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Physical parameter optimization by Response Surface Methodology for lipase production by *Aspergillus niger* and its partial purification

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ABSTRACT

Response Surface Methodology (RSM) is an empirical technique involving the use of Design Expert software to derive a predictive model similar to regression analysis. This present study explains the significant application of RSM in optimization of lipase production by *Aspergillus niger*. The experimental validation of the predictive model reveals 33% increase in lipase production. Furthermore, the molecular weight of the partially purified lipase was found to be 45,000 Da. Moreover, the purified protein can be used as an additive in laundry detergent formulation, enabling laundering at lower temperature pertaining to expenditure reduction.

Keywords: *Aspergillus niger*, Response Surface Methodology, Lipase, Detergent.

INTRODUCTION

Enzymes are unique biocatalysts produced by living cell and no life is possible devoid of it (Scheurwater et al., 2007). Lipases are group of enzymes, specifically hydrolyzing the ester bond of triacylglycerol (Bora and McKalita, 2009). These enzymes are derived from plants, animals and microbes (Anderson et al., 1979), whilst microbes make their presence felt as they can provide an unlimited source of enzymes. Moreover, the production limit can be expanded to meet any level of demand (Kamini et al., 1997). *Aspergillus niger* is an unique fungi of great industrial interest, due to its ability to produce variety of commercial enzymes (Shu-Fei et al., 2008). Work on fungal lipases started as early as 1950's and a comprehensive review on various aspects has been presented by Fukumoto et al. (1963) and subsequently well documented by Brockerholf and Jenson (1974) and many successors. As per the documented studies, both physical and chemical factors affect the production of lipase by microorganisms (Mahanta et al., 2007). The optimum condition for lipase production was significantly demonstrated by Teng and Xu (2008). Response surface methodology (R.S.M) is a widely used statistical method for optimal design of enzymatic reactions and can be applied for the maximization of lipase production (Chen et al., 2008). Hence, the present study was framed with the objectives involving optimization of lipase production medium by R.S.M (Response surface Methodology) and partial purification of the same, enabling improved industrial productivity.

MATERIALS AND METHODS

Microorganism and maintenance of culture

The fungal strain *Aspergillus niger,* isolated from our laboratory was chosen for the present study. The fungal strain was maintained on Czapekdox medium under laboratory condition. The culture of the *Aspergillus niger* for lipase production was carried out in the R.S.M optimized medium, containing Magnesium Sulphate, meat extract, olive oil, urea and water. The optimization of physical parameters was performed by R.S.M, wherein a series of experiments were designed that will yield adequate and reliable measurements of the response of interest. Using the data from the set of experiments, a mathematical model that best fits the data is determined by conducting appropriate tests of hypotheses concerning the optional parameters and determining the optimal settings of the maximum value of the response. The predicted model was validated by ANOVA with $p < 0.05$ level of confidence.

Physical parameter optimization

According to the predicitve design, pH of 6 and 7 was maintained by phosphate buffer, pH of 5 and 7.68 was maintained by citrate-phosphate buffer. Temperature optimization for lipase production was performed by fixing the temperature in a shaker incubator. Inoculum concentration was also varied in accordance to the design by varying the levels in micropipette, pertaining to enzyme activity assay. The enzyme assay was performed by titrimetric method as documented by Ota and Yamada (1962).

Partial Purification

The partial purification of lipase was performed by Ammonium Sulphate precipitation and the presence of suspected protein was confirmed by SDS-PAGE. The 72 hrs old fermentation broth culture was centrifuged at 10,000 rpm for 20 min at 40 ºC. The extracted supernatant was treated with Ammonium Sulphate to obtain final concentration of 20% saturation and equilibrated for 30 min at 4 ºC with overnight stirring. The resultant precipitate was collected by centrifugation at 10,000 rpm for 20 minutes at 4 ºC. The above mentioned precipitation procedure was followed for 40%, 60% and 80% saturation with ammonium sulphate, and the respective precipitates were collected. To study the homogeneity of the enzyme, SDS-PAGE was carried out as described by Laemmli (1971). The resultant bands were confirmed by silver staining.

