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Optimization of Lipase Activity Obtained from Dairy Industry Wastes

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***Abstract*:- Optimisation of media components is very essential for the production of lipase to enhance its activity in order to meet wide variety of industrial applications. The enzyme production by the microorganisms in the environment is very low, hence it is essential to improve under required optimum growth conditions. This study mainly focuses on the enhancement of lipase production and its activity which includes the environmental, nutritional and biochemical factors of the media. In order to accomplish the enhanced activity, media parameters such as Lipid, and Carbon and Nitrogen sources along with other process parameters were altered. The lipase production mainly depends on nitrogen source, carbon source, metal ions, pH, and temperature and incubation time interval for media enrichment. The observations includes the screening of lipolytic bacteria by quantitative estimation of lipase by titrimetric method for isolated bacteria from dairy industry waste, confirmed on tributyrin agar and found to be highest lipase enzyme producer at an optimum pH 8, temperature 37^{0}C with sunflower oil as substrate where as the nitrogen and carbon sources found to be peptone and glucose respectively.**

Key words: Bacteria, Tributyrin agar, Lipolysis, enrichment

I. INTRODUCTION:

Dairy industries around the world, produces wide range of products like cheese, milk, buttermilk etc. During the manufacturing of these products, the industries are involved in the production of solid and liquid wastes. The effluents which were collected from the diary industry consist of wide range of proteins, fats, and possibly other active substances. Micro biota are adapted to the waste water physiochemical conditions and produces wide range of enzymes like lipases, proteases etc. Some new active bacterial strains may be identified in waste water effluents from dairy industries.

Lipases are ubiquitous enzymes which are found in animals, plants, fungi, bacteria. Enzymes from microbial source are more stable than their corresponding plant and animal enzymes and the production is more convenient and safer. Microbial lipases have been produced by yeasts, fungi, and bacteria as extracellular, intracellular, and cell-bound enzyme. It has been estimated that only about 2% of the world's microorganisms have been tested as source of enzymes (sahu etal. 2011).These enzymes are generally involved in increasing free fatty acid [FFA] concentration through the hydrolysis of fats or lipids. They are mainly involved in hydrolysis, which catalyses the breakdown of triacylglycerol to glycerol and fatty acids. Hence these enzymes are used to catalyze interesterification of fats and oils to modify glycerides. Lipase production is also used as a marker for pathogenicity in some medically important bacteria. Many varieties of lipases are produced from both gram positive and gram negative bacteria.

The process of lipolysis is to release free fatty acids [FFA] and partial glycerides which have either deterimental or beneficial effects in the environment. Lipolytic enzymes have wide range of industrial applications in food, dairy, paper, textile, leather, and detergent industries. These lipolytic enzymes are also used in waste water treatment, production of fine chemicals, pharmaceuticals and cosmetics, synthesis of surfactants and polymers, vegetable fermentation and meat curing.

Due to the wide range of industrial applications, lipases obtained from microorganisms are more interesting as they can be produced with better yields. There are many varieties of catalytic activities that can be used in several applications and the genetic manipulations are easily available. The enzymes are heat stable and involved in the spoilage of a various dairy products (subham verma etal 2014.)

II. MATERIALS AND METHODS:

2.1 Collection of dairy waste water samples:

Samples from dairy industry wastes were collected in a sterile container and stored at 4ºC until the analysis was carried out according to the standard methods of APHA [American Public Health Association] and Trivedy and Goel (1984). All experiments in the study were carried out with isolated lipolytic bacteria in triplicates and the averages of the results were displayed. In the present study, dairy waste water samples were collected and screened for the presence of lipolytic bacteria.

2.2 Isolation of lipolytic bacteria:

Selective isolation of lipolytic bacteria from dairy waste water samples were performed by using olive oil emulsion medium in screening medium for primary isolation and later confirmed on Tributyrin agar medium.

