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PRODUCTION OF LIPASE UTILIZING LINSEED OILCAKE AS FERMENTATION SUBSTRATE

Dharmendra K. Parihar

Department of Biotechnology, University Teaching Department, Guru Ghasidas University - A Central University, Bilaspur- 495009, Chhattisgarh, India,
Email: parihardkp@rediffmail.com

Abstract: Agricultural by products such as different oilcakes, bran and baggasse contain not only a good nutrient but also are rich in lipids, which is a very good inducer for microbial lipase production and also carbon source for lipolytic microbes. Linseed and mustard oil cake were used as fermentation substrate for lipase production and was compared with those by synthetic media. Different parameter such as effect of fermentation time and effect of additional carbon and nitrogen source were studied. It was found that both linseed and mustard oil cakes were found useful for lipase production. Addition of nutrient supplement with linseed oil cake did not show increase in lipase production where as it is required in case of mustard oil to produce significantly high lipase.

Key words: lipase production, linseed oil cake, Lipase assay, Solid-state fermentation.

INTRODUCTION

Lipases are hydrolytic enzymes that act in aqueous-organic interfaces, catalysing the cleavage of ester bonds in triglycerides and producing glycerol and free fatty acids. It catalyse esterification, interesterification and transesterification reactions. [1] It has got wide industrial applications such as in detergent, dairy product, bakery foods, pharmaceuticals, cosmetics, leather industries and paper pulp industries. In addition to these, there are several promising fields for lipases like in biodegradation of plastics, *viz.* polyhydroxyalkanoates and polycaprolactane [2], and resolution of recemic mixtures to produce optically active compounds [3]. As in many applications that demand high enzyme yields, lipase production depends on the cost reduction so as to be economically viable. The factor affecting enzyme production cost being the raw materials used as substrate for microbial growth, inducer and down stream processing. Several agro

byproducts including wheat bran, rice bran, dexrin, sugarcane baggase, oil cakes such as coconut oil cake, olive oil cake and gingili oil cakes have been found a potential substrate for growth of microorganisms supplying essential nutrients to them lipase production [4-9]. Solid wastes from the production of vegetable oils (oil cakes) have been widely used for the production of industrial enzymes, antibiotics, biopesticides, vitamins and other biochemicals because they are excellent supports for microbial growth as well as interesting sources of nutrients, requiring low or no supplementation. Furthermore, they are inexpensive and plentiful in countries like Brazil [9]. For last more than one decade such solid substrates have been evaluated for lipase production and shown its potentials as efficient substrate for lipase production has been reported. Coconut cake has been used as a potent substrate for production of lipase by Candida [10]. Gingelly oil cake [8] olive oil cake [11-12] and olive oil cake in combination with wheat bran [13] have been reported as efficient source. Similarly palm oil [14] babasu oil cake [15] used as substrate by tropical marine yeast used in mill effluent

In the last two decades, solid state fermentation (SSF) has attracted increasing attention for the production of enzymes, metabolites, etc., due to several biotechnological advantages such as higher fermentation productivity, higher end-product concentration, higher product stability, and lower catabolic repression [16]. Solid-state fermentation is the fermentation on moist solid substrate in the absence or near absence of free water, thus being close to the natural environment to which microorganisms are adapted [17]. The economic evaluation of lipase production from *P. restrictum* in SSF and submerged fermentation (SmF) [18] For a 100m3/year scale, total capital investment in SmF was 78% higher than that in SSF. Additionally, SSF seemed to be more attractive from the economical viewpoint, because the unitary lipase cost was 47% lower than the selling price in SSF, while 68% higher than that in SmF. More importantly, SSF offers a useful tool for processing agro-industrial residues. [19]

The present research emphasizes on evaluation of ground nut, mustard and linseed oil cake for lipase production through SSF by *Pseudomonas aeruginosa* LB-2, process optimization and characterization of lipase.

