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Research Article

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Effect of stirring speed in lipase production using germinated maize oil

M. Sathish Kumar[1], C. M. Karrunakaran[1], M. Vikram[1] and M. R. K. Rao[2]

[1]Department of Industrial Biotechnology, Bharath University, Chennai
[2]Department of Biomedical Engineering, S.M.K. Fomra Institute of Technology, Chennai

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ABSTRACT

The present study deals with the production of lipase enzyme from Bacillus sp. isolated from the soil samples from around Bharath University, Chennai, India. One of the parameters in the production of lipase is the speed levels of agitation performed in the bioreactor. Germinated maize oil was used as the main carbon source along with other substrates. Different trials at different ranges of agitation speeds were performed. It was observed that maximum lipase production of 48 IU/ml was achieved at 700 rpm agitation speed.

Keywords: lipase, enzyme, bacillus, agitation, rpm.

INTRODUCTION

Maximum enzymes are produced by the fermentation of biological materials [8] for various industrial and domestic purposes. Breakdown of fats are involved by lipolytic enzymes and also involved in the movement of lipids within the cells of individual organisms as well as in the transfer of lipids from one organism to another [9]. Inducible enzymes are mostly produced by bacterial lipases. Very few reports are available of constitutive lipase production by bacteria. [10,11]. In a bioreactor, mixing is a process of two or more substances allowed to enter a chamber and they are combined. Mostly, stirring or agitation is the method to combine compounds. Stirring mechanism is an arm of rotating that is often powered with a motor. In Some types, the chamber itself is rotated to enhance mixing. Different levels of mixing and stirring speeds are required for different substances. Because of these variables, speed of the agitator can play a key role in the mixing process. Aeration and agitation on fermentation broth has been studied in stirred tank reactor for lipase production using *Rhodotorula mucilaginosa* MTCC 8737 [4]. Lipase production from molasses as a sole production medium by varying aeration and agitation were reported. Maximum lipase activity was obtained during 96 hours of fermentation at 2, 200 rpm, pH 7, and 25 ± 2^0C temperature. Indu Bhushan *et al* [2] optimized the fermentation conditions for the production of lipase from *Arthrobacter sp*. The scale of the shake flask was upscaled to 10L fermentor by them. The culture was grown at 30 ^{o}C for 18 – 24 h in an ideally mixed batch fermentor with aeration of 0.7vvm, agitation of 500 rpm and pressure of 0.5 Ibs/in2. Krastanov *et al* [3] attempted to study the effect of the parameters on lipase production .The production of lipase by *Candida cylindracea* NRRL Y-17506 focuses on the effects of aeration, agitation and dissolved oxygen on lipase production by submerged fermentation. The analysis showed that enhanced lipase production can be achieved with 200 rpm, 0.5 vvm and 100% dissolved oxygen concentration. The present study is in continuance of our project dealing with germinated maize oil as the only source of carbon for the production of lipase enzyme. It has already been reported by us on parameters like, effect of different carbon sources, pH, temperature and fed batch studies. [6,7]. The present study deals with the effects of rpm on the production of lipase enzyme by *Bacillus spp*.

EXPERIMENTAL SECTION

Strain isolation

The aerobic strain was primarily isolated from soil sample near to our university, chennai, India. The strain was separated by serial dilution. The bacillus strain was an aerobic and rod-shaped, which could grow within a temperature range of 30°C -55°C with an optimum at 50°C and with an optimum pH of 6.0. Based on its morphological and physiological characteristics, the strain was assigned as *Bacillus spp*. The culture was allowed to grow in a nutrient agar medium. The agar plate was maintained in an incubator at a temperature of 50°C. [6]

Subculture of *Bacillus* strain

A loop full of strain grown in the agar plate was transferred to a100ml of nutrient broth medium through inoculation loop. The culture in the nutrient broth medium was allowed to grow for 24 hours. Here the pH maintained was 6.0 and the temperature was maintained at 45°C. The culture was kept in an incubator shaker at 100 rpm. [6]