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2.2.1 Preparation of enrichment media:

Enrichment media is prepared by using Peptone (0.05% w/v), KH_2PO_4 (0.15% w/v), $Na_2H_2PO_4$ (0.1% w/v), $MgSO_4$ (0.05% w/v), $(NH_4)_2SO_4$ (0.5% w/v), NaCl (0.5% w/v), Olive oil emulsion (1% v/v was prepared by mixing with 1 volume of the olive oil with 4 volumes of 5% Poly Vinyl Alcohol (PVA).This emulsified solution was kept in orbital shaker at 250 rpm for overnight at 37ºC. Then the prepared oil emulsion was autoclaved at 121ºC for 20 min at 15 lbs pressure and the pHwas adjusted to 7.0. Then 5 ml of each sample was added to 50 ml of enrichment media in 150 ml flasks. The flasks were incubated on a shaker at 200 rpm at 37ºC for 72 hrs.

2.2.3 Preparation of confirmatory media:

Further to confirm the lipolytic isolates, the tributyrin broth medium was used as confirmatory media. This medium consists of components like Tributyrin (1% v/v), Yeast extract (0.3% w/v), Peptone (0.5% w/v).

2.3 Screening of lipolytic isolates:

2.3.1 Primary Screening of Lipase producing bacteria:

5ml of each sample collected from dairy industry wastes were suspended in 100 ml of enrichment media and agitated in orbital shaker incubator at 37ºC at 200rpm. The enriched suspensions were used to make the dilutions from 10^{-1} to 10^{-10} by serial dilution method.

2.3.2 Confirmation of lipolytic bacteria:

Lipolytic bacterial colonies were screened by qualitative plate assay method in the presence of tributyrin agar medium for 72 hrs. Zone of clearance was observed due to hydrolysis of substrate tributyrin because of extracellular lipase production by lipolytic bacteria.

3. LIPASE ACTIVITY DETERMINATION:

The better clearance zone formed colony was selected for further studies. Qualitative test was done by hydrolysing the medium to form a clear zone and quantitatively by using titrimetric method. Lipolytic activity was checked quantitavely by titrimetric method.

3.1 Estimation of lipase activity in the lipolytic bacteria:

Extracellular lipase assay was done with crude enzyme which is collected by centrifuging the bacterial culture for 10min. 1ml of the crude enzyme is mixed with 4ml of olive oil emulsion and 5 ml of 0.5M phosphate buffer at pH 7. Then this mixture was incubated for 15min at 37°C. After incubation, reaction was terminated by the addition of 2ml of acetone: ethanol mixture (1: 1). Then 2 drops of phenolphthalein indicator (pp) was added and titrated against 0.1 N NaOH until the color changes to pale pink.

Lipase activity can be calculated by the following equation (1). One lipase unit (U) is defined as the amount of lipids required per minute to liberate 1 mol of fatty acids. In the current study lipase activity is measured in the presence of olive oil as the substrate.

Lipase activity (U / ml) = ((AB) x N (NaOH) x 1000) / (VE) ------- (1)

Description:

A = Volume (ml) of NaOH for titration of the sample, B = Volume (ml) NaOH for blank titration,1000 = conversion to mmol to mol, VE = total volume of the mixture of crude extract.

4. OPTIMIZATION OF THE CONDITIONS FOR LIPASE ACTIVITY:

The media parameters should be altered in order to optimise the lipase activity. For media optimisation the following parameters are considered i.e., varying pH (from 5 to 9), varying temperature (from 27^0C,30^0C,33^0C,37^0C,39^0C,42^0C),Lipid sources (Palm oil, Mustard oil, Sunflower oil, Coconut oil, Tween-20 and Butter), Carbon sources (Glucose, Fructose, Galactose and Sucrose), nitrogen sources (Peptone, Yeast extract, Beef extract and Sodium nitrate).

