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CULTURE MEDIUM OPTIMIZATION OF α-AMYLASE PRODUCING ORGANISM *MOCUR SPP*. USING THE VARIABLE SIZE-SIMPLEX ALGORITHM

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ABSTRACT

Effects of different culture conditions on growth and production of amylase by *Mucor sp*, using the variable size - simplex design, was investigated. The results showed that the optimum condition are pH of 5.5 -7, temperature of 23.8- 25 ^{o}C, shaking rate of 126 -155 rpm, starch at the concentration of 5.6 - 8 g/L and arginin at the concentration of 4.3 - 5.7 g/L.

Keywords: *Mucor spp*, Optimization, Simplex Algorithm, Culture medium

INTRODUCTION

Amylases are among the most important enzymes and are of great significant in biotechnology and are commercially important in various starch processing industries (1). Although amylases are derived from different sources such as plants, animals and micro-organisms, the enzymes from microbial sources generally meet industrial demands. Microbial amylases could be potentially useful in the pharmaceutical and fine-chemical industries if enzymes with suitable properties could be prepared. With the advent of new frontiers in biotechnology, the spectrum of amylase application has widened in many other fields, such as clinical, medicinal and analytical chemistry, textile, food, brewing and distilling industries, as well as starch saccharification (2). Many organisms are capable of amylase production of which fungi are well known because of their abilities to excrete enzymes into the environment. While there are several reports about the control of extracellular amylase production by fungi (3), optimization of cultivation conditions is expected to improve the enzyme production. In this investigation, the effect of different cultivation conditions on growth and amylase production by the isolated organism using the variable size simplex algorithm analyses was investigated.

MATERIALS AND METHODS

Microorganism and inoculum preparation

Mucor spp was isolated from food spoilage and a voucher specimen (M. 700) deposited at the Department of Pharmacognosy in the School of Pharmacy, Shaheed Beheshti University of Medical Sciences.

For inoculum preparation, the organism was grown on malt extract agar at 25 ^{o}C for 3 days. The number of spores ware determined by the neubauer counting chamber and inoculum was adjusted to 1.6×10^{7} spores /ml.

Growth medium and cultivation medium

The fungus was grown in basal liquid medium containing $MgSO_4$, NH_4Cl, NaH_2SO_4 in concentrations of 0.5 g/L and different amounts of starch and arginin (according to the optimization design). The medium was inoculated with 0.5 ml of spore suspension (1×10^{6} spores/mL) and incubated for 72 hours at 25^{o}C. The mycelium was separated from the culture medium by filtration and the filtrate was used to determine the enzyme activity. Dry weight was determined after drying the mycelium at 105^{o}C for 24 hours.

Effect of different factors including: temperature, initial pH medium, shaking rate, starch and arginin concentration, on growth and amylase production were determined.

Enzyme assay

Amylase activity was determined (4) on the basis of the amount of reducing sugar which was released following the dinitrosalyicilic acid (DNS) method. One unit of amylase activity was defined as the amount of enzyme that released 1 µmol of reducing sugar equivalent to glucose per min under the assay condition.

Statistical analysis

The study was carried out and presented in the Variable – size simplex algorithm described previously by Spendly (5).

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Table 1. The summary of all simplex moves obtained from growth and amylase activity of *Mucor spp.*

Vertex[a]	pH	Temp. (°C)	Shaking rate (rpm)	Starch (g/L)	Arginin (g/L)	Biomass (g/L)	Response[b] (U/ml)
1	5.5	25	200	8	5	1.9	10
2	5.5	25	150	5	4	0.8	6
3	5	20	100	4	3	0.6	5
4	4.5	30	200	6	3	0.3	4.6
5	6	25	100	7.2	5	0.3	3.9
6	4	20	150	3.6	2	0.28	3.6
7	6.6	30	150	8.4	6	1.7	15
8	4.8	27	200	5.2	3.4	0.9	7
9	6.46	20.8	120	6.2	5.4	2.1	20
10	6.6	31.2	200	9.2	7	0.4	3.7
11	5.4	22.8	132	5.2	3.8	1.8	23.6
12	5.5	25.05	155	5.6	4.3	2	26.1
13	6.8	23.5	126	7.3	5.2	2.1	27
14	6.9	23.8	135	8	5.7	2	26

[a] Experiment number, [b] One unit of amylase activity is defined as the amount of enzyme that released 1 µmol of reducing sugar equivalent to glucose per min under the assay condition

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The simplex methods are based on an initial design of k+1 trials, where k is the number of variables. A k+1 geometric figure in a k-dimensional space is called a simplex. The corners of this figure are called vertices.

Five variables were selected and the initial simplex design was based on six trials. After initial trials, the simplex process continued by the addition and evaluation of one new trial. The simplex searches systematically the best levels of the control variables. The optimization process ends when the optimization objective is reached or when the responses cannot be improved further.

RESULTS AND DISCUSSION

The summary of all sequential simplex moves and results, are shown in Table1. Since comparison of results indicated that results from the last three experiments are almost similar and simplex has converged adequately, no further experiments were carried out. The differences in enzyme concentration in vertex #10 in comparison with the vertexes #12, #13 and #14 may be due to higher shaking rates. The effect of mechanical stress and physical damage to the culture medium may results in mycelial fragmentation and lower production of the enzyme. This is in agreement with the report that liquid shear is a major factor in bringing physical damages to filamentous fungi (6). However, low shaking rate also resulted in the low growth and production of amylase. This observation is supported by comparison of results in vertex #5 with those in the last three experiments. One possible explanation for this observation may be the interference of the size of the pellets which makes it difficult for oxygen molecules to diffuse into the centre of pellets. This is in good agreement with the reports (7,8) that specific oxygen consumption of pellets decrease with increase in the pellet size.

The concentration of starch as substrate had influences on the production of the enzyme. It was also observed that increase in the concentration of starch in the culture medium enhanced fungal growth and enzyme production. The differences in the enzyme activity which was observed in vertex #7, where the starch concentration is 8 g/L, may be attributed to the medium temperature.

Our results indicate that the effect of nitrogen concentration, compare to starch concentration, on amylase production is insignificant which is in agreement with results of other reports (9).

CONCLUSION

The results of this study show that there is not a single end point for termination of optimization process. However the simplex algorithm has converged sufficiently to make a decision about the best composition of variable in order to optimize conditions for growth and production of amylase.

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