Carbon Sources for Lipase Production

Different carbon sources were used for the production of lipase like maize oil, coconut oil, olive oil, soybean oil and ground nut oil from local markets. It was decided to germinate the maize seeds and to check its production. Maize seeds from the local market was taken and allowed to germinate for a period of three days. Other than maize different carbon sources were used for the production of lipase where glucose, lactose, sucrose, maltose, fructose, olive oil, oleic acid, linoleic acid, linoleinic acid, maize oil, germinated maize oil, castor oil, coconut oil, olive oil, soybean oil ground nut oil, sesame oil, Tween 20 and Tween 80.[6]

Germination & Extraction of seeds

Maize Seeds were germinated by covering the seeds with a wet cotton cloth and periodically the moisture was maintained in the cloth. Then after the fourth day the germinated seeds were allowed to dry in the outside atmosphere. Then the dried seedswere size reduced for enough distribution in the Soxhlet's apparatus for oil extraction.[6]

Medium for Lipase Production

The shake flask experiments were done for screening of suitable carbon source in modified Tryptone Yeast Extract Medium (TYEM). Screening for higher lipase production using various carbon sources was carried out using batch experiments. Lipase was produced from *Bacillus spp* using FB medium supplemented with either carboxylic acids (fatty acids) or carbohydrates as the sole carbon source (10 g/L). Different carbon sources were used for the production of lipase like glucose, lactose, sucrose, maltose, fructose, oleic acid, linoleic acid, linoleinic acid, maize oil, germinated maize oil, castor oil, coconut oil, olive oil, soybean oil, ground nut oil, sesame oil, Tween 20 and Tween 80. The cell mass was maximum for groundnut oil but the lipase activity was less when compared to the olive oil and germinated maize oil as a substrate. The lipase activity from olive oil and germinated maize oil showed maximum at their late logarithmic phase or stationary phase. Instead of using olive oil, germinated maize oil was taken as a sole carbon sources for all studies for cost effective production.[6]

Batch reactor studies:

Batch culture was carried out with a working volume of 1.5 L in a 2.4-L bioreactor (Bioengineering AG, Switzerland), the medium composition for batch fermentation was 4 g/L K2HPO4, 7 g/L Na2HPO4.12H2O, 1.2 g/L (NH4)2SO4, 0.2 g/L NH4Cl, 10 mg/L MnSO4.7H2O, 2 mg/L ZnSO4.7H2O, 1 mg/L AlCl3.6H2O, 1 mg/L CuCl2.2H2O, 0.5 mg/L H3BO3, 1 g/L MgSO4.7H2O, 40 mg/L FeSO4.7H2O and 40 mg/L CaCl2 (pH 6.5). The inoculum size of 10 % was transferred from the subculture. The growth 70 temperature was maintained at 37°C and the pH was maintained at 6.5 throughout the cultivations. The DO value was maintained above 25% air saturation in order to prevent oxygen limitation. Every three hours, the samples were collected from the fermentor to find and lipase activity.[6]

Lipase Activity:

For titrimetry assay of lipase, 20 ml olive oil was added to 80 ml of 20 g/l polyvinyl alcohol solution and sonicated using Branson sonifier 450. The reaction mixture composed of 5 ml olive oil emulsion, 4 ml glycine-NaOH buffer (0.1 M, pH 9.0), and 1 ml of enzyme sample. This mixture was incubated at 30 °C in shaking water bath at 180 rpm for 1 h. At the end of the 48 incubation, the emulsion was broken down by addition of 20 ml acetone: ethanol mixture (1:1) and the liberated fatty acids were titrated with 0.05 N NaOH [5].

RESULTS AND DISCUSSION

The Effect of Agitation on Lipase Production:

