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Comparison studies on lipase production using immobilized *Exiguobacterium aestuari* by repeated batch bioreactor

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Abstract
Enzymes play a pivotal role in a number of industries. In recent years there has been potential increase in the use of lipase as industrial catalysts. *Exiguobacterium aestuari* has been found to be a potent producer of lipase. This study is focused at the optimization, extraction and purification of Lipase from the organism. Maximum activity was obtained at pH 7.0 and temperature 37^0C. Carbon source glucose gave a maximum activity. A medium containing yeast extract and 1% olive oil also generated maximum activity. The lipase enzyme was precipitated using Ammonium sulphate and purified by using a dialysis membrane. The activity of purified enzyme was increased thre-fold compared to crude enzyme. The enzyme isolated was subjected to characterization studies. Solid state fermentation of lipase was carried out in coconut, sesame and ground nut oil cakes. Maximum activity of lipase was sesame oil cake as the substrate.

Keywords: *Exiguobacterium aestuari*, cell immobilization, Bioreactor, solid state fermentation

1. Introduction
Lipase (triacylglycerol acyl hydrolases EC 3.1.1.3) is one of the most prominent industrial enzymes that catalyze the hydrolysis of triacylglycerol into fatty acids and glycerol, mono and diacyl gylcerol at the water-lipid interface and the reversible reaction of lipids in non-aqueous solvents (Jaeger *et al.* 1998; Sharma *et al.* 2001; Stergion *et al.* 2013) [24, 46, 48, 50].

The versatility of lipases allows applications in reactions such as hydrolysis, esterification, interesterification and transesterification, alcoholysis, acidolysis and aminolysis. Several modern bioprocesses use lipases, such as: biodiesel production (Hwang *et al.* 2014; Fukuda *et al.* 2001) [23, 16], food industry (Aravindan *et al.* 2007; Ferreiradias *et al* 2013) [6, 15], medicine (Gopinath *et al.* 2013; Mo *et al.* 2013) [19, 35], pharmaceutical and cosmetic industries (Wang *et al.* 2009; Jeganathan *et al.* 2006) [32, 25], pre-treatment of industrial wastewaters (Kim *et al.* 1998) [28], detergents formulation (Aaslyag *et al* 1991; Li *et al* 2014) [1, 32], and many others (Batista *et al* 2014) [7].

Immobilized cells have some advantages over free cells. For example, immobilized cells have higher density in per volume of reactor and their separation from the reaction medium is easier. Use of immobilized cells not only shortens the adaptation phase (lag phase) and optimal reaction time but also provides a higher substrate conversion (Duarte *et al.* 2016). On the other hand, sensitivity of immobilized cells against the effect of inhibitory compounds and nutrient depletion is less. Immobilization protects the cells against environmental stresses, reduces susceptibility to unwanted contaminants and increases productivity (Ahmed 2008; Nedović *et al.* 2015). Furthermore, this process can make possible the reuse of immobilized cells in repeated batch cultures (Aydogan *et al.*, 2014). Due to these potential advantages, immobilized cells of thermophilic or mesophilic microorganisms have been used for the production of lipases (Pollero *et al.* 1997; Ellaiah *et al.* 2004; Bhushan *et al.*, 2008; Bisht *et al.*, 2013). Similarly, some studies have demonstrated that lipase production can be also performed at low temperatures using immobilized cells of cold-adapted bacteria and filamentous fungi (Ferrarezi *et al.* 2014; Joseph *et al.* 2006) [26]. In contrast, to our best knowledge, there is no study on lipase production by immobilized whole cells of cold-adapted yeasts. In this point, we consider that more studies should be performed to develop an effective industrial process in lipase production by immobilized cells of cold-adapted yeasts.