The activity of lipase enzyme from isolated bacteria present in dairy industry wastes was optimized by considering the effect of following conditions:

(a)Effect of pH on lipase activity:

The optimum pH for maximum lipase activity for the isolated bacteria was determined by considering the effect of different pH in the Tributyrin broth ranging from pH-5, pH-6,pH-7,pH-8,pH-9 where as the other parameters remain unaltered.

(b)Effect of temperature on lipase activity:

To select the optimum temperature for the maximum lipase activity bacteria, the effect of temperatures was studied ranging from 27^0C, 30^0C, 33^0C, 37^0C, 39^0C, 42^0C by keeping the remaining parameters unaltered.

(c)Effect of different carbon sources on lipase activity:

In order to optimize the activity of lipase obtained from isolated bacteria, different carbon sources like Glucose, Fructose, Galactose, Sucrose were added to enrichment broth at a final concentration of 1% (w/v) where as other parameters remain unaltered.

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(d)Effect of different nitrogen sources on lipase activity:

To optimize the activity of lipase obtained from isolated bacteria, different nitrogen sources like Yeast extract, Sodium nitrate, Beef extract and Peptone were added to the enrichment broth at a final concentration of 1% (w/v) where as other parameters remain unaltered.

(e)Effect of different oils as Lipid source on lipase activity:

Lipid sources for the production of lipase was optimized by replacing the Olive oil present in the enrichment media with different oils like Palm oil, Coconut oil, Sunflower oil, Mustard oil, Tween20, Butter at a final concentration of 1%(w/v) and where as other parameters remain unaltered.

(f) Effect of different metal ions on lipase activity:

In order to optimize the activity of lipase obtained from isolated bacteria of dairy industry wastes, different metal ions like Mg^{+2},Na^{+},K^{+},Ca^{+2},Zn^{+2}.Fe^{+2} were added at 1mM concentration and incubated for 72hrs.

Finally Mg^{+2},Na^{+} were tested at different concentrations 1mM,2mM,5mM,10mM and 20mM and incubated again for 72hrs keeping the other parameters unaltered.

III RESULT AND DISCUSSION:

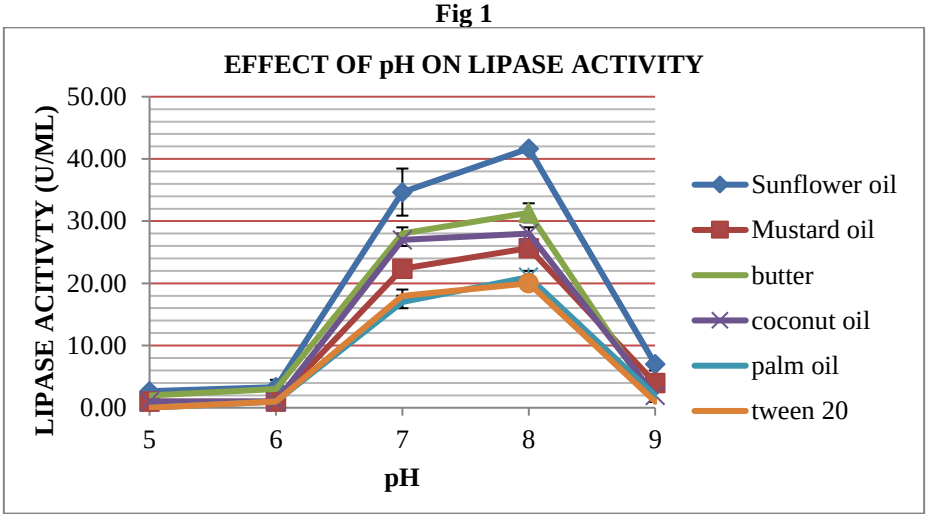
3.1Optimisation of media parameters:

The media parameters should be optimised in order to enhance the lipase activity. For the media optimisation the following parameters are considered i.e., varying pH of (5,6,7,8 and 9) varying temperature(from 27^0C, 30^0C, 33^0C, 37^0C, 39^0C, 42^0C), lipid sources like palm oil, mustard oil, sunflower oil, coconut oil, tween-20,butter. Carbon sources like glucose, fructose, galactose and sucrose were used. Effect of Na^{+} and Mg^{+2} metal ions at different concentrations such as 1mM,2mM,5mM,10mM,20mM were analysed on lipase activity, after which the different nitrogen sources like peptone, yeast extract, beef extract and sodium nitrate were also considered. All the experiments were done in triplicates and the averages of each experiment is analysed.

