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Optimization of Extracellular Lipase Production by *Penicillium chrysogenum* Using Factorial Design

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ABSTRACT

The effect of oxygen on lipase production by *Penicillium chrysogenum* was studied under two operating modes, controlled aeration rate tested and controlled agitation at dissolved oxygen concentration (DO) 1.00 vvm. Lipase production and cell dry weight were tested in a stirred batch fermenter 5 L. Improvement in oxygen transfer rate (OTR) either by aeration or agitation resulted in an increase in lipase production. Growth curves and lipase activities of *P.chrysogenum* were examined at agitation rates (200,400,600 rpm), aeration rates (2,4 vvm) at different fermentation periods (24,48,72,96,120 h). Response Surface Methodology (RSM) using Design Expert software was used to study the effect of aeration, agitation, and fermentation time on lipase activity and cell dry weight. These factors were analyzed using 2^1. 3^2 level factorial design. An optimal set of conditions that maximize lipase production: (2 vvm aeration; 600 rpm agitation after 72 h) was obtained. The maximum lipase activity obtained was 240 U/mL. Beside lipase activity, this paper also studies the optimal combination of the controllable factors (aeration; agitation and fermentation time) that will maximize the cell dry weight.

Keywords: lipase activity, optimization, fermentation, factorial design, Design-Expert software.

INTRODUCTION

Lipases (EC 3.1.1.3) are enzymes, the biological function of which is to catalyze the hydrolysis of tri-acyl glycerol to fatty acids, mono- and di-acylglycerols and glycerol. Lipases act specifically at oil/water interfaces (Cihangir and Sarikaya, 2004; Javed *et al*., 2007). Lipolytic enzymes can be found in animals, plants and microorganisms (Kamimura *et al*., 2000; Burkert *et al*., 2004). They form an important enzyme group due to their remarkable levels of activity and stability in both aqueous and non-aqueous media, which enables several reactions catalysis such as acidolysis, alcoholysis, aminolysis, esterification and transesterification. Lipases also show unique properties of chemo-, regio-and enantio- selectivity (Gutarra *et al*., 2009).

The interest in microbial lipase production has increased in the last decades, because of its large potential in industrial applications as additives in food, fine chemical, detergents, waste water treatment, cosmetics, pharmaceuticals, leather, and medicines (Kamini *et al*., 2000; Sharma *et al.*, 2001).

Microbial lipase fermentations are affected by pH, temperature, medium composition, aeration, agitation etc (Chen *et al.*, 1999). It was reported that the presence of air was essential for lipase production by *Staphylococcus aureus* (Vadehra and Harmos, 1969) and *Pseudomonas putida* (Lee and Rhee, 1993). It was previously summarized, that improvements in aeration of liquid by agitation or air sparging is beneficial for lipase production in all cases for single cell organisms and in most cases for filamentous moulds (Frost and Moss, 1987).

Most lipases used in biotechnological applications are produced by fungi and bacteria. Filamentous fungi are interesting sources of lipases because they produce extracellular enzymes (Hölker *et al.*, 2004). As in any application that demands high enzyme quantities such as treatment of oily wastewater and biodiesel production, lipase utilization depends on the reduction of its costs to become economically feasible (Cammarota and Freire, 2006). For the optimization, RSM was used as a tool since it enables the evaluation of multiple parameters alone or in combination on response variables (Mutalik *et al.*, 2008).It is also a statistical technique useful for designing experiments, building models and analyzing the effects of independent variables (Garrido-Vidal *et al*., 2003; Nemukula *et al.*, 2009).

Our objectives were to better understand the relationship between the factors (aeration, agitation and time) and the response values (lipase activity and cell dry weight) and to determine the optimal conditions that maximize lipase production/cell dry weight.

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MATERIALS AND METHODS

Microorganism and culture conditions

Penicillium chrysogenum used in the present work was kindly obtained from Chemistry of Natural and Microbial Products Dept., National Research Center, Cairo, Egypt. The slants of *P. chrysogenum* were incubated on potato-dextrose agar medium (PDA) at 30 °C for 7 days.

