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Influence of various bioprocess factors on production of lipase from *Pseudomonas aeruginosa*

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Abstract

This study was conducted in the laboratories of biology department, faculty of Science, which deal with isolation of lipase and optimization of process parameters to achieve maximum yield of lipase by *Pseudomonas aeruginosa* which carried out for enhanced production of lipase using olive oil as the substrate of enzyme , maximum activity of the enzyme was achieved under best growth conditions , the best conditions were the isolated of lipase from *Pseudomonas aeruginosa* in synthetic medium , it gave high titer of lipase activity , the glucose as carbon source , peptone as nitrogen source , incubation period 48 h , incubation temperature 40 °C and pH = 8 .

Key Words: Lipase; *Pseudomonas aeruginosa*; production

Introduction

Lipases are water soluble enzymes which have the capacity to hydrolyses triacylglycerol to release free fatty acids and glycerol, lipases create a main group of biocatalysts that have huge biotechnology uses , lipases have been insulated and purified from bacteria, plant, fungi, yeast, , and animal sources , of all these, bacterial lipases are more stable and cheap [1 ,2] .

Bacterial lipases are used widely in diet and dairy industry for the hydrolysis of cheese ripening , milk fat, flavor enrichment and lipolysis of butter fat and cream [3]. Lipases are also used in cleansing agent industry as additive in washing powder , textile industry to rise fabric absorbency [4, 5] , for creation of biodegradable polymers or mixtures [6] , and diverse transesterification reactions [7].

In addition , the lipase is used as a promoter for manufacture of diverse products used in beautifying industry [8], in paper and pulp industry [9], in production of biodiesel [10], degreasing of leather [11] and in pharmaceutical industry [12]. Now bacterial lipases are great demand because of potential industrial uses, in favor of the lipase importance , the present research has been accomplished to lipase isolation and optimization of route parameters to employment lipase highest give way via *Pseudomonas aeruginosa* .

Materials and Methods :

1. Bacterial isolates :

bacterial isolates were obtained from the hospital of Al-Hakem Hospital in Najaf city , and identification of bacterial isolates were based on biochemical tests which described by (13) .

2: Preparation of *P. aeruginosa* inoculum and enzyme isolation (14) :

Pure culture of newly isolated *P. aeruginosa* was maintained on nutrient agar slants at 4 °C, the synthetic medium (Table 1) was autoclaved for 15 min, after cooling the flasks, inoculum was arranged 3 colony of *P. aeruginosa* were mixed in sterilized test tube with sterilized conditions, inoculum (5 ml) was added to each flask containing 100 ml of growth

medium with the help of sterilized disposable syringe and flasks was incubated at 35°C on a rotary shaker at 125 rpm for 3 days , The cell was detached by centrifugation at 10000 rpm and 4 °C for 20 min. The supernatants were collected and used to define the activity of lipase. The separation expressed the maximum activity was designated for further studied .

(Table 1) : Structures of synthetic medium of *P. aeruginosa*.

S/N	Ingredients	Quantity(g/100 ml)
1	$CaCl_2$	0.0 1
2	$(NH_4)_2SO_4$	0.025
3	Yeast extract	0.
4	K_2HPO_4	0.07
5	$MgSO_4$	0.05
6	peptone	0.1
7	$FeSO_4.7H2O$	0.01
0.01		$MnCl_2$ 8
9	olive oil	1%
10	KH_2PO_4	0.03
11	Distilled water Up to	100 ml mark

3: lipase Assay : activity of lipase was determined titrimetrically using olive oil hydrolysis (15), after which 1 ml of solution of enzyme was added to the assay substrate containing 10 ml of 10% homogenized olive oil in 10% gum acacia, 2 ml of 0.6% CaCl2 solution and 5 ml of 0.2 mol/L phosphate buffer, with pH 7.2. The substrate – enzyme was incubated at 35°C on an orbital shaker at 125 rpm for 1 h (16) , after which 20 ml acetone - ethanol (1:1 v/v) was added to end the reaction. Liberated fatty acids were titrated with 0.1 mol/L NaOH using phenolphthalein as an indicator. The reaction mixture without the enzyme was titrated in the same way and used as blank. One lipase unit was defined as the enzyme that released (1 µmol) of fatty acid per min under standard assay conditions .