(a)Selection of pH optima for lipase activity:

It is necessary to determine the pH optima of enzymes as the enzymes are sensitive to the pH of their reacting medium. The maximum efficiency of enzyme activity can be obtained only at pH- 8 with different substrates like Sunflower oil, Mustard oil, Butter, Coconut oil, Palm oil and Tween 20. The maximum lipase activity was found to be 42IU/ml for sunflower oil at pH 8 as indicated in Fig 1.

Fig 1



(b)Selection of temperature optima for lipase activity:

The enzyme activity is found to be optimum with all substrates at 37ºC exhibited. However the activity remains less at pH 6 and 7. The lipase activity was almost the same at all other temperatures taken under study and the optimum activity is observed with sunflower oil as substrate. The maximum lipase activity was found to be 30 IU/ml at 37ºC as indicated in Fig 2

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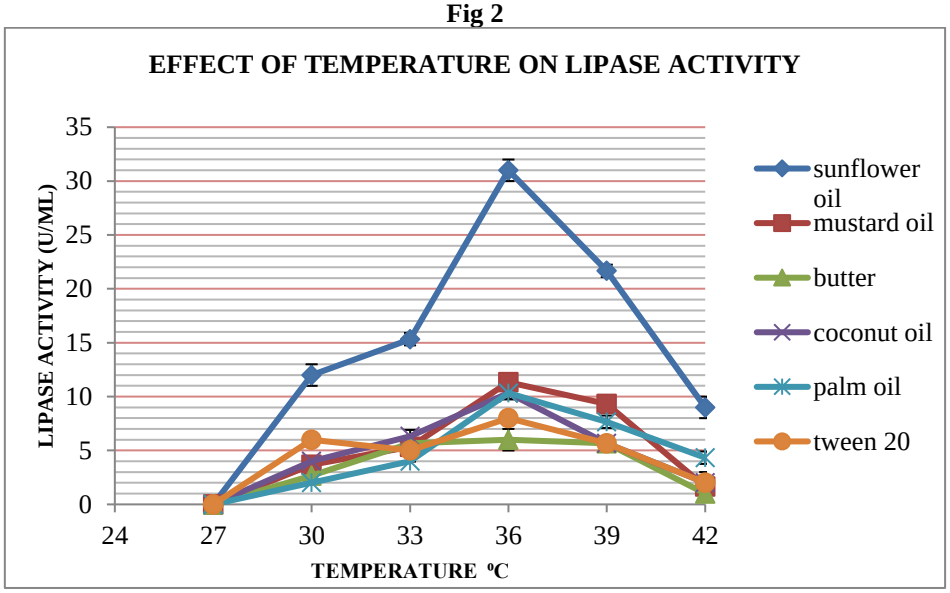
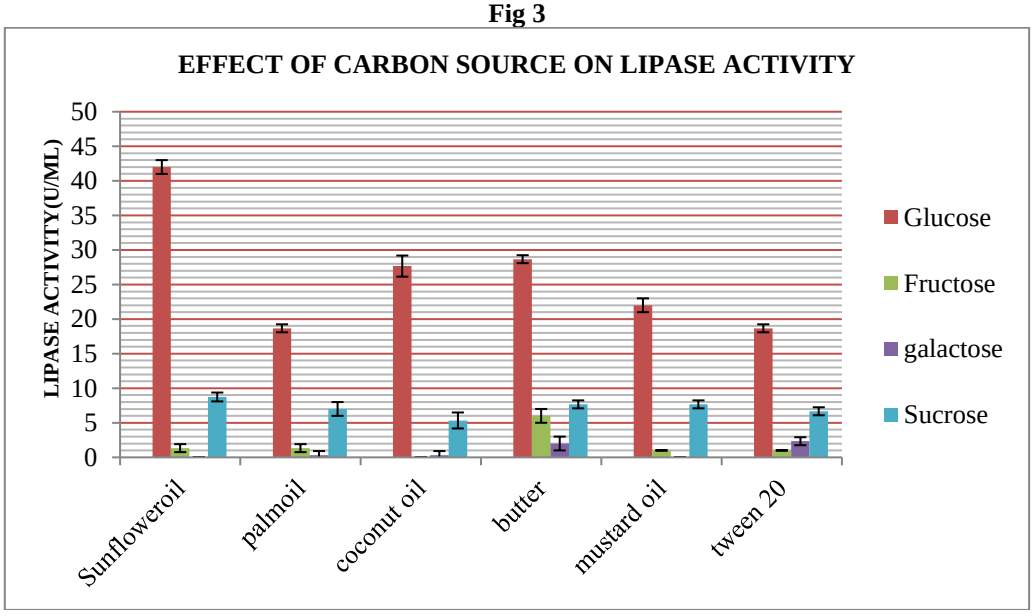
Fig 2