Lipase have been isolated from various microorganisms, which include bacteria (Gram positive and negative), filamentous fungi and yeasts. These lipase producing microorganisms have been isolated from various sources, like palm fruit, raw milk, raw meat, fermented sausages, oil-contaminated areas, hot springs, soil samples, contaminated water samples, crude oil, contaminated soil sample, spoiled coconut, etc. Lipases are reported to be produced by many species of animals, plants, bacteria, yeasts and fungi (Sztajer *et al*., 1998). Among these, microbial lipases have gained wide industrial applications in view of high yields, rapid growth of microorganisms and ease of genetic manipulation (Andualema and Gessesse 2012). Extracellular lipase production has been observed in several species of *Mucor*, *Rhizopus*, *Geotrichum*, *Pencillium*, *Acremonium*, *Candida*, *Humicola*, *Cunninghamella*and *Aspergillus* (Abrunhosa *et al.* 2013; Cihangir and Sarikaya, 2004; Gopinath*et al.*, 2000, 2002, 2003; Prabhakar*et al.*, 2012). Filamentous fungi are preferred sources of lipase production because they are easily extracted from fermentation processes, are considered safe and easy to hand, and can potentially be used as whole cells (Alberton *et al*., 2010; Singh and Mukhopadhyay,

2012; Andrade *et al*., 2014). Filamentous fungi have proven to be the most convenient biosystem for industrial applications because of their strong cell walls. *Rhizopusoryzae*, *Rhizopuschinensis*, *Aspergillus Niger*, and *Mucorcircinelloides*have all been studied as whole cell biocatalysts by several research groups (Fukuda *et al*., 2009; Andrade *et al*., 2014) [37].

In several cases, immobilization of cells producing lipase increases the extent of reaction and facilitates the downstream processing. This is mainly due to the following reasons: (1) avoiding washout of the cells at high dilution rate, (2) higher cell concentration in the reactor, and (3) easy separation of the cells from the product containing solution.

Different types of bioreactors used for lipase production are: Batch reactors, Continuous stirrer tank reactors, Airlift Bioreactors (Bannarme and Jeffries, 1990), Bubble Column Bioreactors (Laugero *et al*, 1996), Fluidized-bed Bioreactors (Moreira *et al*, 1996), Biofilms on Flat Sheets Various Materials (Fillis, 1997), Packed Bed Bioreactors (Moreira et ai, 1997)

One of the problems encountered with all bioreactors is the control of the biomass produced. In this regard washout, cell retention, cell recycle and waste are all operating variables peculiar to a given type of reactor or operating configuration. The nutrients must be effectively and/or efficiently utilized. Plugging of the reactor components or active support device is a major consideration when control of biomass is dealt with in the suspended culture type reactors by cell recovery and recycles using settlers or ultra- or micro-filtration steps. In the case of immobilized cell systems it is necessary to provide some form of shear or possibly scraping or other mechanism to release excess biomass to prevent plugging. A successful bioreactor system would be one in which this aspect is adequately managed by the process operators.

The present paper deals with the immobilization of *Exiguobacterium aestuari* has been used as the microorganism source for lipase production and also investigates the optimization, extraction and purification of lipase from *Exiguobacterium aestuari*.

2. Materials and Methods

2.1 Isolation and identification of bacterial strain

Exiguobacterium aestuari used in the present study was isolated from Domestic waste from Thiruchirappalli, Tamilnadu, India and identified by 16S rRNA sequencing. The lipase producing ability was tested by the presence of clear hydrolytic zone on Tributyrin agar (Sirisha *et al*., 2010) [49]

2.2 Enzyme Production media preparation and inoculation

Na_2HPO_4 0.7%, KH_2PO_4 0.3%, NH_4Cl 0.1%, NaCl 0.5%, Sucrose 0.2% were added together (pH at 7.0) and made up to 200ml using nutrient broth. The medium was sterilized and 5ml of 16hr old inoculum was added to the production medium. This inoculated production medium was kept under shaking conditions at 37^0C, 120rpm at 3days followed by centrifugation at 8000 rpm for 10 min at 4°C to get cell free supernatant for estimation of enzyme activity.

2.3 Preparation of immobilized cells

- **Sodium alginate entrapment method**

20 ml of 0.2M $CaCl_2.2H_2O$solution and 3% Sodium alginate solutions were prepared. 5ml of inoculum was added to the sodium alginate solution and mixed. This solution was taken in a syringe and added drop by drop to the $CaCl_2$ solution. The beads formed were then cured in a refrigerator at 4^0C for 1 hr. These beads were added to the nutrient broth for the production of lipase.