RESULT AND DISCUSSION

Physical parameter optimization and partial purification

The 3D counter plot (Figures 1 et 2) clearly portrays that at an inoculum volume of 150 µl with the temperature of 25 ºC, maximal lipase activity is produced. Furthermore, the second 3D plot clearly indicates that a pH of 5 with the temperature of 25 ºC exhibits maximum lipase activity. Thus, the maximal pH of 5 with temperature of 25 ºC and inoculum volume of 150 µl are the optimal combination, as per the resulted 36 units of enzyme activity, in comparison with the previously optimized media. This reported activity is 10% higher than the previous studies (Dutta et al., 2004). Thus, the experimental design by R.S.M for lipase production from *Aspergillus niger* has resulted both in a good predictive model ($p<0.05$) which has proven significant rise in enzyme production by optimization of physical parameters in the present study. This falls in line with the study on *Pseudomonas aeruginosa* by Ruchi et al. (2008). The molecular weight of ammonium sulphate precipitated lipase was determined with the standard protein molecular weight marker. The approximate molecular weight of the sample was found to be 45,000 Da, confirming the presence of lipase (Figure 3).

The major application of lipase is confined to degreasing of leather in tannery industry (Hasan et al., 2006). Degreasing is a process wherein the fat particles embedded in the skin are hydrolyzed using chemical substance like chromium. The use of these chemicals pollutes the environment heavily. Alternative usage of lipases for leather degreasing has been proven to be eco-friendly (Thanikaivelan and Rao, 2004). The present study was initiated with an intention to accelerate eco-friendly degreasing of leather. Hence, we report the significant optimization of physical parameter of media in lipase production from *Aspergillus niger* by R.S.M.

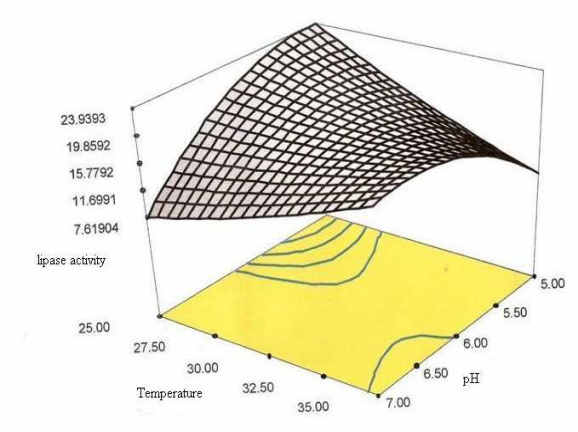

Figure 1: Plot showing the effect of temperature and pH on lipase activity.

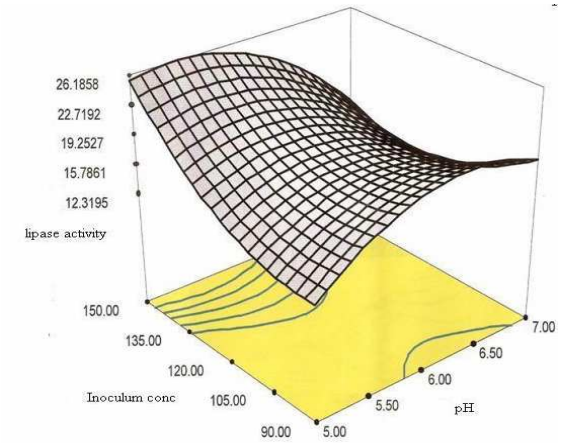

Figure 2: Plot showing the effect of inoculum concentrations and pH on lipase activity.

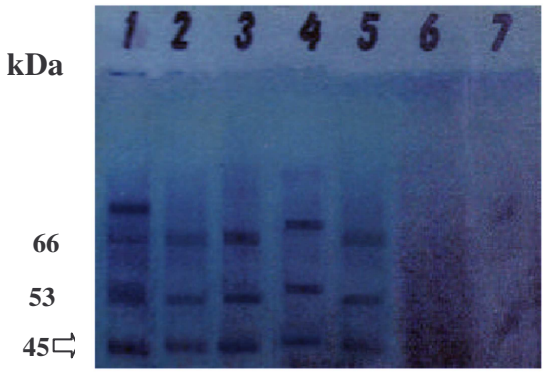

Figure 3: SDS-PAGE analyses of the purified lipase from *A. niger*. The arrow (►) indicates the position of the purified lipase. Lane 1; Protein molecular mass markers. Lane 2; Ammonium sulphate precipitated sample. Lane 3; Dialysed sample. Lane 4-7; Crude sample.

In conclusion, the study on lipase as per our experiment has resulted in optimal productivity; the strategy implemented in this study shall be applied for varied types of commercial enzymes leading to optimized productivity. Moreover, we present the results of our study to the leather research community for further validation during mass production of lipase.

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