4:factors affecting lipase production : according to (17)

4-1 : Culture media : The optimum culture medium of the lipase production was determined by assaying activity using 1% olive oil as the substrate of enzyme using different culture media include [synthetic medium , M.R.V.P broth, and yeast extract broth] .

4-2 :Incubation periods

The reaction mixture was incubated at diverse incubation period (24, 48, 72, 96) hours at pH = 7 and 37 °C.

4-3 : Incubation temperature

The optimum incubation temperature of the lipase production was determined by assaying activity using 1% olive oil as the substrate of enzyme using temperatures different values (20,30,40, 50) °C .

4-4 : pH values

The optimum pH of the enzyme production was also determined by assaying activity using 1% olive oil as the substrate of enzyme using diverse pH values(4, 7, 8, 10) at 40 °C .

4-5 : Influence of diverse sources of carbon on production of lipase

The effect of several carbon sources on lipase production was done as designated by using the previously described production medium , the carbon sources studied are glucose, lactose , sucrose ,galactose and fructose , the pH of the medium was adjusted to 8 and cultures were incubated at 40 °C.

4-6 : Influence of different sources of nitrogen on production of lipase

The effect of several nitrogen sources on lipase production was done as designated by using the previously production medium , the nitrogen sources studied include, organic sources such as peptone and yeast extract and inorganic sources such as urea, ammonium chloride and $(NH_4)_2SO_4$, the pH of the medium was adjusted to 8 and cultures were incubated at 40 C .

Results and Discussion:

1: Identification of bacterial isolates :

The results of biochemical tests which used for identified the *P. aeruginosa* isolates , this results found all the bacterial isolates they ferment glucose and fructose , they are oxidase , citrate , gelatin hydrolysis and catalase positive , As well as all the *P. aeruginosa* isolates are nitrate reduction positive but the result for other biochemical tests negative (13) .

2 : Factors affecting lipase production :

2-1 : Optimum culture medium for a lipase production :

The highest enzyme production by *P. aeruginosa* was occurred using the synthetic medium , it gave high titer of lipase (0.932U/ml) followed by M.R.V.P broth (0.514 U/ml) while yeast extract broth gave low titer of lipase activity (0.082 U/ml) , Figure (1) . The select of the appropriate fermentation medium is a essential factor for microbial development and enzyme creation , the growth of an organism in culture medium is inclined by the nutrient composition of the medium (18) .