2.4 Optimization of production of lipase using Immobilized and non-immobilized cells

2.4.1 Effect of Incubation Period on Lipase Activity

Production medium, pH 7 (100ml in 250ml flask) was inoculated with 1% 16 hr old seed culture of *Exiguobacterium aestuari* immobilized cells and incubated at 37^0C under shaking (150rpm) for 3 days. For every 24hrs, samples were collected and centrifuged at 10000 rpm; 4ºC for 10min.The enzyme activity was calculated by the titrimetric assay method.

2.4.2 Effect of pH on lipase production

Immobilized and non-immobilized cells were grown individually in production media with different pH values ranging from 5 to 9. They were incubated at 37ºC for 3 days and for every 24hrs, samples were collected and centrifuged at 10000 rpm; 4ºC for 10min. titrimetric assay method was used to calculate the activity.

2.4.3 Effect of Temperature on lipase production

Immobilized and non-immobilized cells were inoculated in production medium and incubated at different temperatures (22, 27, 37, 45ºC) for 3 days. For every each 24hr, samples were collected and centrifuged at 10000 rpm; 4ºC for 10min.Enzyme activity was calculated by the titrimetric assay method.

2.4.4Effect of age of inoculums on lipase production

Non-immobilized cells were inoculated into the production medium at different time intervals 24, 48, 72 hrs. Activity was measured by spectrophotometric method.

2.4.5Effect of different Carbon sources on lipase production

Immobilized and non-immobilized cells were inoculated in production medium containing different carbon sources like glucose, maltose and sucrose (2%) at pH 7 and incubated at 37ºC for 3 days. After every 24 hr interval, the culture was collected and centrifuged at 10000 rpm; 4ºC for 10 min. The Activity was calculated by spectrophotometric assay method.

2.4.6Effect of Oil source on lipase production

Cells were inoculated in the production medium containing different oil sources like olive oil, coconut oil (1%) along with surfactant gum acacia. They are incubated at 37ºC for 3 days and for every 24hrs, the culture was collected and centrifuged at 10000 rpm; 4ºC for 10mins.Activity was estimated by the spectrophotometric assay method.

2.4.7Effect of Nitrogen sources on lipase production

Immobilized and non-immobilized cells were inoculated in production medium with different nitrogen sources like peptone, beef extract, yeast extract and KNO_3 and incubated at 37ºC for 3 days. For every 24 hr period, the culture was collected and centrifuged at 10000 rpm; 4ºC for 10min.Activity was calculated with spectrophotometric assay method.

2.4.8 Effect of bead porosity on lipase production

Different concentrations of sodium alginate (3, 4 and 5%) were used for the immobilization of cells for lipase production. Cells were isolated from the nutrient broth and added to the sodium alginate solution (3, 4 and 5%). Those mixtures were added to the 0.2M $CaCl_2.5H_20$ solution through pipette. The beads were cured at 4^0C and added to the production medium. The conical flasks were incubated in shaking incubator at 130 rpm. After each 24hr interval, the samples were collected and centrifuged at 10000 rpm. Supernatants were collected and activity was found using the spectrophometric method at 510nm.

2.4 Lipase assay

The lipolytic activity of the purified enzyme was estimated by a spectrophotometric method using 4-nitrophenyl palmitate (4-NPP) as substrate, following established procedures by Winkler & Stuckmann (1979). One activity unit of lipase (LU) was defined as lmole of 4-NP released from the hydrolysis of 4-NPP/ml/min by one ml of enzyme at 45°C under standard assay conditions.

2.6 Response Surface Methodology (RSM) Studies with two parameters

RSM studies with two factors namely inoculum and glucose (1, 2, 3, 4 and 5%) concentration were carried out by Central composite method. Using the minitab software the optimized combination of the inoculum and glucose concentration was found out and the activity of the lipase measured.

