ISSN 2250-3153

Effect of some factors on lipase production by Bacillus cereus isolated from diesel fuel polluted soil

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Abstract- Optimization the production of extracellular lipase in culture of *Bacillus cereus* has been investigated. From 10 samples of diesel fuel polluted soil it was found that 3 samples was positive for the presence of *B. cereus*. Lipase production by *B. cereus* isolated from diesel fuel polluted soil was investigated and optimized. The enzyme production was increased with increasing time and maximum enzyme activity was obtained after 72 hrs of incubation. Effect of pH and temperature indicated that, the lipase production was maximum at pH 8.0 (60.2 U/ml) and at 35°C (55.25 U/ml). The lipase production was optimized in shake flask experiments. With a selected carbon sources, maltose (65.5 U/mL) and nitrogen source, peptone (66.25 U/mL) was suitable substrates for accelerating lipase production. The present study indicates that the various factors influenced enzyme production by the bacteria, it appears that the nature of the supplements had significantly influenced on the production.

Index Terms- Lipase, B. cereus, Production factors.

I. INTRODUCTION

Lipase is a potential enzyme employed in industries for decades to hydrolyse fats and for the production of desirable esters [1]. The production of lipase by microorganisms depends largely on the species, strains and culture conditions that are responsible for the hydrolysis of lipids. Lipases have been isolated from microorganisms especially from fungi, bacteria, and yeasts [2]. Lipase occurs widely throughout the world's flora and fauna. In eukaryotes, lipases are involved in various stages of lipid metabolism including fat digestion, absorption, reconstitution, and lipoprotein metabolism. In plants, lipases are found in energy reserve tissues. Numerous species of bacteria, yeasts and moulds produce lipases with different enzymological properties and specificities [3]. Similarly, many authors reported that, the common lipase-producing bacterial strains are *Pseudomonas aeruginosa, P. fluorescens, Bacillus coagulans, B. cereus, Staphylococcus aureus, S. hyicus* [4]. Lipases are used as additives in detergent formulations, in cleaning solutions and in waste treatment cocktails for downstream industrial processes and for domestic use also [5]**.** Lipase production is influenced by the type and concentration of carbon and nitrogen sources, the culture pH, the growth temperature and the dissolved oxygen concentration [6]**.** Considering the information given above the present study was undertaken to optimize the lipase production by *Bacillus cereus* isolated from diesel fuel polluted soil using different substrates, pH, temperature and metal ions.

II. MATERIALS AND METHODS

Sampling

Diesel fuel polluted soil samples were collected in sterilized polystyrene bags of one liter capacity from different 10 areas, Baghdad, Iraq. Samples were transferred for microbial analysis.

Isolation of Lipolytic bacteria

For the isolation of lipolytic microbes, 1.0 gm of sample was dissolved in 100 ml of double distilled water. It was then serially diluted ($10^{-1}$to 10^{-6}) and the diluted samples were plated on tributyrin agar plates. The formation of clear zone around the colony on the plate was considered as lipolytic microbes [7].

Identification

Bacilli bacteria which formed large clear zone around the colony were identified based on morphological, biochemical and physiological characters according to Bergeys manual of determinative bacteriology [8] which were identified as *Bacillus cereus*, the isolated organisms were maintained on nutrient agar slant supplemented with 1% olive oil.

Lipase Production

The bacterium was initially cultured using medium containing (w/v): yeast extract (0.15%), peptone (0.5%), sodium chloride (1.0%) and olive oil (0.5%), at pH 7, and 32°C for 24 h. Then, 5% of enriched seed culture was inoculated into a 50 ml medium (w/v) containing potassium peptone 0.5%, dihydrogen orthophosphate 0.1%; sodium chloride 1% and magnesium sulphate 0.01%. Then it was incubated at 32°C at 150 rpm. After incubation it was centrifuged at 10000 rpm and the supernatant was used for lipase activity determination [7].