Lipase production

P. chrysogenum was cultured on the following medium (g/L in distilled water): yeast extract 5; olive oil 10; KNO_3 2; $MgSO_4 \cdot 7H_2O$ 0.5; K_2HPO_4 1; $ZnSO_4 \cdot 7H_2O$ 0.44; $FeSO_4 \cdot 7H_2O$ 1.1; $MnSO_4 \cdot 7H_2O$ 0.2; $(NH_4)_2SO_4$10 at pH 7.0 using phosphate buffer (Lima *et al.*, 2003).

Fermenter culture

Fermenter experiments were carried out in a 5 L jar fermenter (Braun biotech, International microdcu 200) which was adopted for bench-scale cultivation under the following conditions: the inoculum was prepared in 5 Erlenmyer flasks 250 mL each with 100 mL medium. The cultures were inoculated with 4 mL of spores suspension (10^8 spores/ mL) and the flasks were agitated at 120 rpm at 30 °C. After 48 h, the whole volume was transferred into the fermenter. The submerged cultivation was performed in 5 L glass fermentor with working volume 3 L and the pH was automatically controlled at pH 7.0 using 0.1 N NaOH or 0.1 N HCl. The dissolved oxygen concentration (DO) was maintained at 1.00 vvm. Samples (25 mL) were daily collected for analysis. The experiments were repeated with variously stirred agitation speed (200; 400; 600 rpm), aeration (2.0; 4.0 vvm) during different fermentation time (24, 48, 72, 96, 120 h) corresponding to the growth phase of the microorganism.

Lipase activity

Lipase activity was determined using an emulsion of 10% olive oil in 10% gum Arabic. The emulsion was prepared by treating the mixture of olive oil and gum Arabic solution in a top drive homogenizer for 10 min. The reaction mixture contained 3 mL of the substrate, 2.5 mL of deionized water, 1 mL of 0.1 M Tris-HCl buffer (pH 7.5) and 1mL of culture filtrate. The mixture was incubated for 2 h at 35 °C in shaking water bath after which 10mL of 99% acetone (absolute) was added. The resulting mixture was then titrated against 0.5 N NaOH using thymolphethalein indicator. Blanks were obtained by boiling the culture filtrate. The lipase activity was defined as that produces 1 µmole of free fatty acids under assayed conditions (Parry *et al.*, 1966).

Biomass estimation

The mycelial biomass obtained after growth, was separated by filtration, washed by distilled water, dried in an oven 60 °C and reweighed till constant weight.

Table 1: Factors examined as independent variables effecting lipase production and cell dry weight by *P.chysogenum* and their levels.

	Factors					
	Aeration (vvm)		Speed (rpm)		Time (h)	
Level	Real Value	Coded Value	Real Value	Coded Value	Real Value	Coded Value
	2	-1	200	-1	72	-1
	4	+1	400	0	96	0
			600	+1	120	1

Table 2: Table 2: The experimental design for 3 variables and its results on the responses (lipase production and cell dry weight).

No. trial	Aeration (vvm)	Speed (rpm)	Time (h)	Lipase activity (U/ mL)	Cell dry weight (g/100 mL)
1	-1	-1	-1	96.67	3.126
2	-1	-1	0	140	3.345
3	-1	-1	+1	80	3.382
4	+1	-1	-1	101.667	3.704
5	+1	-1	0	160	3.86
6	+1	-1	+1	120	3.57
7	-1	0	-1	175	3.990
8	-1	0	0	155	3.954
9	-1	0	+1	110	3.954
10	+1	0	-1	205	4.593
11	+1	0	0	180	4.408
12	+1	0	+1	161.667	4.600
13	-1	+1	-1	240	4.974
14	-1	+1	0	200	4.755
15	-1	+1	+1	170	4.810
16	+1	+1	-1	138.33	3.776
17	+1	+1	0	95	3.50
18	+1	+1	+1	80	3.631