2.7 Mechanical Design of column for lipase production using *Exiguobacterium aestuari*

A glass Column molded with inner diameter 5 cm, outer diameter 5.5 cm and height 17 cm with inlet and outlet was placed on column holder. Aquarium pump was used for the air supply with a membrane filter. Medium was passed into the column using a peristaltic- pump. Glass beads were used for supporting the immobilized cells. Immobilized cells were packed into the column in aseptic conditions and the medium was passed at different time intervals (1, 2, 3 days)

2.8 Purification of the extracted lipase

Lipase was purified to homogeneity by ammonium salt precipitation. The culture broth (500ml) was centrifuged (10,000*g*, 20 min) and filtered through a Whatman No. 1 filter paper. The filtrate was then precipitated with ammonium sulphate and dialyzed extensively against 0.1M Tris–HCl buffer; the protein concentration was determined in the dialysed sample. The column was eluted with phosphate buffer 0.02M, pH 8.5, containing 1M KCl. All eluted fractions were assayed for lipase activity. The fractions showing highest lipase activity were pooled and assayed for protein content. The specific activity of the purified enzyme was compared with that of crude enzyme and fold purification was calculated. The relative molecular mass of the purified lipase was estimated by SDS–PAGE (12%). Proteins were stained with Coomassie Brilliant Blue R-250.

3. Results

3.1 Effect of pH for production of lipase using free and immobilized cell in Repeat batch fermentation

The results of present investigation showed that the pH of the medium significantly influenced the lipase activity. pH has great influence on lipase production.Lipase production decreased with the increase of acidity or alkalinity. Production media is maintained at different pH values (5, 6, 7, 8, and 9). The highest Lipase production by the *E. aestuari* strain was observed at pH 7.0, 48 hr.Lipase maximum activity was 610 U/ml using free cells and 520 U/ml using immobilized cells (Figure 1 & 2).

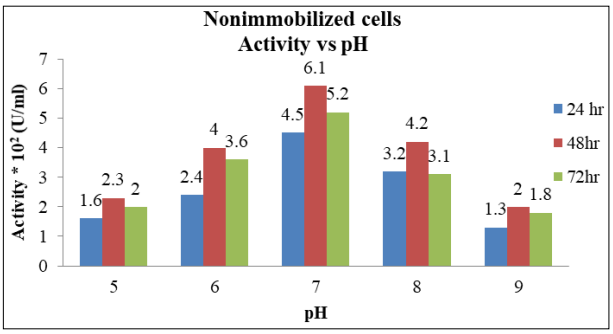

Fig 1: Lipase activity of Non-immobilized cells with different pH

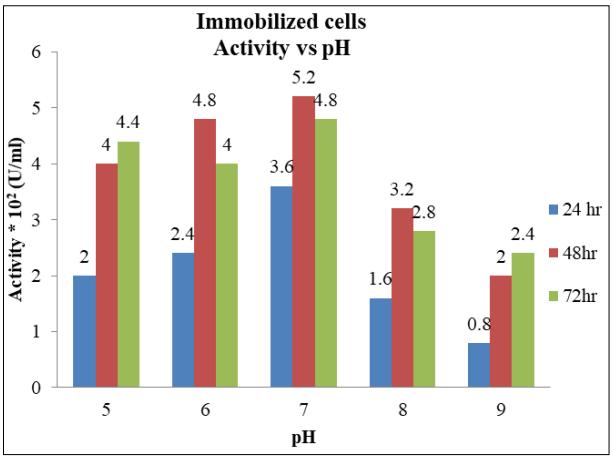

Fig 2: Lipase activity of immobilized cells with different pH

Several authors consider pH to be an important variable in the production of lipase by submerged fermentation. Muralidhar *et al.* used an initial pH of 6.5 in the culture of C. cylindracea. Teng and Xu studied the effect of pH on the production of lipases by *Rhyzopuschinensis* between pH 5 and 7, and the best results were achieved at pH 5.5. In this study, pH proved to be an important variable for the production of lipases, with the optimal value being approximately 7.0. This result is in agreement with Gopinath *et al.*, who studied pH4, 7 and 10 in the production of lipase by *Geotrichumcandidum*. Among the factors studied by Wang *et al.* for the production of lipases by *R. chinensis*, pH was the variable that had a lower effect on production. Each microorganism has a pH for optimal growth. In this case, pH could have affected lipase synthesis beyond microorganism growth.