Lipase Assay

Lipase activity was assayed through spectrophotometric method by using p-nitrophenol palmitate as substrate. The reaction mixture containing 100 l of 50 mM Tris buffer (pH-7.0), 50 l of substrate solution (1mM p-NPP containing 1% Triton X-100), 350 l of H2O and the reaction was initiated by adding 100µl of enzyme solution. After incubation of 10 min, the reaction was stopped by adding 1 ml of 2% sodium dodecyl sulphate (SDS) solution. The absorbance was red at 420 nm using UV–vis–spectorophotometer. One unit of lipase activity was defined as the amount of enzyme releasing 1 µmol of p-nitrophenol per minute [9].

Optimization of Lipase Production [10].

Effect of Incubation time on lipase activity

The effect of incubation time on lipase activity was carried at various time periods such as 24,48,72,96,120 and144 hours in medium and incubated at 32°C. The production time (24 h) was kept as constant for the consecutive experiments.

Effect of Incubation temperature on lipase activity

For selection of optimum temperature for the production of lipase the temperatures varying from 25-45°C were selected .

Effect of pH on lipase activity

To study the effect of pH, the lipase activity was measured at various pH ranging from 3 to 9. The pH was varied using different buffers (citrate buffer for pH 3-6.phosphate buffer for pH 7-8 and borate buffer for pH 9-10).

Effect of Carbon supplements on lipase activity

Substrate is supplemented with different carbon supplements like glucose, maltose, lactose, sucrose and fructose at a final concentration 5% (w/w).

Effect of Nitrogen supplements on lipase activity

Substrate is supplemented with different nitrogen supplements such as yeast extract, beef extract, peptone and urea at a concentration of 3% (w/w) were used.

III. RESULTS AND DISCUSSION

From different 10 area in Baghdad, 10 sample of soil contaminated with diesel fuel were obtained. 7 isolates identified as rod of spore former (*Bacillus* spp.) by morphological and microscopic characteristics, and 3 of these isolates (DB.1, DB.2, and DB.5) were identified as species (*B. cereus*) according to there biochemical characteristics [11]. The previous results indicated that *B. cereus* strains isolated from refinery field have great potential for in situ remediation of diesel contaminated soil in oil refinery site [12]. *B. subtilis* and *B. cereus* were found to be hydrocarbon degrades in polluted soil with diesel oil [13]. Tributyrin agar plates were used and screened for the production of lipase. The presence of clear zone of 30mm diameter around the colony was observed as lipase producers by the isolate DB.5 in contrast with the other 2 isolates and used for the other experiments .

The production of lipase was studied by conducting the incubation of *B. cereus* for different time intervals (24, 48, 72, 96, 120 and 144 hrs). The enzyme production was increased with increasing time and maximum enzyme activity was obtained after 72 hrs of incubation(figure 1).

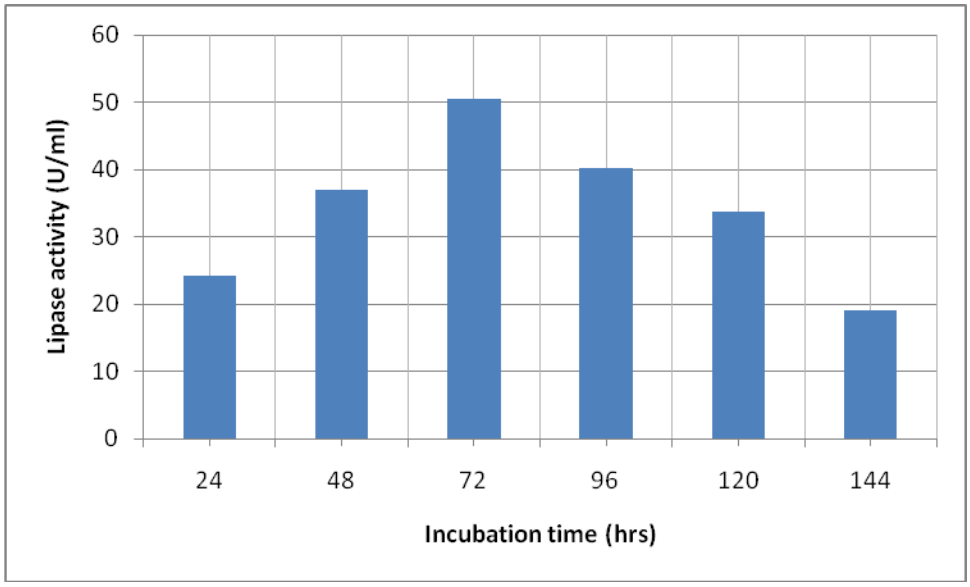

Figure1. Effect of incubation time on lipase production by *B. cereus* .