Experiments to assess the most favourable agitation for higher lipase production were carried out in a 2.4-L bioreactor (Bioengineering AG, Wald, Switzerland) with a working volume of 1.5 litres. The agitation plays a key role in the fermentation, since the optimal agitation will increase the dissolved oxygen concentration that triggers the higher growth of cells and also maximal amount of the product. When *Yarrowia lipolytica* was used for lipase production at a constant air flow rate (Q = 1 dm3/min, 0.8 vvm) and different stirring speeds, the highest lipase levels (4,680 and 5,300 U/L, for the titrimetric and spectrophotometric methods, respectively) were obtained at 240 hours and 200 rpm. When stirring speed was raised to 300 rpm, an early enzyme release into the medium was observed, with maximum activity levels (2,390 and 1,500 U/L, for the titrimetric and spectrophotometric methods, respectively) at 120 hours and considerably lower levels of 960 and 830 U /L were seen at 100 and 400 rpm, respectively, for the titrimetric method. Protease enzyme was also detected from the beginning of cultivation for all stirring speeds. The lowest cell growth (4.3 g cell dry weight/L) was observed at 100 rpm, suggesting limitation of oxygen. Biomass concentration (8.7 g cell dry weight/L) was highest at 200 rpm. The decrease in biomass concentration after 100 hours at 200 rpm was possibly due to cell removal from the medium caused by the formation of foam. Increase in stirring speed to 300 and 400 rpm resulted in lower biomass concentrations (5.9 and 5.3 g cell dry weight/L, respectively), probably caused by mechanical stress. Parallel profiles for viability and cell dry weight were found at 300 and 400 rpm. Viability remained unaffected at 300 rpm, while at 400 rpm a drastic drop was seen after 24 hours [1]. In the present study the fermentation was carried out in a batch mode with the germinated maize oil as a sole carbon source, the temperature and the pH of the fermentation also kept constant 35°C and pH 6.5 respectively. The range of agitation was used from 400 rpm to 1000 rpm.

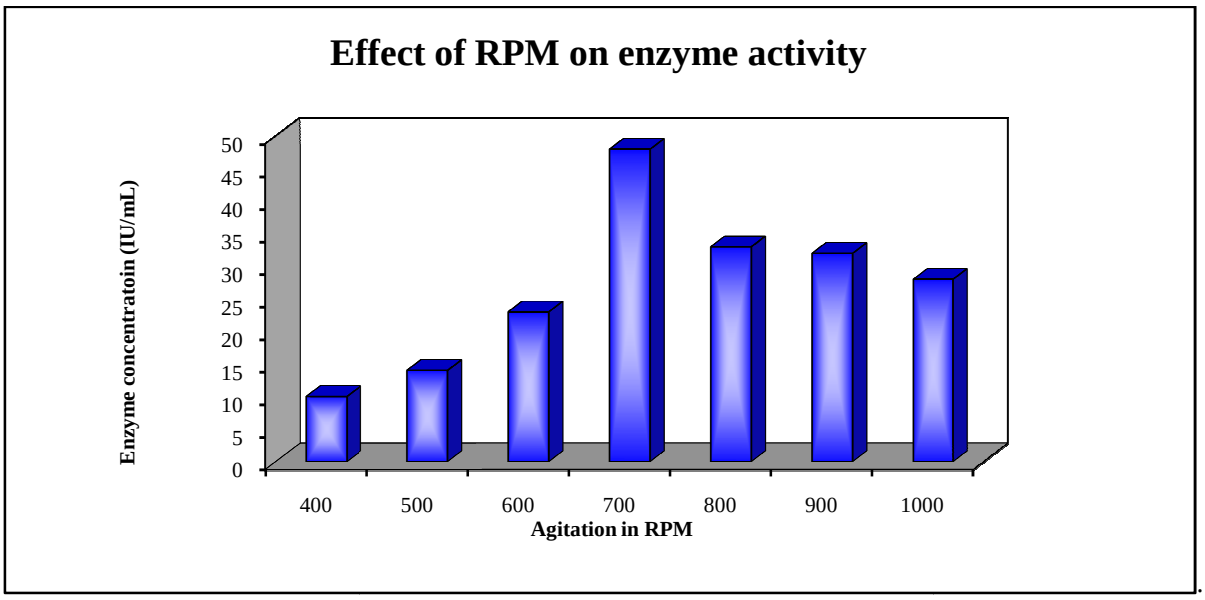

Figure 1

As represented in figure 1 it was observed that the enzyme production keep on increasing when the agitation increase and it attained the maximum of 48 IU/mL at 700 rpm and kept on decreasing on further increase of agitation. The agitation was given at 700 rpm and maintained as the working agitation for further studies.

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