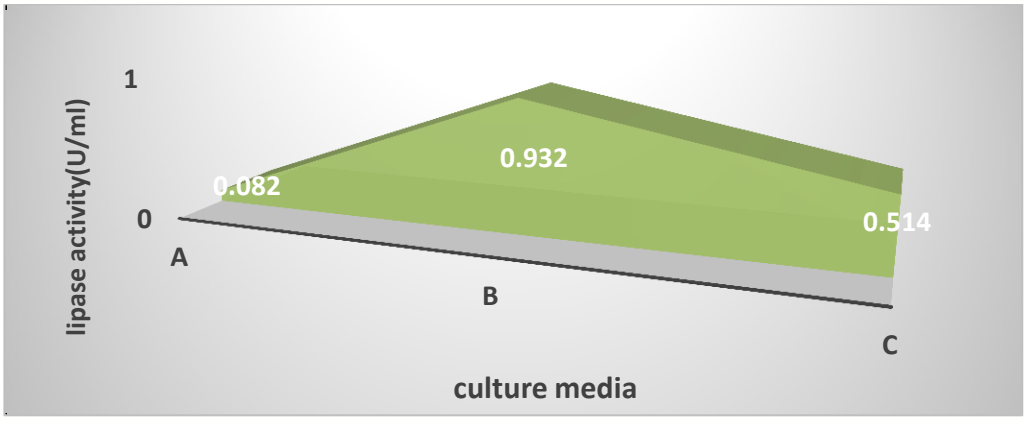
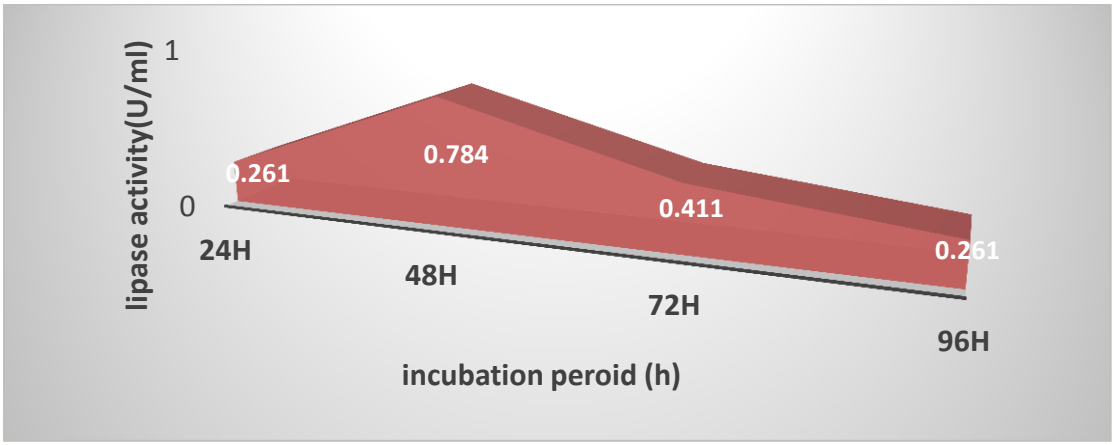


Figure (4-1) : Effect culture media A: yeast extract broth, B: synthetic media , C: M.R.V.P broth on the lipase production from *P. aeruginosa*.

2-2 : Optimum incubation period for Lipase production:

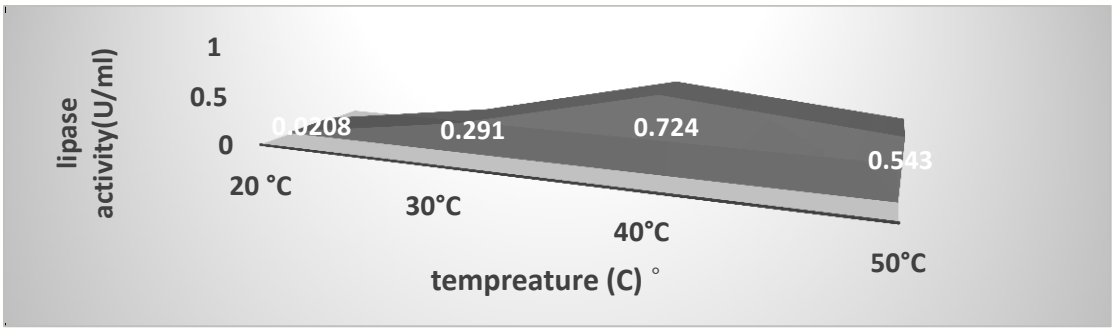
The figure (4-2) shown the increasing of enzyme activity with increasing the incubation period until reach to maximum activity (0.784 U/ml) in second day (48 hours) using the olive oil as a substrate of enzyme , then it began to decreased (0.411U/ml) in 72, then it began to decreased more (0.082U/ml) in 96 , rise in incubation period resulted in decrease in the lipase production by *E. coli*, this may be due to the information that after maximum creation of lipase enzyme there was manufacture of other by products and a nutrients diminution .Similar results were reported that the maximum lipase production from bacteria BLP2 *Pseudomonas gessardii* (19) and from the *staphylococcus sp* (20) occurred in the same incubation period , but other study (21) report the maximum activity for lipase production from *Bacillus cereus* occurred in 72 h .