3.2 Effect of temperature for production of lipase using free and immobilized cell in Repeat batch fermentation

Temperature is one of the important factors that affect the growth of microorganism and its enzymes production. Most species have a characteristic range of temperature in which they can grow, but they do not grow at the same rate over the whole of temperature range. In this case, the total enzyme activity is related to initial cell concentration, cell

growth, enzyme production and enzyme stability in the process of fermentation and the highest lipase activity was obtained at 37°C (figure 3).

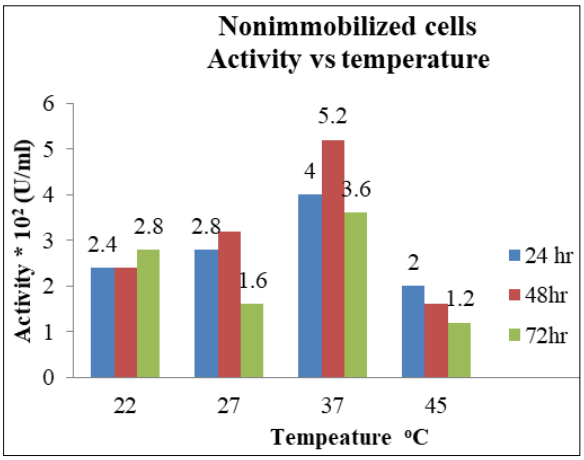

Fig 3: Lipase activity of Non-immobilized cells with different temperature

The temperature influences secretion of extracellular enzymes by changing thephysical properties of the cell membrane. In general, several studies conducted for the optimization of temperature shows that the bacteria produces lipase in wide range of temperature from 20 °C to 45 °C. The lipases are produced at different ranges of temperature of incubation. However, the optimum temperature for lipase production corresponds with growth temperature of respective microorganisms. The results in the Figure 3 & 4 were elucidated that the enzyme productivity was increased with the rising of incubation temperature to reach optimum temperature for lipase production was at 37°C which give maximum lipase activity about 520U/ml using free cells.Maximum Activity of immobilized cells was 410 U/ ml at 37ºC, 72 hr. For immobilized cells activity was quite less compared to the non-immobilized cells.

The lipase production was decreased with the increasing of temperature ranges above 37 °C to 45 °C. The decreasing in lipase productivity may be due to inadequate temperature for the growth and enzyme production by the microorganism because the most organism's enzymes rapidly become denatured at extreme temperatures, therefore the temperature played an important role in the production of the enzyme through its impact on the solubility of oxygen and the kinetic energy of the particles.