(c)Selection of carbon sources for optimum lipase activity:

For selection of optimum carbon source, different types of 1% carbon sources like glucose, fructose, galactose, sucrose were used in the media preparation in which the maximum peak activity can be seen only for glucose as carbon source with all different substrates. The maximum lipase activity was found to be 42 IU/ml as sunflower oil as substrate as indicated in Fig3

Fig 3



(d)Selection of nitrogen sources for optimum lipase activity:

Generally, microorganisms produce high amounts of lipase when provided with organic nitrogen sources such as peptone and yeast extract as nitrogen sources for lipase production. In the present study, when inorganic nitrogen source such as sodium nitrate is used, but didn't show any lipase activity. Optimum activity is observed only with peptone when used as a nitrogen source and with sunflower oil as a substrate when compared to other substrates. At these conditions the maximum lipase activity was found to be 32 IU/ml as indicated in Fig 4.

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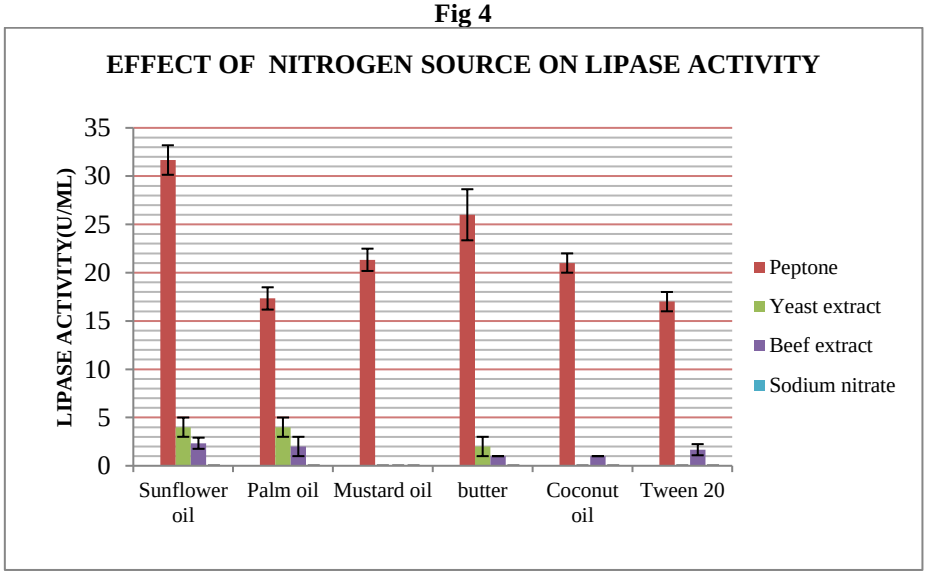
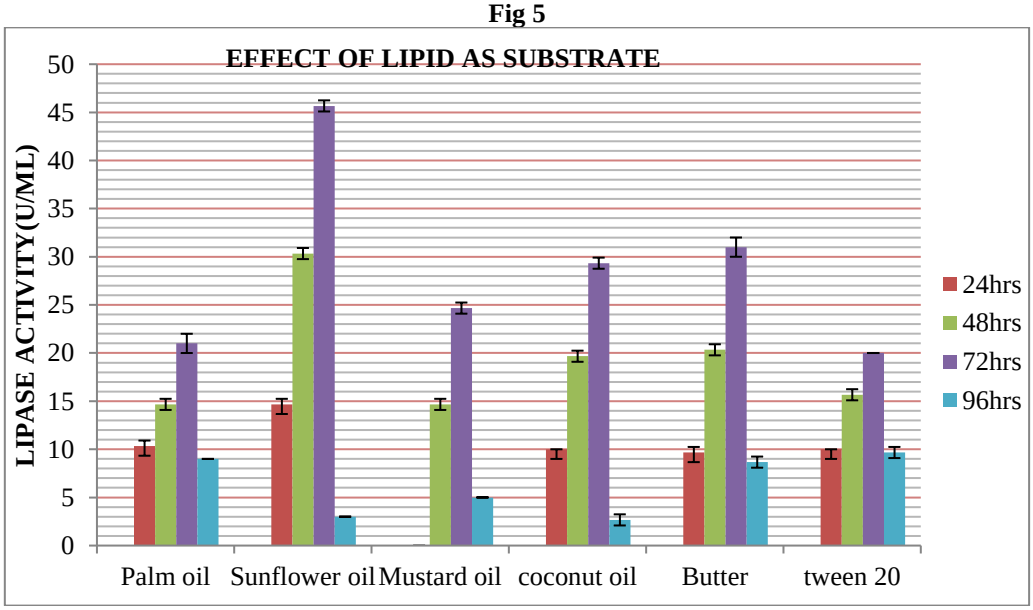
Fig 4



(e) Selection of lipid sources as substrates for optimum lipase activity:

For selection suitable substrates for optimum enzyme activity sunflower oil, palm oil, mustard oil, coconut oil, butter and tween 20 were used as a source of substrates, in which the sunflower oil is found to be a better substrate with highest enzyme activity when compared to other substrates at 72hrs of enrichment. Maximum lipase activity was found to be 46 IU/ml with sunflower oil at as indicated in Fig 5

Fig 5



Based on the results, Lipase activity is enriched with the isolated bacteria in presence of glucose as carbon source, peptone as nitrogen source for 72hrs and activity is checked repeatedly in all conditions, where sunflower oil is found to be a best substrate at optimum pH 8 and at 37^0C temperature. The average activity lipase activity was found to be 38.4±6.2 IU/ml.

(f) Effect of metal ion on lipase activity:

For selection of suitable metal ions for optimum enzyme activity, different metal ions like Mg^{+2},Na^{+},K^{+},Ca^{+2},Zn^{+2}.Fe^{+2} were checked for the optimum lipase activity in which the maximum lipase activity was found to be for sodium and magnesium ions at 1mM concentration after 72hrs of incubation. From which the two metal ions that is Mg^{+2}, Na^{+} which has shown the maximum lipase activity that is 10.9U/ml and 14 U/ml respectively. Hence they were again tested at different concentrations 1mM, 2mM, 5mM, 10mM and 20mM concentrations. In which the maximum activity was found to be 17.6 U/ml at 10mM of Na^{+} and 15.7 U/ml at 20mM of Mg^{+2} metal ion concentration as shown in the Fig 6 &7.