Figure(4- 2) : Effect of incubation period on the lipase production from *P. aeruginosa* .

2-3 : Optimum temperature for enzyme production:

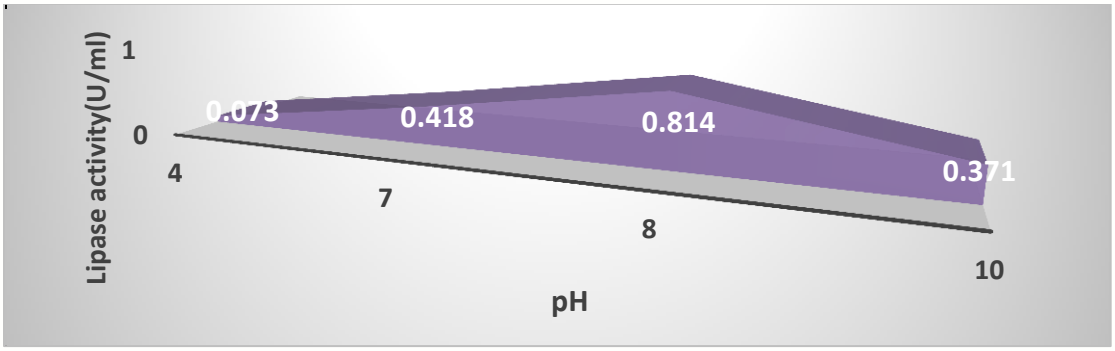
The effect of temperature on lipase production showed that enzyme activity improved gradually with increase in temperature from 20 °C reaching a maximum in 40 °C (0.724U/ml) above this temperature there was a reduction in the lipase activity (0.543U/ml) in 50°C respectively (Figure 4-3) . Growth heat is a very significant factor which varies from organism to organism and minor changes in growth temperature may change enzyme creation (22) . other research (23) was reported that the maximum lipase production occur at 37°C by *Pseudomonas xinjiangensis*



Figure(4-3) : Effect of incubation temperature on the production of lipase from *P. aeruginosa*.

2-4 : Optimum pH for enzyme production:

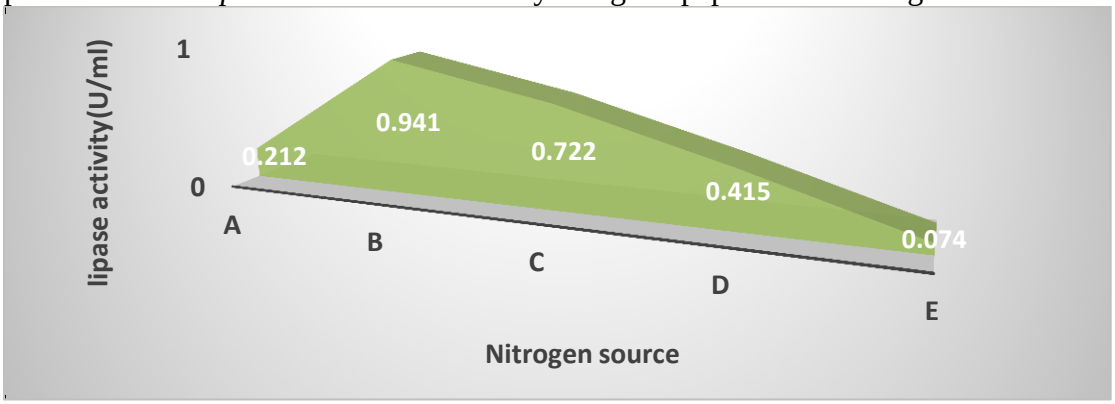
The maximum activity of lipase occurred in pH = 8 (0.814 U/ml) using the olive oil as a substrate, then it began to decreased in higher pH values (0.372 U/ml) in pH= 10 . pH is important feature that effects the microbial physiology by affecting morphology of cell membrane by product formation and oxidative reductive reactions , nutrient solubility and uptake and enzyme activity , the pH alteration observed during the organism growth also affects product steadiness in the medium, pH can have deep effects on both the rate of manufacture and the synthesis of enzymes (24) . other study (25) they reported the optimum pH for lipase production from *Pseudomonas spp* occurred in the pH = 7 .