MATERIALS AND METHODS

Bacterial strain and its maintenance: lipolytic bacterial strain *Pseudomonas aeruginosa* LB-2 was used from our own culture collection. The bacterial isolate was maintained on tributyrin-nutrient agar slants containing peptone; 0.5%, beef extract; 0.3%, NaCl; 0.5% and Tribyutyrine 20% (sterilized separately).

Fermentation of oil cake: Ground nut Linseed and mustard oil cake were obtained from the local oil mills of Bilaspur Chhattisgarh.

Fermentation substrate was prepared according to the method described by Mahanta *et al* [20] with slight modifications. Oilcakes were dried in air, crushed in order to get a mixture of coursed and powered oil cake. A 25g of oilcakes was taken in a 500 ml Erlenmeyer flask and moisturized with 25 ml of distilled water. The contents of the flask were mixed and autoclaved at 121°C for 20 min. twenty five milliliter of sterilized water was added to the autoclaved medium.

The beakers were incubated for 6 days in a climatic chamber at different temperatures, with humid air injection. Relative humidity of the air inside the chamber was kept at about 99%. Samples were taken periodically to monitor pH, cake moisture, and lipase activity.

Moisture and pH determination: Two 0.5-g samples were taken from each beaker for moisture and pH determination. Moisture content was measured by gravimetry, and pH was measured after adding 5 ml of deionized water to the sample, using a pH meter.

Enzyme extraction: Enzymes extraction was carried out by the method of Ramachandran et al. [21] Crude enzymes were extracted by mixing a known quantity of fermented substrate with 10ml of 0.1 M Sorenson phosphate buffer, pH 8.0, and then shaking the mixture in an orbital shaker at 250 rpm for 2 min. The suspension was then centrifuged at 12,000 rpm for 10 min and the supernatant used for lipase assay.

Lipase assay: The lipase activity measured in the enzyme extract by the method described by Winkler and Stuckmann, [22] using *p*-nitrophenyl palmitate as substrate, where one unit of lipase activity was defined as the amount of enzyme releasing 1µmol of *p*-nitrophenol per minute under the assay condition.

Protein estimation: The protein content in the enzyme solution was determined by the method of Bradford [23] using bovine serum albumin as standard.

RESULT AND DISCUSSION

Lipase production has been shown to influence largely by the presence of triglyceride content in the medium. It is assumed that oil induce lipase production [24-25]. Oil cakes contain significant amount of oil along with the seed proteins that become available to the lipolytic organisms as carbon and nitrogen source for their growth. Apart from this some essential nutrients and minerals are also found in oilcakes.

In an experiment the SSF substrate was supplemented with olive oil to study requirement additional of triglyceride for lipase production (Fig. 1). Supplementation of the substrate with oil led to an increase in lipase activity. This might be owing to acceleration of the metabolism of the microorganism. However, the total enzyme production per grams of oil cake could not be assessed and must be analyzed carefully, since factors such as inoculums age and physiologic state may directly affect this result.

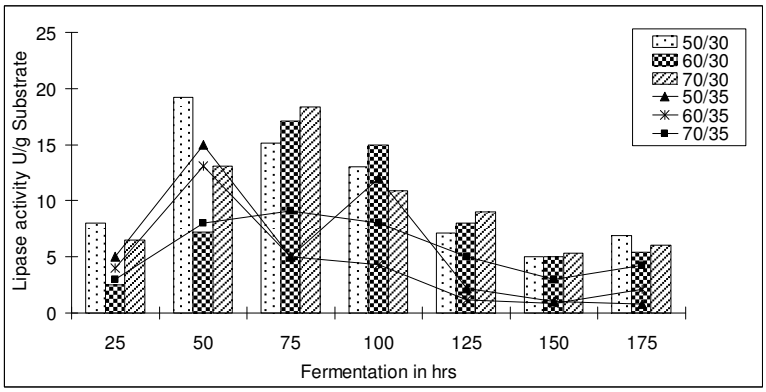

Figure: 1. Effect of olive oil on lipase production

The moisture content in ssf is essential for proper growth of microorganism [26] An experiment was designed to study relation between lipase production and moisture content of substrate. The moisture level of fermentation medium was monitored during the process in all experiments. In most of the studied conditions, the moisture was quite constant with time until 100 h of fermentation.