The enzyme activity declined in subsequent hours. The fall of enzyme activity might be due to the absorption of the enzyme by the substrate or by the proteolytic activity. To study the effect of incubation temperature, the inoculated flasks were incubated at different temperatures to determine the optimum temperature for lipase production. The enzyme production was carried out by at 25-45°C temperature range. The optimum incubation temperature of the production of lipase 55.25 U/ml was found at 35°C (figure 2). Enzyme production was reduced when the incubation temperature was increased above 35°C.

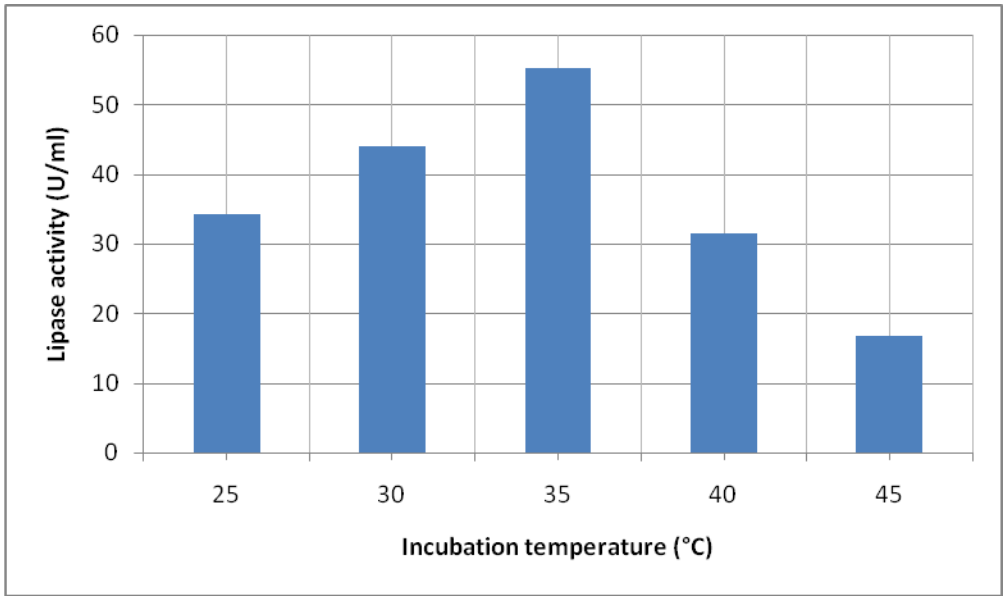

Figure 2. Effect of incubation temperature on lipase production by *B. cereus*.

This indicated that *B.cereus* was mesophilic organisms and that couldn't tolerate higher or lower temperature. Optimization of temperature is vital for cell growth and enzyme production. The present result was supported by the report of Shariff *et al.,* (2007) [10] for optimum lipase production by *Bacillus* sp. strain L2 at 37- 40°C. Also Baharum *et al.,* (2003) [14] found that lipase production by *Pseudomonas* sp. strain S5 was maximum at 37°C.

Lipase production of *B. cereus* was observed between pH 5.0-9.0. The results suggested that there was a stimulation of an enzyme production at a pH range of 8.0 (figure 3).