Factorial design

A general factorial design $2^1 . 3^2$with three controllable and two response factors was studied resulting in 18 run each replicated three times to estimate the experimental error. The aim of the study was to find the optimum fermentation time that maximizes the lipase activity and the cell dry

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weight. The aeration was tested at two levels (2 and 4 vvm) while the speed was tested at three levels (200, 400 and 600 rpm) at 3 fermentation times (72, 96 and 120 h). The response factors were the lipase activity (U/mL) and the cell dry weight (g/100). The statistical analysis of the observed data was performed using the Design Expect software. The test factors and their levels are shown in Table 1, followed by the experimental design and response values in Table 2.

RESULTS AND DISCUSSION

P. chrysogenum lipase production and cell dry weight

The effect of aeration, speed and fermentation time on lipase production and cell dry weight of *P. chrysogenum* were tested. The experiments were carried out in 5 L fermenter with working volume 3.0 L. Lipase activity and the corresponding growth curve were studied at aeration (2, 4 vvm) agitation (200, 400, 600, rpm) at different incubation periods (Figures 1 and 2).

Figure 1 shows the growth curve and the time course at different agitation speeds (200, 400, 600 rpm) at controlled aeration 2 vvm. The lipase production started from early stages and reached maximum in late log phase after 72 h for both agitation speeds 400 and 600 rpm. It

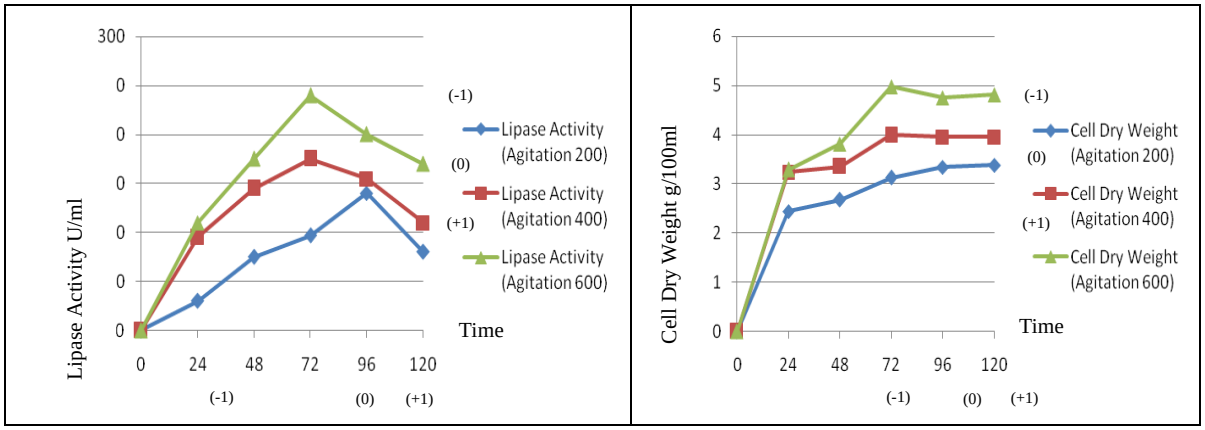

Figure 1: Time course of lipase production by *P. chrysogenum* under controlled aeration (2 L /min "-1").