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Fig 6

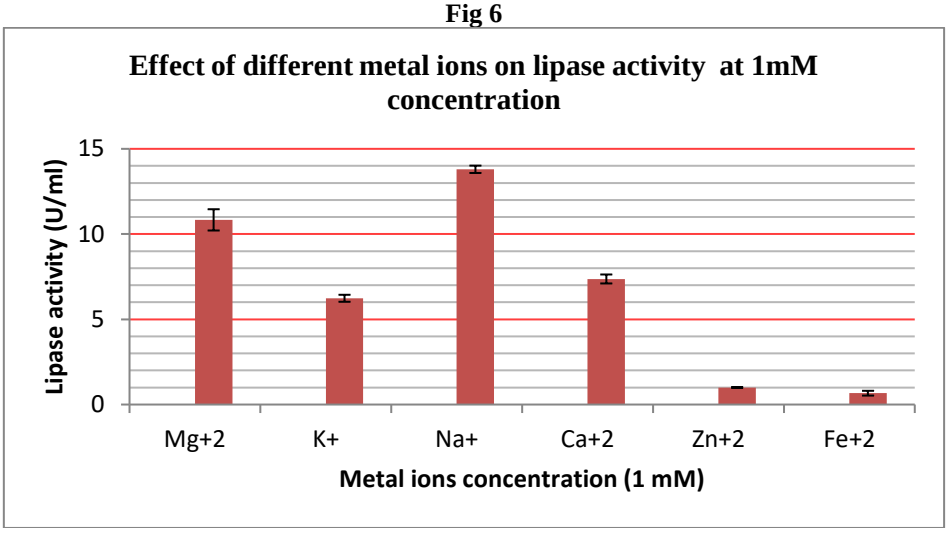
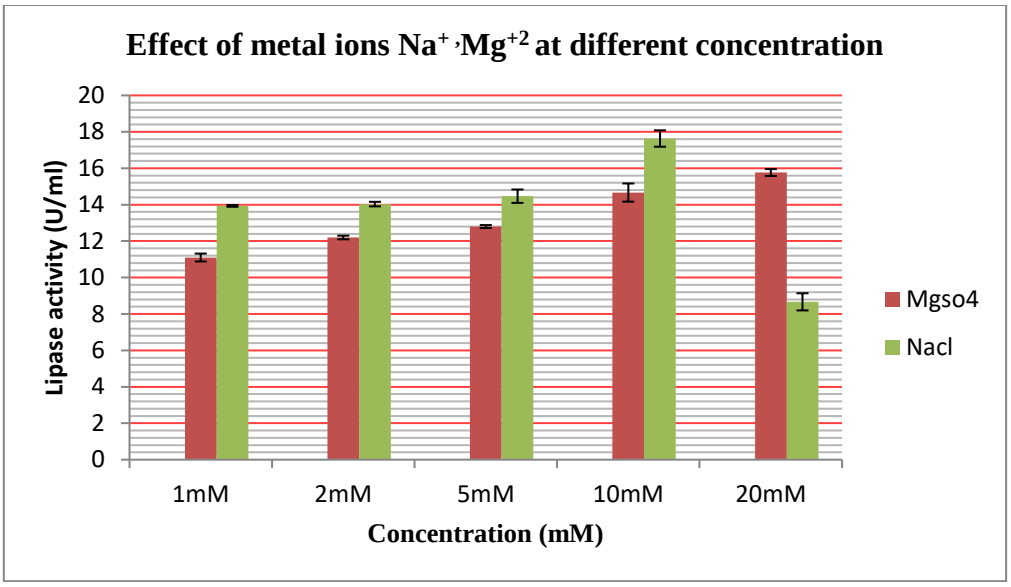


