Theme No:VI Sub topic: Cleaner Production & Emerging Sustainable Practices

Optimization of production of lipase from *Staphylococcus hominis* using response surface methodology

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Abstract

Lipases, versatile hydrolytic enzymes are gaining more importance in environmental applications such as treatment of fat, oil and grease (FOG) containing wastewater, pretreatment of solid waste/industrial wastewater for anaerobic treatment etc. The use of lipase enzyme in pre/treating such wastewaters and solid wastes are considered as greener and sustainable approach compared to physical and/or chemical methods. The maximum production of lipases is indispensable for economical point of view. Therefore, the present study attempts to improve the production of lipase from *Staphylococcus hominis* MTCC 8980 by optimizing physical factors namely pH, temperature and agitation in batch shake flasks. The experiments were designed using full factorial central composite design (CCD) and the results were analyzed using response surface methodology (RSM). Total twenty experiments were conducted to optimize the pH, temperature and agitation speed for maximum production of lipase. The regression model could explain the variation of 94.09% (R^2 = 94.09). The analysis of variance (ANOVA) from response model indicates that temperature and agitation significantly influence the lipase production (p value <0.05). The interaction term for pH and agitation was found to have positive significant effect on lipase production. The model predicted 1.5 fold increase in lipase activity (1.85 U) under optimized conditions of pH (8.0), temperature (33.1°C) and agitation speed (178 rpm).

Keywords: Lipase, Central composite design, optimization, enzyme activity, *Staphylococcus hominis*

Table 1: A 2^4 full-factorial central composite design matrix of pH, temperature and agitation in coded and actual values with experimental and predicted values of lipase production.

Table 2: Analysis of variance (ANOVA) for quadratic model.

Table 3: Model coefficient estimated by multiple linear regressions.

Fig. 1: Growth Curve of *Staphylococcus hominis*.

Fig. 2: Effect of incubation period on lipase activity.

Fig. 3: Response surface plots for lipase production showing the interactive effects of parameter studied.

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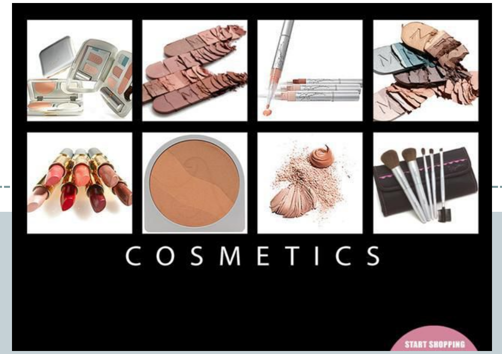
Introduction

- Enzyme mediated processes have been under usage from ancient times, out of approximately 4000 known enzymes only 200 enzymes are commercially used.
- Among microbial enzymes, lipase has been studied extensively.
- Lipase is a versatile enzyme which can catalyze hydrolysis, esterification and transesterification reactions.
- Several industries may utilize this various kind of reactions for production of different products
- Since each industrial application may require specific properties of the enzyme, there is an interest in finding new lipases that could create novel applications.

Dairy Industry

cosmetic Industry



Food and beverages Industry

Applications

Detergent Industry

Biodiesel

Pharmaceutical Industry

OBJECTIVE

- Optimization of physical process parameters for lipase production from *Staphylococcus hominis*.

Materials and methods

Table. 1 Basal Mineral Medium composition

Components	Composition (g/100ml)
$(NH_4)_2SO_4$	0.5
$NaNO_3$	0.05
K_2HPO_4	0.1
KH_2PO_4	0.05
KCl	0.1
$MgSO_4.7H_2O$	0.03
$CaCO_3$	0.05
Yeast extract	1
Olive oil	2

Table. 2 Trace elements solution composition

Components	Composition (g/100ml)
H_3BO_3	0.5
$CuSO_4.5H_2O$:	0.5
$MnSO_4.H_2O$	0.5
$MoNa_2O_4.2H_2O$	0.06
$ZnSO_4.7H_20$	0.7

100 ml of the basal medium was supplemented with 0.05 ml of trace elements solution