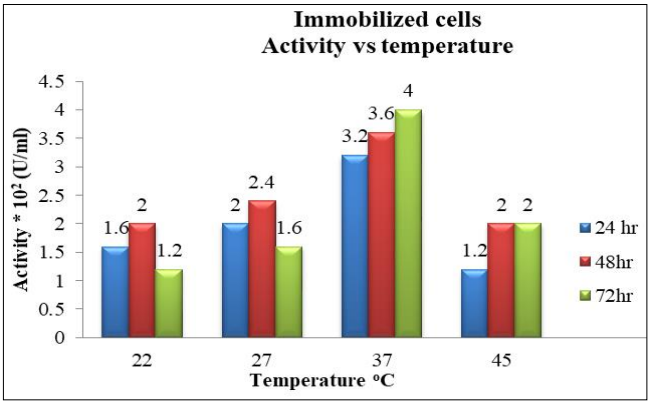

Fig 4: Lipase activity of immobilized cells with different temperature

Similar results were reported that the maximum lipase production was at 35 °C by *Pseudomonas aeruginosa* as study of Zouaoui *et al*. (2012) [59] while other studies were showed that the growth and lipase production by *Pseudomonas fluorescens* was maximum at temperature 36 °C (Khemikalomthaesong *et al*., 2012) and the optimum lipase production at temperature 37 °C for *Pseudomonas gessardii* (Veerapagu *et al*., 2013). The study of Patel *et al*. (2016) was suggested that the maximum lipase activity for *Bacillus* sp. was at temperature 37 °C. While the study of Kulkarni and Gadre (2002) [29] was reported that maximum lipase production was at 25 °C for *Pseudomonas* spp. also the study of Adan (2009) [4] and the study of Abdul Faisal *et al*. (2014) [4] reported that the 25 °C temperature consider as the optimum temperature for the growth of bacteria *Pseudomonas* sp. and lipase production. While the study of Abdul Hammid *et al*. (2013) [2] showed that the maximum productivity for *Pseudomonas cepaciea* was at 40°C.

3.3 Effect of different Carbon sources on lipase production

The carbon source has been reported as the major factor that affects lipase production, ever since lipases are inducible enzymes (Treichel *et al*., 2009). In the case three different carbon sources (glucose, maltose, sucrose) were used for finding maximum activity. The results were showed that the maximum lipases production was attained in present of glucose in fermentation medium with lipase activity about 480 U/ml using free *E. aestuari* cells at 48 hr and 440 U/ml using immobilized *E. aestuari* cells at 72 hr (figure 5).

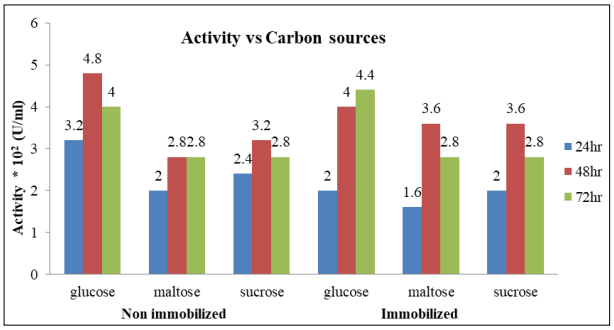

Fig 5: Lipase activity of immobilized and non-immobilized cells with different carbon sources

Kiran *et al* (2008) was reported that starch was the best carbon source for lipase production by the isolate *Pseudomonas fluorescens*. Several studies have shown that lipase production was influenced by different carbon sources that present in fermentation media (Immanuel *et al*., 2008). In general the required specific carbon source and its concentration differ among organisms. Different studies conducted on the effect of sugars that supplied the medium as additional carbon source have not enhanced the lipase production when compared to control medium. The reduction in the in the presence of sugars as carbon sources could be due to catabolite repression by readily available carbon sources in the medium (Kiran *et al.*, 2008).

3.4 Effect of nitrogen sources

Among the organic and inorganic nitrogen sources, organic nitrogen source showed higher lipase activity. In the case of non-immobilized cells, medium containing yeast extract shows maximum activity 150 U/ml. Effect of inorganic

nitrogen source on immobilized cells (KNO_3) showed high activity 290 U/ml, which was followed by yeast extract medium.

The sources of organic nitrogen have been used for the production of lipases by fungi, as reported by several authors. Peptone was used by Kaushik *et al.* to produce lipases in submerged fermentation using *Aspergillus carneus* and by Tengand Xuand Wang *et al.* using *Rhizopuschinensis*. Yeast extract and peptone were used in combination by Mahadik *et al.* for the production of lipase by *A. niger*. Miranda *et al.* evaluated the production of lipases using oil refinery wastein the presence of nitrogen sources such as ammonium sulfate, urea and ammonium chloride, with the best results being obtained using ammonium chloride. For lipase production by *Antrodiacinnamomia*, Lin *et al.* used organic and inorganic sources of nitrogen, including ammonium and nitrate salts, proteins, peptides and amino acids, and higher activities were obtained with sodium and potassium nitrates, ammonium chloride and asparagine. In our study, yeast extract generated higher lipolytic activities compared to other nitrogen sources, a result that could be explained because yeast extract contains other components besides a nitrogen source that can act as coenzymes of the aerobic metabolic pathway, for example, carbon skeletons and complex B vitamins.