Fig 7



DISCUSSION

The results showed for optimization of media in order to enhance the activity was observed only after enrichment with sunflower oil for 72 h, glucose as Carbon source and peptone as a nitrogen source. Further it is observed that the enzyme possesses optimum pH at 8, Temperature at 37^0C. Based on the results we conclude that isolated bacteria from dairy industry wastes has optimum lipase activity with sunflower oil as substrate with maximum activity at 46 IU/ml after 72 hours of incubation, whereas,peptone as a nitrogen source showed maximum activity with 32 IU/ml and glucose as carbon source was shown maximum activity of 42 IU/ml at optimum pH of 8 it showed 42IU/ml and at its optimum temperature,37^0C maximum activity is found to be 30 IU/ml. Finally the optimization for lipase activity is done repeatedly for several times at the optimal conditions and the activity is calculated as 38.4±6.2 IU/ml

The activity of enzyme is very susceptible to media composition and other paramaters. Formerly, lipase production from *Aspergillus niger* was acquire by Solid Substrate Fermentation by from gingelly oil cake as substrate (Kamini et al 1998), however, we observed sunflower oil as substrate which has shown highest lipase activity in submerged fermentation process. Further it was already investigated that Lipase Production from milk isolate *Serratia rubidaea* and observed pH (7–8) and temperature (30–40°C) range optimum for maximum lipase production (Immaneul et al 2007.).Casein is selected as a nitrogen source ((6.5±0.015) U/mL) and soytone as a suitable substrate ((9.4±0.02) U/mL) for accelerating lipase production and activity for lipase obtained from *Serratia rubidaea (*Immaneul et al 2007.*)*,It was also noticed that the lipase production by *Pseudomonas sp. S34* was maximum with two nitrogen sources, namely tryptone and soytone. Even some of the studies reported that lipase production by *Penicillium aurantiogriseum* was high when inorganic nitrogen source was used. Basically, organic nitrogen such as peptone and yeast extract as suggested by our observations, have preferably been used as nitrogen

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source for lipase production by various *Bacillus* spp. (viz. *Bacillus* strain A30-1, *B. alcalophilus*, *B. Licheniformis*strain H1), different pseudomonas (viz. *Pseudomonas* sp., *P. fragi*, *P. fluorescens*BW 96CC) and *Staphylococcushaemolyticus*(Oh et al., 1999; Ghanem et al., 2000; Lanser et al., 2002; Sharma et al., 2002), while tryptone and yeast extract have been used for *S. Haemolyticus* L62 (Oh et al., 1999).

In our study, isolated bacteria shown similarity to all *Bacillus* sp. in the maximum production of lipase at 72 hr of enrichment and similar results were examined by Sarkar et al. (1998). In contrast, 12 h of the optimum incubation period was observed for *A.calcoaceticus*and *Bacillus* sp. (Mahler et al., 2000) and 16 h the optimum incubation period for *B. sthermocatenulatus (*Dannert et al., 1997). However, the maximum lipase activity was shown to be 72 and 96 h in the case of *P. fragi*and *P. fluorescens*, respectively (Pabai et al., 1996; Dong et al., 1999). Optimum temperature for lipase production was observed with respect to optimum temperature for growth. In the case of *B. subtilis* PCSIRNL-39 *it is observed* the optimum temperature at 45°C which is in agreement with the result of Alabras et al. (2017).

CONCLUSION:

Experimental results suggest that various media components influence the lipase activity by the isolated bacterial strain from the dairy industry waste. Optimization of media parameters for enhanced activity was observed at varying pH, Temperature, Carbon sources, nitrogen sources and different substrates, which has shown significant effect on lipase activity. Based on the results we concluded that isolated bacteria from dairy industry wastes has optimum lipase activity with sunflower oil as substrate after 72 hours of enrichment, whereas peptone as nitrogen source and glucose as carbon source along with increased activity with metal ions like Na^{+} and Mg^{+2} at an optimum pH 8 &optimum temperature at 37^{0}C.The maximum lipase activity was found to be 38.4±6.2 IU/ml.

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