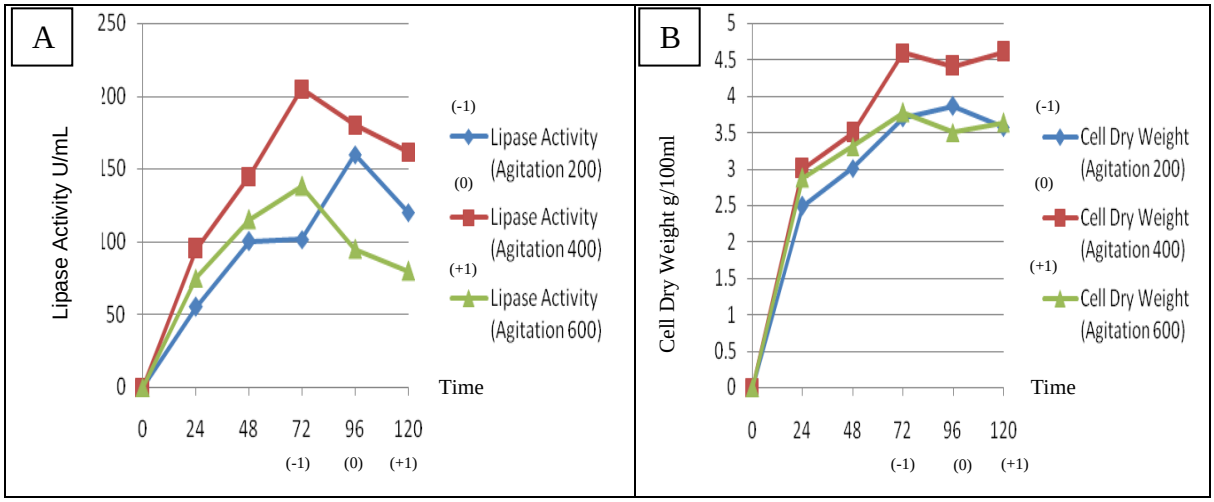

Figure 2 (A, B): Time course of lipase production by *P. chrysogenum* under controlled aeration (4L /min "1").

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should be noted that at agitation speed 200 rpm, lipase production was maximum. When aeration increased from 200 to 600 rpm, the lipase production decreased. Similar results were obtained by Elibol and Ozer (2000) who reported that lipase production only occurred in the presence of air and, therefore, indicated that the critical demand of oxygen was higher for lipase production than for growth.

Figure 2 shows the growth curve and the lipase production at different agitation speeds (200, 400, 600 rpm) under controlled aeration (4.0 vvm). It can be observed that maximum lipase activity (160 U/mL) was obtained after 96 h at 200 rpm and on increasing the agitation speed from 200 to 400/rpm the lipase activity was 205 U/mL at 72 h. On increasing the agitation speed to 600 rpm lipase activity decreased once more. This may be attributed to the fact that the degree of aeration is critical in some cases. Similarly Ebrahimpour *et al.* (2008) showed that shallow layer (static culture) where aeration is moderate produced much more lipase than shake cultures (high aeration).

Analysis of variance (ANOVA) and adequacy test of the model

Model coefficients, F values and P values, which were generated by the Design Expert software (2009), can be used to evaluate the model. If $p < 0.05$, this indicates that the controllable factor or their interactions has a significant effect on the response factor (Amin and Anggaro, 2004; Zheng *et al.*, 2009).

Tables 3 and 4 showed that the probabilities for the regression quadratic models of both lipase activity and cell dry weight were significant ($p < 0.0001$) and the lack of fits were insignificant ($p > 0.05$). This means that both models were statistically good, and the models had no lack of fit. The diagnostic examination for the residual revealed no violations of the ANOVA underlying assumptions, which mean that the ANOVA results can be trusted. Therefore, as indicated in Tables 3 and 4, the fitted model is significant with a *p*-value of < 0.0001.

The results indicated that the aeration, agitation, and the fermentation time with their interactions have a significant effect on the lipase activity. Higher orders of the controllable factors and the products of the controllable factors also have a significant effect on the lipase activity. With the exception of the fermentation time, the above factors also have a significant effect on the cell dry weight.

Table 3: Analysis of variance (ANOVA) for the lipase activity.