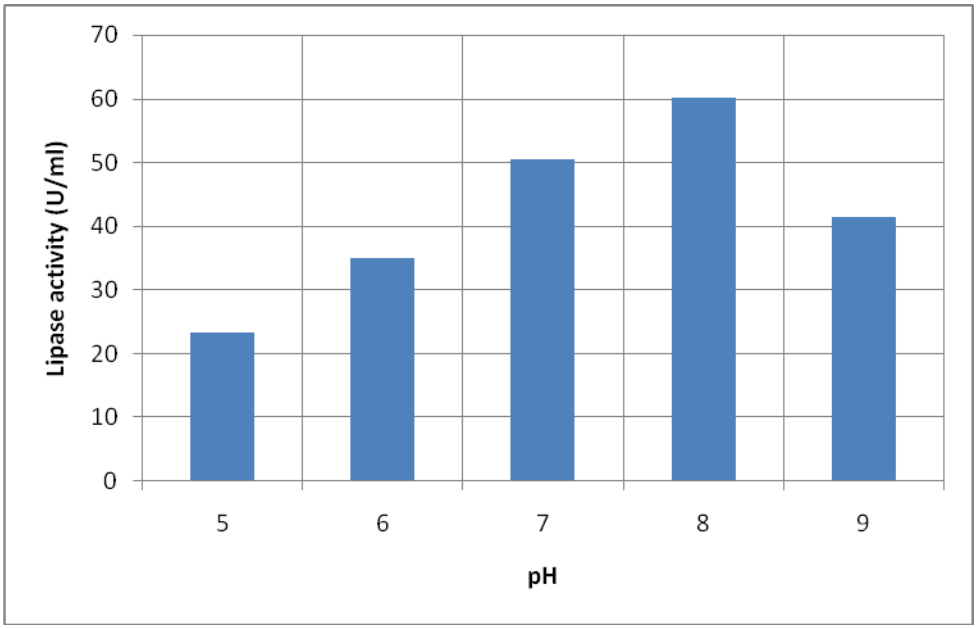

Figure 3. Effect of pH on lipase production by *Bacillus cereus*.

It reflected that the organism required an alkaline pH for the production of lipase. Likewise, the high lipase production was obtained from *Acinetobacter sp.* RAG-1 in a production medium at pH 8.0 through submerged fermentation [15]. The results obtained were similar to that of typical characteristics on *Bacillus* sp. [16]. This was supported by the study of Sekhon *et al.* (2005)[17] *on Bacillus megaterium* AKG-1, which has optimum activity at pH 7.

The culture environment has a dramatic influence on enzyme production especially carbon and nitrogen sources play a crucial role in enzyme induction in bacteria [18]. In the present study, the effect of various supplementary carbon sources namely

maltose, lactose, sucrose and fructose 5% (w/w) to production medium. Of the four different carbon supplements used as enrichment, maltose was a good carbon supplement which gave maximum lipase activity 65. 5 U/ml (figure 4).

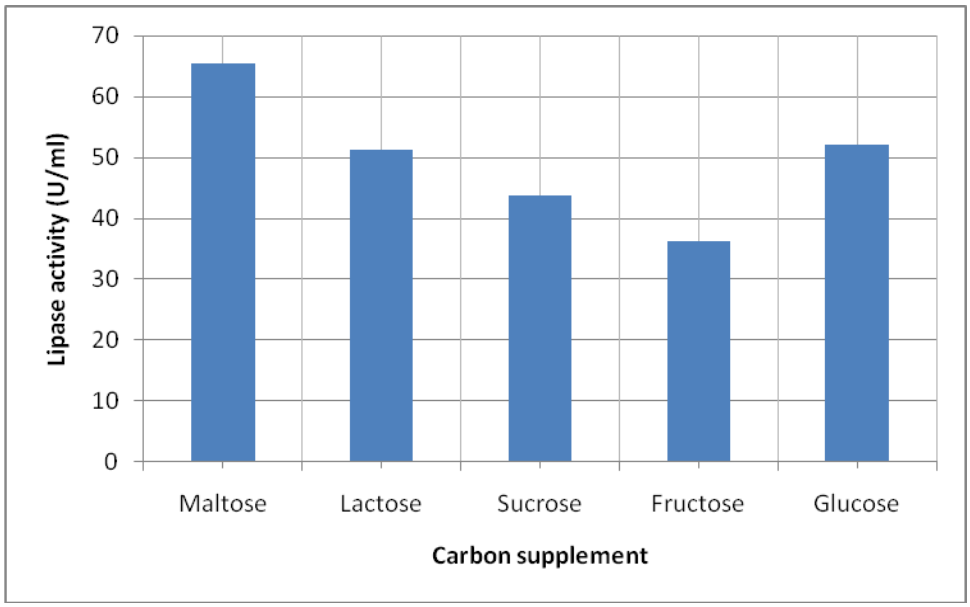

Figure 4. Effect of carbon supplements on lipase production by *B. cereus*.