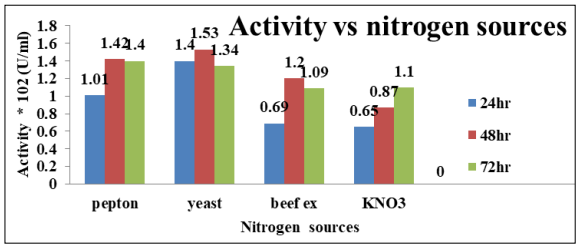

Fig 6: Lipase activity of non-immobilized cells with different nitrogen sources

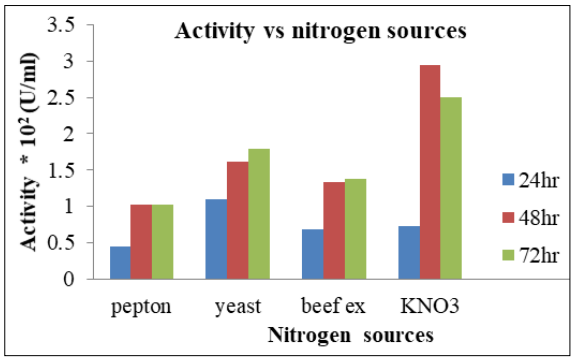

Fig 7: Lipase activity of immobilized cells with different nitrogen sources

3.5 Effect of Oil sources

The addition of lipids substrates is a known strategy to increase the lipase activity for different microorganisms. Long-chain fatty acids have been used as inductors for lipase production from *E. aestuari*. Addition of oil sources to the production medium enhances lipase production from *E. aestuari*. 1% of oil sources like coconut and olive oils were added to the production medium. Olive oil showed more activity 5.1U/ml ((µmol fatty acid /ml sample)/ time).

Figure 8 shows the activity of lipase produced by immobilized and normal cell with addition of oil sources.

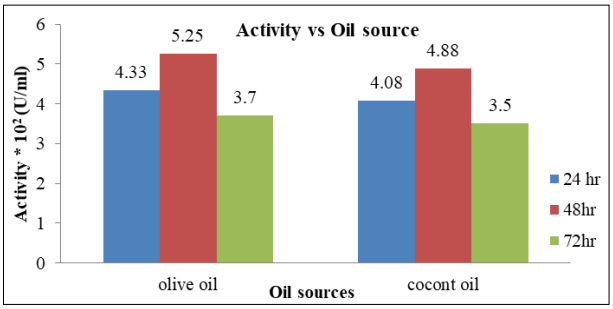

Fig 8: Lipase activity of non-immobilized cells with different oil sources

3.6 Effect of bead porosity on lipase production

Different sodium alginate concentrations were used for the preparation of beads and to study their effects on the production of lipase (figure 9). The production of lipase was maximal with 4% alginate (136 U/ml) in comparison with the other concentrations used. These results are in accordance with the results of Jamuna *et al.* Although 4% alginate gave more stable beads, the yield of lipase was lower. The decrease in production of lipase with 4% alginate could be due to the diffusional resistance offered by the beads. Based on this result 3% alginate was selected for subsequent studies.Activity of the 4% sodium alginate cells yield more lipase compared to 3% and 5%. When the sodium alginate solution was autoclaved the viscosity was reduced, hence a heating mantle was used for getting a good mixture. Also as the viscosity was increased beyond the optimum level passing of the medium through the pipette was very difficult and getting spherical beads became difficult. When the $CaCl_2$ solution was agitated with the magnetic stirrer better spherical beads were obtained.

