol Biotechnol 63: 691-697.
17. Hang YD, Woodams EE (1986) A solid state fermentation of apple pomace for citric acid production using Aspergillus niger. J Appl Microbiol Biotechnol 2: 283-287.
18. Ismail A, Linder M, Ghoul M (1999) Optimization of butylgalactoside synthesis by β-galactosidase from Aspergillus oryzae. Enzyme Microb Technol 25: 208-213.
19. Khuri AI, Cornell JA (1987) Response surfaces: design and analysis. New York Marcel Dekker.
20. Kuo LH (1967) Animal feeding stuffs compositional data of feeds and concentrates (Part 3). J Malaysian Agric 46: 63-70.
21. Lonsan BK, Ghildyal NP, Budiatman S, Ramakrishna SV (1985) Engineering aspects of solid-state fermentation. Enz Microb Technol 7: 258-265.
22. Mashburn LT, Wriston JC (1964) Tumor inhibitory effect of L-asparaginase from Escherichia coli. Arch Biochem Biophys 105: 450-452.
23. Mishra A (2006) Production of L-Asparaginase, an Anticancer Agent, from Aspergillus niger using agricultural waste in solid state fermentation. Appl Biochem Biotechnol 135: 33- 42.
24. Mukherjee J, Majumdar S, Scheper T (2000) Studies on nutritional and oxygen requirements for production of L-Asparaginase by Enterobacter aerogenes. Appl Microbiol Biotechnol 53: 180-184.
25. Peterson RE, Ciegler A (1969) L-Asparaginase production by Erwinia aroideae. Appl Microbiol 18: 64-67.
26. Plackett RL, Burman JP (1946) The design of optimum multifactorial experiments. Biometrika 33: 305-325.
27. Ramachandran S, Singh SK, Larroche C, Soccol CR, Pandey A (2007) Oil cakes and their biotechnological applications – A review. Bioresour Technol 98: 2000-2009.
28. Rowley B, Wriston JC (1967) Partial purification and antilymphoma activity of Serratia marcescens L-asparaginase. Biochem Biophys Res Commun 28: 160-165.
29. Sarat Babu I, Sita Kumari K and Hanumantha Rao G (2010) Optimization of process parameters for the production of Lipase in Solid State Fermentation by Yarrowia Lipolytica from niger seed oil cake (Guizotia Abyssinica). J Microbial Biochem Technol 2: 28-33.
30. Singh B, Satyanarayana T (2006) A marked enhancement in phytase production by a thermophilic mould Sporotrichum thermophile using statistical designs in a cost-effective cane molasses medium. J Appl Microbiol 101: 344-352.
31. Sita Kumari K, Narasimha Rao M (2010) Application of Doehlert Experimental Design for the optimization of medium constituents for the production of L-Asparaginase from palm kernal cake (Elaeis guineensis). J Microbial Biochem Technol 2: 7-12.
32. Tran CT, Sly LI, Mitchell DA (1998) Selection of a strain of Aspergillus for the production of citric acid from pineapple waste in solid-state fermentation. World J Microbiol Biotechnol 14: 399-404.
33. Vanot G, Val´erie D, Guilhem MC, Phan-Tan-Luu R, Comeau LC (2002) Maximizing production of Penicillium cyclopium partial acylglycerol lipase. Appl Microbial Biotechnol 60: 417-419.
34. Vellard M (2003) The enzyme as drug: application of enzymes as pharmaceuticals. Curr Opin Biotechnol 14: 1-7.