Source	Sum of squares	DF	Mean square	F-value	*p*-value
Model	104863	9	11651.44	29.785	< 0.0001
(A)-aeration	5688.88	1	5688.88	14.54	0.0004
(B)-Speed	18.75	1	18.75	0.04	0.827
(C)-Time	13806.25	1	13806.25	35.29	< 0.001
AB	32700.69	1	32700.69	83.59	< 0.0001
BC	6337.5	1	6337.5	16.201	< 0.0002
B^2	10305.79	1	10305.79	26.34	< 0.0001
C^2	2750.23	1	2750.23	7.03	0.011
AB^2	16502.08	1	16502.08	42.18	< 0.0001
BC^2	7200	1	7200	18.40	< 0.0001
Residual	17211.57	44	391.17		
Lack of fit	3394.90	8	424.36	1.10	0.382
Pure Error	13816.67	36	383.79		
R^2	0.859				
Total	122074.5	53			

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Table 4: Analysis of variance (ANOVA) for the cell dry weight.

Source	Sum of squares	DF	Mean square	F-value	*p*-value
Model	15.56	5	3.11	36.76	< 0.0001
(A) Aeration	1.45	1	1.42	17.13	< 0. 001
(B) speed	4.98	1	4.98	58.89	< 0.001
AB	6.02	1	6.02	71.10	< 0.001
B^2	1.73	1	1.73	20.44	< 0.001
AB^2	2.75	1	2.75	32.55	< 0.0001
Residual	4.06	48	0.08		
Lack of fit	00	12	0.04	0.42	0.94
Pure error	3.5.56	36	0.098		
R^2	0.80				
Total	19.62	53			

Model fitting

A model fitting was accomplished for the experiment based on the data shown in Table 2 below. The quadratic regression models were obtained by using coded values from the estimation of data was as follows:

Lipase activity (U/mL) = -983.2-70.3*A +1.5 *S +25.6 *T+ 0.6* A*S -0.05* S* T + 2.0E – 003 *S^2 – 0.1* T^2 – 9.3E - 004* A* S^2 +2.6E-004*S* T^2 ……..Eq. (1)

Cell dry weight (g/100 mL) = 4.4- 0.8*A – 0.01* S + 7.5E -003* A*S + 2.6E – 005* S^2 – 1.2E – 005 * A*S^2 ……..Eq. (2)

Where A, S and T represents aeration, agitation and fermentation time respectively. Utilizing Eq. (1), the lipase activity can be estimated for any combination of the controllable factors (aeration, speed and time). It may be worth saying that Eq. (1) and (2) could be used only if the aeration is between 2 and 4 vvm, the agitation is between 200 and 600 rpm and fermentation time is between 72 and 120 h. Model Eq. (1) reports a coefficient of determination R^2 of 0.8590 which implies that the model can explain 86% of the variation in the lipase activity. The values of the adjusted R^2 and the adequate precision are 0.8302 and 20.039 respectively. Both values indicated that the model can be used to navigate the design space and that the model fitness is very good. Similarly the fitted regression model that describes the cell dry weight is shown in Eq. (2). This model reveals R^2 equal to 0.8 meaning that the fitted model explains 80% of the variability in the cell dry weight. It should be noted that a model can be considered predictive if the coefficient of determination (R^2) is close to 1.0 (Gutarra *et al.*, 2009)

The data were fitted to the response surface model to effectively evaluate the true relationship between the lipase activity/cell dry weight and the controllable factors. These investigations are based upon the optimization of cultural conditions for the lipase production and cell dry weight. The optimization was observed by varying three factors: aeration, speed and fermentation time with their respective levels shown in Table 2. The aeration was tested at 2 levels (2 and 4 vvm), agitation was tested at 3 levels (200, 400, 600 rpm). The experiment was performed at 3 different fermentation periods (72, 96 and 120 h) to find the optimum combination of these factors that maximizes the lipase activity as well as the cell dry weight. Eq. (1) and (2) were chosen to fit the experimental data for lipase activity (U/mL) and the cell dry weight (mg/100mL).