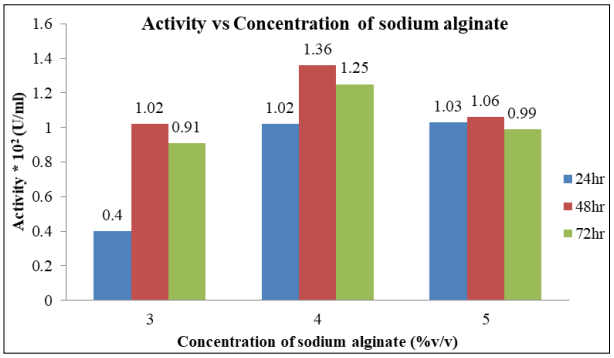

Fig 9: Lipase activity vs concentration of sodium alginate

3.7 Response Surface Methodology (RSM) Studies

RSM studies with two factors such as inoculums and glucose (1, 2, 3, 4 and 5%) concentration was carried out by Central Composite method. During the RSM studies 13 trials were done based on the data generated by the software. Based on the values of activity of lipase, contour plots were plotted. From the plot maximum activity of lipase was at 5% glucose and 5% inoculums concentration.

From the contour plot the intensity was maximum at intercept of 1.0 of A and B. 5% of inoculums and glucose concentration yield maximum activity (Table 1)

Table 1: Activity of lipase in RSM studies with two factors of inoculums and glucose concentration

S. No	Run order	Blocks	Inoculums	Glucose	Activity
1	1	1	1.414214	0	5.27
2	2	1	0	0	5.4
3	3	1	-1	1	3.71
4	4	1	1	1	4.27
5	5	1	-1.41421	0	2.29
6	6	1	0	0	2.49
7	7	1	0	0	3.26
8	8	1	0	0	3.35
9	9	1	1	-1	3.64
10	10	1	0	0	2.127
11	11	1	0	-1.41421	2.84
12	12	1	-1	-1	2.33
13	13	1	0	1.414214	5.22

3.8 Mechanical Design of column for lipase production using *E. aestuari*

A glass column molded for packing of calcium alginate immobilized cells was used. By using a peristaltic pump, the production media was passed through silicon tube (2.5mm) into the column. The medium was passed through the top of the column at a rate of 6ml/min. After adding the medium filtered air was passed into the column through the bottom side opening. Samples were collected. After a two day interval fresh media was added. The activity was determined following centrifugation.

Column specification

Inner diameter = 5cm Packing height = 6 cm

Outer diameter = 5.6cm Void volume = 21ml

Height = 17cm Void Fraction =0.105

Volume = 333ml Working volume=200 ml

3.9 Lipase purification

Lipase purification was done to get a protein of interest and to remove unnecessary one. Purification process of lipase occurs in sequential manner. The enzyme produced over 48 hours of culture was purified by ammonium sulphate precipitation for salting out the proteins. For increased enzymatic activity desalting was performed for removing the traces of salt. According to Pabai *et al.* increased lipase activity depends on the concentration of ammonium sulphate. An extracellular lipase from *E. aestuari* was purified by ammonium sulphate precipitation and Sephadex G-100 column chromatography with a total yield of 20.8 %- and 2.1-fold purification (Table 2).

Table 2: Purification of lipase from *E. aestuari*

Purification stage	Total activity (U/ml)	Total protein (mg)	Specific activity (U/mg)	Purification fold	Yield (%)
Crude sample	355	150	2.3	1	100 %
Ammonium sulphate precipitation	243	68	3.5	1.5	68%
Sephadex G-100	74	15	4.9	2.1	20.8%

It was also found that the total yield and purification fold were 35.6 % and 3 respectively in case of *Microbacterium luteolum*. A low yield of the enzyme may be due to difficulty in removal of the high content of lipopolysaccharide present in *E. aestuari* and coupled with lipid hydrolysis.

4. Conclusion

E. aestuari is one of the extra cellular lipase producing microorganisms. Lipase production by the organism was optimized in terms of media and physical parameters. Calcium alginated immobilized cells had also been used for lipase production. The advantage of immobilization helps in (1) avoiding the washout of the cells at high dilution rate, (2) higher cell concentration in the reactor, and (3) easy separation of the cells from the product containing solution. Production media for lipase production was optimized with physical parameters like pH and temperature. Different variations in the study include the use of packed bed reactor and solid-state fermentation to determine the enzyme activity. To conclude a reasonably high activity was obtained in all the different types of modified systems.

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