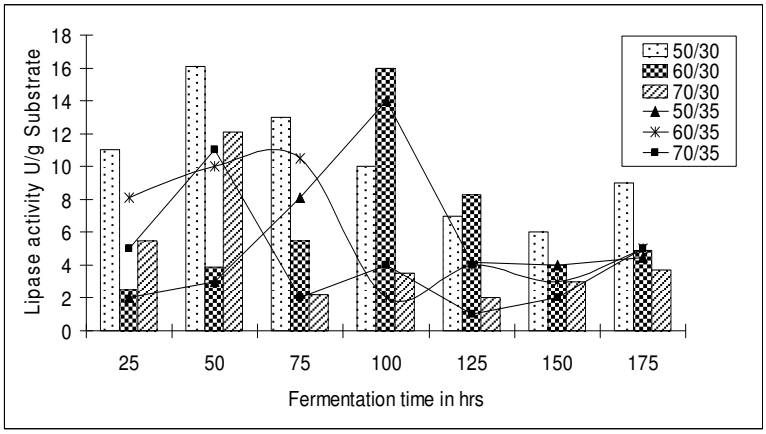

Figure: 2. Effect of moisture on lipase production

The only exception to this behavior occurred at low initial moisture conditions. In such experiments, there was a strong decrease in moisture of the oil cake during the course of fermentation. The increase in incubation temperature caused a stronger decrease in cake moisture, probably owing to the higher water uptake rate by the microorganism and the increase in water vapor pressure in the system at higher temperatures.

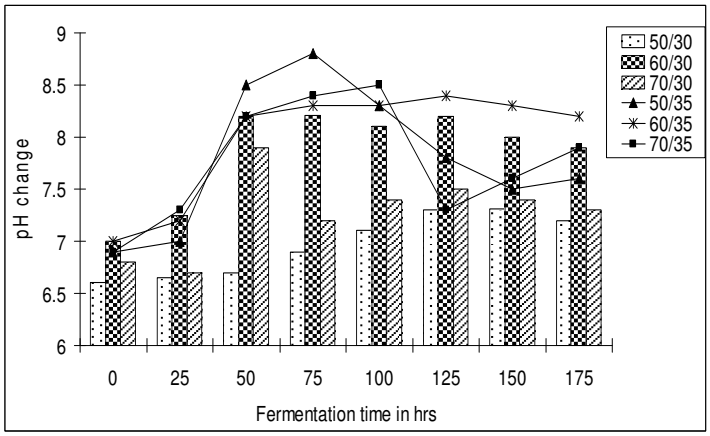

Figure: 3. pH Change during fermentation

Lipase production at 30°C temperature has been seen during 50 to 100hrs of fermentation then it decreases considerably. At the end of the process it again increases. At 35°C lipase production is low as compare to low temperature. The reason behind such behavior may be correlated with the increased in protease production at 35°C lead deactivation of lipase as explained by Gombert *et al.* [27] and Palma *et al*. [28] The slight increase in lipase production at the end may be deactivation of protease [29].

Table: 1. Optimum condition for lipase production

Substrate	Lipase activity U/g	Temp. °C	Time hrs	Moisture %
Linseed oil cake	16.1	30	100	50
Linseed oil cake + olive oil	19.2	30	50	50

The elevation in pH has been noticed during fermentation in all experiments and it exceeds 8.5 pH in one case (fig. 3) The increase in pH can be correlated with proteolysis activity which yields ammonia in to the medium that causes hike in pH [30].

Although the addition of olive oil increased the production of lipase up to 19.2U/g substrate, 16.1U/g substrate is also a significant figure when compared economically (Table 1). It seems that the addition of olive oil has induced lipase production and reduced the fermentation time.

CONCLUSION

From the result presented in this work it may be concluded that the linseed oil cake is a potential substrate for lipase production. However, intensive study on process optimization at large scale lipase production is necessary.

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