Response Surfaces Methodolgy (RSM)

In order to study the effects of the three factors as well as their interaction on lipase activity response surfaces and contour plots are generated as shown in Figure 3. It denotes the three dimensional response surface plots of agitation and aeration on lipase activity of *P. chrysogenum* generated at 72 h fermentation time (found to produce the highest lipase activity). As can be seen, enhancing the agitation from 200 to 600 rpm could increase the lipase activity while the aeration must be kept at 2 vvm. However the lipase activity decreased by further increasing the aeration and decreasing agitation. As indicated, higher agitation and lower aeration were more favorable for improving lipase activity. This may be attributed to the fact that variation in agitation results in a change in oxygen transfer rate, which in turn affected the rate and extent of cell growth and lipase production (Elibol and Ozer, 2004). It should be noted that agitation accelerates oil hydrolysis (Hwan *et al.*, 2007). On the contrary, static condition had resulted in comparatively high lipase production for *Syncephalastrum racemosum* (Chopra and Chander, 1983), *Pseudomonas* sp. strain S5 (Rahman *et al.*, 2006) and *Pseudomonas aeruginosa* (Nadkarni, 1971). Generally, suitable agitation leads to sufficient supply of dissolved oxygen in the media (Kumar and Takagi, 1999).

The coded model shown in Eq. 2 is used to generate the response surface shown in Figure 4 assists the analysis of the effect of the controllable factors on the cell dry weight. Figure 4 showed that on increasing the speed of agitation to 600 rpm and

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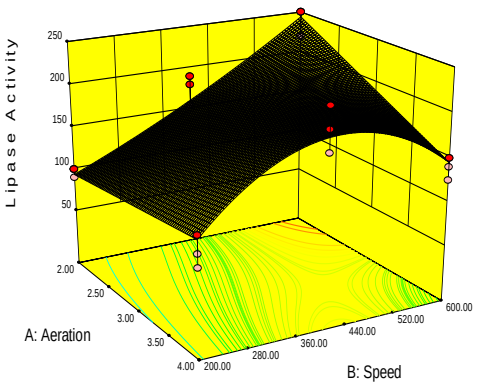

Figure 3: Response surface for the lipase activity of *P. chrysogenum* after 72 h of fermentation time.

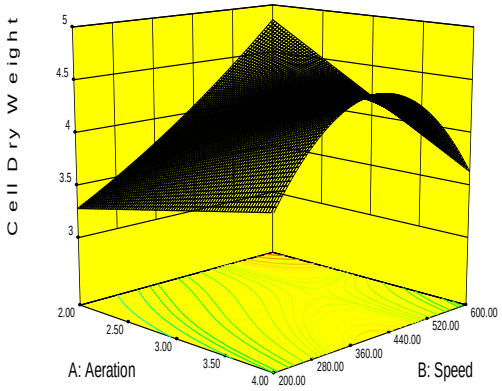

Figure 4: Response surface for the cell dry weight of *P. chrysogenum*.

reducing aeration to 2 vvm lead to a suitable growth after 72 h which in turn showed maximum lipase activity. Hoffman and Cleffman (1981) noticed that higher dissolved oxygen concentration in the medium did not significantly improve cell density. However, an increased biomass was obtained when aeration was 2 vvm. In the same way, aeration and agitation were previously found to influence growth and protease production (DeConink *et al*., 2000 and 2004).

Model optimization

According to the discussion above, it is possible to obtain high lipase activity using *P. chrysogenum* and a suitable cell dry weight through searching for the optimum point. Non-linear optimization was used to find the optimum value for aeration, speed and fermentation time that will express high lipase activity and cell dry weight simultaneously. The optimization model shows that aeration should be kept at a lower level of 2; the speed should be higher level of 600 while the fermentation time should be 72 h.
However, it was found out that at different levels of relative importance, the maximum values of the response factors did not change. There is also high positive correlation between the lipase activity and the cell dry weight which was found to be equal to 0.815693.The experimental values (240 U/mL for lipase activity and 4.810 g/100 mL for cell dry weight) were in agreement to the predicted ones (244.12 U/mL for lipase activity and 4.8464 g/100mL for cell dry weight). This confirmed the validity and adequacy of the predicted models.