The major factor for the expression of lipase activity has always been carbon, since lipases are inducible enzymes and are thus generally produced in the presence of a lipid source such as oil or any other inducer, such as triacylglycerols, fatty acids, hydrolysable esters, tweens, bile salts and glycerol. However, their production is significantly influenced by other carbon sources such as sugars, polysaccharides, whey and other complex sources [19]. Novotny *et al.*, (1988) [20] reported that olive oil in combination with glucose increased the lipase activity and the glucose was important carbon source for lipase production.

Beside carbon source, the type of nitrogen source in the medium also influenced the lipase titers in production medium. Generally, microorganisms provide high yields of lipase when organic nitrogen sources are used, such as peptone and yeast extract, which have been used for lipase production by various *Bacillus* sp. and *Pseudomonads* sp. [21]. Influence of organic nitrogen supplements on enzyme production was studied by adding various supplements namely yeast extract, peptone, beef extract and urea 3% (w/w) to fermentation media. Of the four different nitrogen supplements used as enrichment, it was observed peptone a good nitrogen supplement which gave maximum lipase production 66.25 U/ml (figure 5).

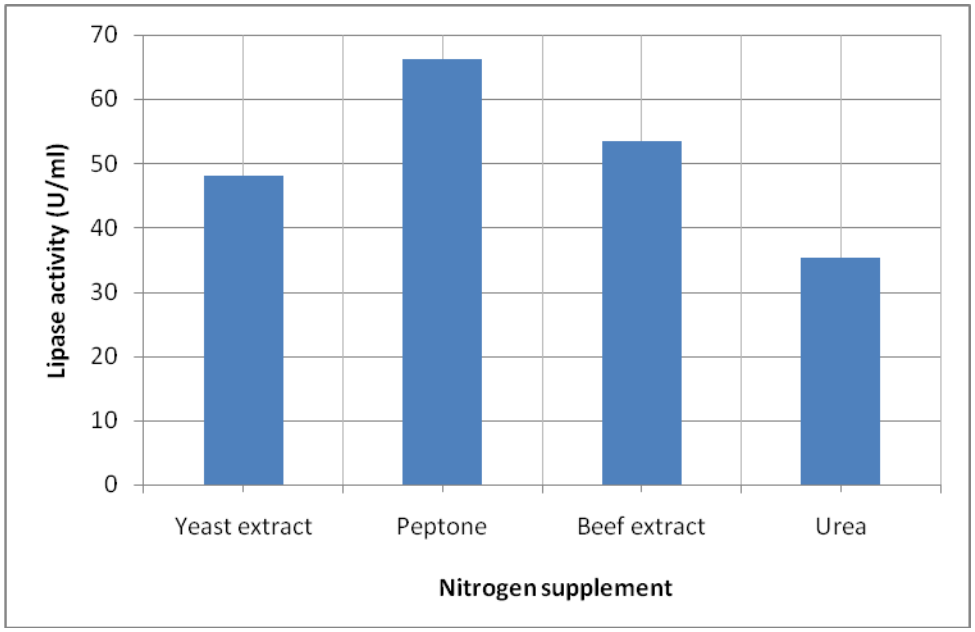

Figure 5. Effect of nitrogen supplements on lipase production by *B. cereus*.

The results of Prasad and Rekha (2013) [22] was not agree with our results who found that high *B. Subtilis* lipase activity in tryptone, *B. Licheniformis* and *Bacillus amyloliquefaciens* activity high in casein as nitrogen source. While other results showed that *Bacillus cereus* strain MSU AS revealed that highest lipase production was observed in nitrogen source peptone supplemented medium[23].

IV. CONCLUSION

Lipases have found a wide range of applications in various industries such as chemical industry, food industry, leather and pulp industries, synthetic chemistry and detergents etc. The present study indicates that the various factors influenced enzyme production by the bacteria, it appears that the nature of the supplements had significantly influenced on the production. The incubation time of 72 hrs, temperature 35 °C, pH 8, maltose as carbon supplement and peptone as nitrogen supplement were found to be the optimum conditions for lipase production and the maximum lipase activity was found to be 66.25 U/ml.

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