Table. 3 Operating condition

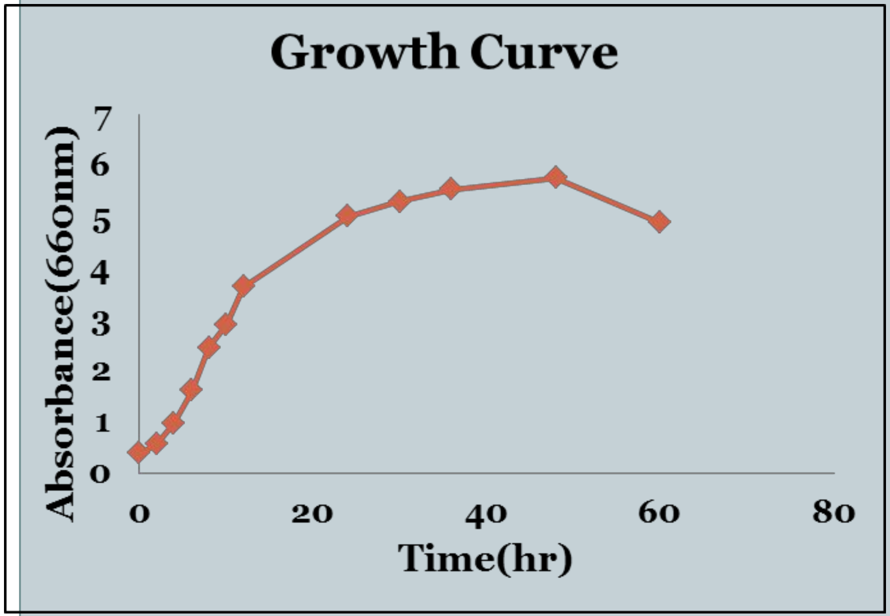
Parameters	Condition
Agitation (rpm)	150
Temperature (°C)	37
pH	7

Analyses

- Growth of the bacterial culture was measured at 660 nm.

- Lipase activity was measured using olive oil as substrate. One unit of lipase activity was defined as 1.0 µmol of free fatty acid liberated per min.

Growth and production of lipase from S. hominis



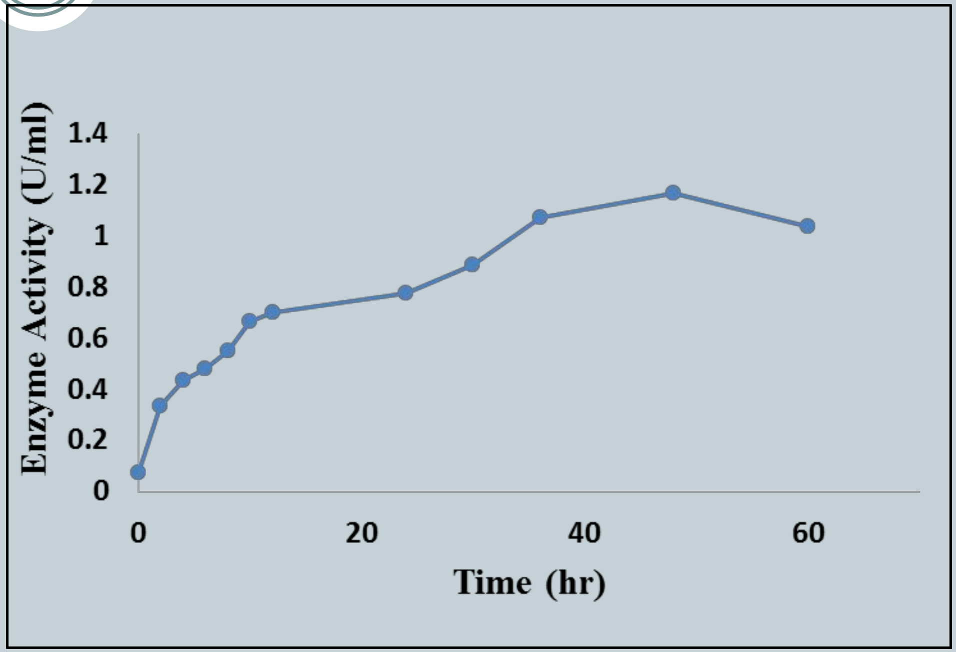


Fig. 1. Growth profile of S. hominis

Fig. 2 Lipase activity profile of S. hominis

Optimization of process parameters by Central composite design

Central Composite Design (CCD)

- No. of factors : 3
- Total no. of runs = 20 (in duplicates)
- Response : Enzyme activity (U/ml)

Table 4. Experimental codes, ranges and levels of the independent variables for response surface methodological experiment (α =1.633)

Variables	Symbol coded	Range and levels				
		-α	-1	0	+1	+α
pH	X_1	5.5	6	6.8	7.5	7.9
Temperature	X_2	27.5	30	34	38	40.5
RPM	X_3	31.5	60	105	150	178.5

Conclusion

- The interaction term for pH and agitation was found to have positive significant effect on lipase production.
- The model predicted 1.5 fold increase in lipase activity under optimized conditions of pH temperature and agitation speed.