Figure(4-4) : Effect of pH on the production of lipase from *P. aeruginosa*.

2-5 : Optimum nitrogen source for lipase production:

The highest enzyme production by *P. aeruginosa* was occurred using the peptone as nitrogen source , it gave high titer of lipase activity (0.941 U/ml) followed by ammonium sulfate (0.722 U/ml) , while the other sources ammonium chloride give (0.415U/ml) , yeast extract give (0.212U/ml) and urea give (0.074U/ml) respectively figure (4- 5) . During the fermentations of microbes , the source of carbon not only acts as a chief constituent for building of cellular substantial , but is also used in the creation of polysaccharide and as source of energy, nitrogen sources such as inorganic nitrogen is supplied as ammonia gas , ammonium salts or nitrates , ammonia has been used for pH control ,ammonium salts such as ammonium sulphate typically creates acidic settings as the ammonium ion gets utilized and the free acid is liberated .Additional study (26) also showed the maximum activity of lipase production from *pseudomnas* achieved by using the peptone as a nitrogen source .



Figure(4-5) : the Effect of nitrogen sources (A:yeast extract, B:peptone, C: ammonium sulfate , D:ammonium chloride , C:urea) on the lipase production from *P. aeruginosa*.

2-6 : Optimum carbon source for lipase production :

The figure (4-6) shown the highest enzyme production by *P. aeruginosa* was occurred using the glucose as the carbon source , it gave high titer of lipase activity (0.814U/ml) followed by fructose and sucrose (0.677 U/ml , 0.512U/ml) respectively, while galactose gave low titer of lipase activity (0.075U/ml) . Also (27) reported the optimum lipase production from *Pseudomonas sp*. BWS-5 occurred by using the same carbon source , whereas (28) reported the optimum lipase production from *Bacillus pumilus* occurred by using the sucrose as the carbon source

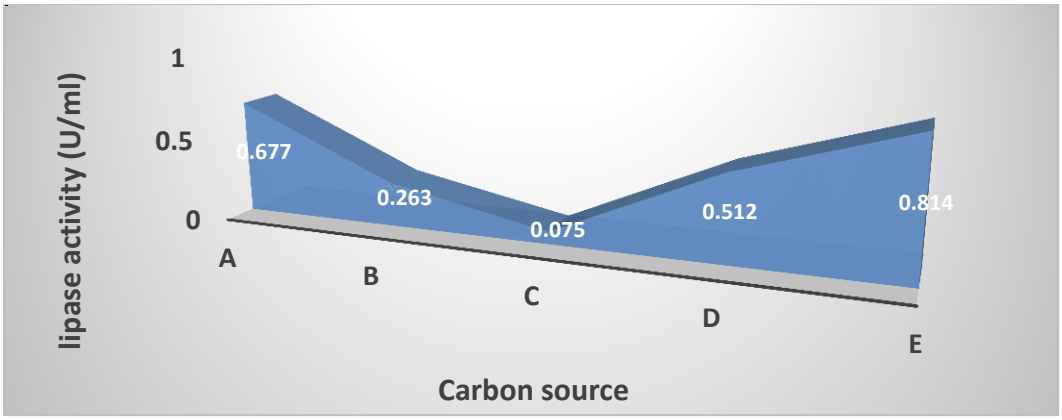


Figure (4-6) : the Effect of Carbon sources (A: fructose, B: lactose , C: galactose , D: sucrose , E: glucose) on lipase production from *P. aeruginosa*.

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