CONCLUSION

In this study, RSM was successfully applied to determine the influential factors that lead to maximum lipase activity/cell dry weight of *P. chrysogenum*. Two sets of quadratic model were obtained to describe the relationship between the parameters (aeration, agitation and fermentation time) and responses (lipase activity and cell dry weight). Two response surface plots and two contour plots were used to analyze the conditions for the maximum lipase activity and cell dry weight. Furthermore, under optimized conditions the experimental values agreed well with the predicted data. The optimal experimental conditions from this design allowed a fast, quantitative and maximum lipase activity. The results from these experiments could contribute to the development and use of this system on industrial scale.

REFERENCES

Amin, N. A. S. and Anggaro, D. D. (2004). Optimization of direct conversion of methane to liquid fuels over Cu loaded W/Z SM-5 catalyst. *Fuel* **83, 487-498.**

Burkert, J. F. M., Maugeri, F. and Rodrigues, M. I. (2004). Optimization of extracllular lipase production by *Geotrichum* sp. using factorial design. *Bioresource Technology* **91, 77-84.**

Cammarota, M. C. and Freire, D. M. G.(2006). A review on hydrolytic enzymes in the treatment of wastewater with high oil and grease content. *Bioresource Technology* **97, 2195-2210.**

Chen,J. Y., Wen, C. M. and Chen, T. C.(1999). Effect of oxygen transfer on lipase production by *Acinetobacter radioresistens* .*Biotechnology Bioengineering* **62, 311-316.**

Chopra , A. K. and Chander, H. (1983). Factors affecting lipase production in *Syncephalastrum racemosum. Journal of Applied Bacteriology* **54,163-169.**

Cihangir, N. E. and Sarikaya, E. (2004). Investigation of lipase production by a new isolate of *Aspergillus*

ISSN (print): 1823-8262, ISSN (online): 2231-7538

sp. *World Journal of Microbiology Biotechnology* **20, 193-197.**

DeConink, J., Leclercq, B., Exbrayat, M. J. and Duyme, F.(2004). Factorial design: An efficient approach to choosing the main factors influencing growth and hydrolase production by *Tetrahymena thermophila. Journal of Indian Microbiology Biotechnology* **31, 204-208.**

DeConink, J., Verdier-Denantes, I., Duyme, F., Bouquelet, S. and Dumortier, V. (2000). Modeling *Tetrahymena thermophila* growth and protease production. *Journal of Indian Microbiology Biotechnology* **25, 57-61.**

Design Expert Software Version 8.0.0, Statease Corporation(2009).

Ebrahimpuor, A., Rahman, R., EanChing, D. and Salleh, A. (2008). A modeling study by response surface methodology and artificial neural network on culture parameters optimization for thermostable lipase production from a newly isolated thermophilic *Geobacillus* sp. Strain ARM. BMC *Biotechnology* **8, 96-111.**

Elibol, M. and Ozer, D. (2000). Influence of oxygen transfer on lipase production by *Rhizopus arrhizus*. *Process Biochemistry* **36, 325-329.**

Frost, G. M. and Moss, D. A. (1987). Production of enzymes by fermentation. *In:* Biotechnology. Rehm, H. J. and Reed, G. W. (eds.). VCH VerlagsgessellschaftmbH Germany, **7a, 65-211.**

Garrido-Vidal, D., Pizarro, C. and Gonzalez–Saiz, J. M. (2003). Study of process variables in industrial acetic fermentation by a continuous pilot fermentor and response surfaces. *Biotechnology Program* **19, 1468-1479.**

Gutarra, M. L. E., Godoy, M. G., Maugeri, F., Rodrigues, M. I., Freire, D. M. G. and Castilho, L. R. (2009). Production of an acidic and thermostable lipase of the mesophilicfungus *Penicillium simplicissium* by solid state fermentation. *Bioresource Technology* **100, 5249-5254.**

Hoffmann, E. and Cleffman, G. (1981). External factors limiting the multiplication. Potential of *Tetrahymena*. *Journal of Cell Science* **50,407-418.**

Hölker, U., Höfer, M. and Lenz, J. (2004). Biotechnological advantages of laboratory- scale solid state fermentation with fungi. *Applied Microbiology Biotechnology* **64, 175-186.**

Hwan, L. D., Kim, J. M., Shin, H. Y. and Kim, S. W. (2007). Optimization of lipase pretreatment prior to lipase immobilization to prevent loss of activity. *Journal of Microbiology Biotechnology* **17(4), 650-654.**

Javed, M. M., Khan, T. S. and Ul-Haq, I.(2007). A 2^3 level full factorial design to optimize cultural conditions for lipase production by consortium of *Aspergillus niger* and *Trichoderma viride*. *Research Journal of Microbiology* **2 (8), 639-644.**

Kamimura, E. S, Medieta, O., Rodrigues, M. I. and Maugeri, F. (2000). Studies on lipase affinity adsorption using response – surface analysis. *Biotechnology Applied Biochemistry* **33, 153-159.**

Kamini, N. R., Fujii, T., Kurosu, T. and Lefuji, H.(2000). Production, purification and characterization of an extracellular lipase from yeast *Coryptococcus* sp. S2*. Process Biochemistry* **33, 153 -159.**

Kumar, C. G. and Takagi, H. (1999). Microbial alkaline proteases from a bioindustial viewpoint. *Biotechnology Advances* **17, 561-594.**

Lee, S. Y. and Rhee, J. S.(1993). Production and partial purification of lipase from *Pseudomonas putida. Enzyme Microbiology Biotechnology* **15, 617-623.**

Lima, V. M. G., Krieger, N. Sarquis, M. I. M., Mitchell, D. A., Ramos, L. P. and Fontana, J. D. (2003). Effect of nitrogen and carbon sources on lipase production by *Penicillium aurantigoriseum* . *Food Technology Biotechnology* **41, 105-110.**

Mutalik, S. R., Vaidya, B. K., Joshi, R. M., Desai, K. M. and Nene, S. N.(2008). Use of response surface optimization for the production of biosurfactant from *Rhodococcus* spp. MTCC 2574. *Bioresource Technology* **99, 7875-7880.**

Nadkarni, S. R. (1971). Studies on bacterial lipase. II. Study of the characteristics of partially purified lipase from *Pseudomonas aeruginosa*. *Enzymologia* **40, 286-301.**

Nemukula, A., Mutanda, T., Wilhelmi, B. S. and Whiteley, C. G. (2009). Response surface methodology synthesis of short chain fractooligosaccharides with a fructosyltransferase from *Aspergillus oculeatus*. *Bioresource Technology* **100, 2040-2045.**

Parry, R. M., Chandan, R. C. and Shahani, K. M.(1966). Rapid and sensitive assay for milk lipase. *Journal of Dairy Science* **49, 356-360.**

Rahman, R., Baharum, S. N., Salleh, A. B. and Basri, M. (2006). S5 lipase: An organic solvent tolerant enzyme. *Journal of Microbiology* **44, 583-590.**

Sharma, R., Chisti, Y. and Banerjee, U. C. (2001). Production, purification, characterization and application of lipases. *Biotechnology Advances* **19, 627-662.**

Vadehra, D. A. and Harmos, L. G.(1969). Factors affecting production of *Straphylococcol* lipase. *Journal of Applied Bacteriology* **32, 147-150.**

Zheng, Y., Wu, X. M., Branford-white, C., Quan, J. and Zhu-Li, M. (2009). Dual response surface-optimized process for feruloylated diacylglycerols by selective lipase. Catalysed transestesification in solvent free system. *Bioresource Technology* **100, 2896-2901.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538