

*Full Length Research Paper*

# Screening for lipase-producing *Enterobacter agglomerans* for biodiesel catalyzation

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**This paper reports a comprehensive study of screening rapeseed soil sample in Victoria blue solution solid medium, with olive oil as sole carbon source. The lipase of *Enterobacter agglomerans* which has the highest activity was identified. Under the optimum conditions determined, the enzyme activity of this strain reached 39.09 U/mL. The optimal conditions were found to be: initial pH 7.0, agitating at 30 °C for 48 h, with 2.0% (w/v) lactose as carbon source, 1.5% (w/v) beef extract and 1.0% (w/v) yeast extract as nitrogen source, and 0.1% (w/v) MgSO<sub>4</sub>·7H<sub>2</sub>O. Olive oil, sesame oil and tea oil as raw materials can be catalyzed to biodiesel by the lipase of this strain at 30 °C and 180 rpm. And the yield reached 54.51% with sesame oil as raw material, even when they contained 92.4% (w/v) water in the starting materials. This strain will potentially serve as a promising alternative lipase for biodiesel production with raw materials containing water.**

**Key words:** Biodiesel, lipase, enzyme activity, culture condition.

## INTRODUCTION

Biodiesel (fatty acid methyl esters), derived from triglycerides by transesterification with methanol, has attracted considerable attention as a renewable, biodegradable and nontoxic fuel (Hideki et al., 2001). Additional environmental benefits of biodiesel also include lower exhaust emissions of particulate matter and greenhouse gases such as CO, CO<sub>2</sub> and SO<sub>x</sub>. Several methods have been developed to produce biodiesel, including chemical, lipase catalysis and super critical methods. Conventionally the synthesis of biodiesel is accomplished by chemical transesterification from which short reaction times and high yields are obtained. However, pretreatment of the substrate is required when water is present, and difficulties in the recovery of catalyst and glycerol and high energy requirements are also disadvantages in chemical catalyzed processes (Ana and Enoch, 2003).

Although biodiesel is already produced in volume, its application on an industrial scale has been limited mainly due to the high feedstock price. So, using low-priced substrates can make the biodiesel price competitive and expand biodiesel production. And the potential cheap

substrates may include disposals from restaurants and household, waste vegetable oil from crude oil refining industries (Ana and Enoch, 2003), waste activated bleaching earth (Enoch and Masahiro, 2005), and so on. However, these substrates often contain a lot of water, which is not suitable for biodiesel production by chemical transesterification. Fortunately, as efficient biocatalysts, lipases possess the unique feature of acting at the interface between an aqueous and an organic phase, which is very suitable for treating high water-content raw materials. Activation of the enzyme involves unmasking and restructuring of the active site through conformational changes of the lipase molecule, which requires the presence of oil-water interface (Noureddini et al., 2005). In such a process, the presence of water can help prevent the inactivation of lipase by methanol (Masaru et al., 2001); with the increased addition of water the available interfacial area increased (Noureddini et al., 2005). So, lipases can be widely used as biocatalysts for the synthesis of biodiesel from natural oils. And the enzymatic method becomes more and more attractive (Mohamed and Soumanou, 2003; (Watanabe et al., 2002; Funda et al., 2007).

Since waste oil used as the substrate inevitably contains a certain amount of water, it is necessary to find a lipase that efficiently catalyze methanolysis even in the

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presence of water (Masaru et al., 1999). Moreover, since the composition of the medium can also influence lipase production dramatically, it should be considered carefully (Masaru et al., 1999). A cheap and effective medium can decrease the production cost, so it is crucial to the industrialization of the enzyme method (Rodriguez et al., 2006). Therefore, in order to benefit most from biocatalysts, it is important to determine the optimum cultivation conditions.

In this paper, we report a study to determine the optimum conditions required for the lipase production of *Enterobacter agglomerans* in submerged shake flask cultures. From a series of experiments, we comprehensively investigated the factors that affect the production of lipase of *E. agglomerans*, including the concentrations of the components of the medium, the initial pH, the temperature and time, the choices of the carbon and nitrogen sources and metal ions. Thereafter, the lipase of *E. agglomerans* was used to catalyze biodiesel production with tea oil, olive oil and sesame oil as raw materials.

## MATERIALS AND METHODS

To make the experimental results reliable and reproducible, each experiment in this study was carried out in triplicates.

### Chemicals

All chemical compounds used in this study were purchased commercially at the highest purity possible.

### Medium

The solid flat medium contains (w/v): olive oil 1.0%,  $(\text{NH}_4)_2\text{SO}_4$  0.5%, peptone 2.0%,  $\text{K}_2\text{HPO}_4$  0.5%,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.1%, and agar 1.5%. pH was adjusted to 7.0. 4.0 mg·100 mL<sup>-1</sup> Victoria blue solution was added and the medium was emulsified for 1 min in asepsis condition when the medium was cooled to 60°C after sterilization.

The enrichment medium contains (w/v): olive oil 1.5%, peptone 1.0%,  $\text{K}_2\text{HPO}_4$  0.1%,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.05%, KCl 0.05%, and  $\text{KH}_2\text{PO}_4$  0.3%. The slant peptone medium contains (w/v): peptone 0.5%, yeast extracts 0.5%, NaCl 0.3%,  $\text{K}_2\text{HPO}_4$  0.1%, and agar 1.5%. The duplicate screening medium contains (w/v): olive oil 1.0%, peptone 1.0%,  $\text{K}_2\text{HPO}_4$  0.1%,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.05%, KCl 0.05%, and  $\text{KH}_2\text{PO}_4$  0.3%. The fermentation medium contains (w/v): olive oil 2.0%, peptone 2.0%,  $\text{K}_2\text{HPO}_4$  0.1%,  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  0.1%,  $\text{KH}_2\text{PO}_4$  0.3%, and  $(\text{NH}_4)_2\text{SO}_4$  0.5%. The final pH was adjusted to 7.0 using 0.1 M  $\text{Na}_2\text{CO}_3$  or 0.1 M HCl.

### Assay of lipase activity

Lipase activity was determined by the olive oil–PVA emulsion method, which is a partial modification of the method reported by Abramic et al. (1999). 3.0% polyvinyl alcohol solution and olive oil were blended by 3:1 (v:v) and emulsified for 15 min, then the mixture was centrifuged at a stirring speed of 5000 rpm for 30 min, and the supernatant (olive oil) was used as substrate.

The reaction mixture containing 4.0 mL of olive oil was emulsified in polyvinyl alcohol, 5.0 mL of 0.05 M glycine-NaOH

buffer (pH 10.30) and 1.0 mL of the lipase solution. Incubation was performed at 30°C for 15 min and terminated by adding 15 mL of ethanol. The free fatty acid produced was titrated against 0.1 M sodium hydroxide solution with phenolphthalein as an indicator. One unit of lipase activity (U) was defined as the amount of enzyme which liberates 1.0 μmol of fatty acid per minute at 30°C.

### Screening of strains

All the strains used in this study were isolated from soil samples obtained from rapeseed field. The screening process is as follows:

- (1) 1.0 g sample was dilute to 10<sup>-4</sup> g·L<sup>-1</sup>, then 0.5 mL of diluted solution was used to daub the solid flat medium and incubated at 28°C for 72 h.
- (2) All the strains that can positively produce lipase were diverted to the enrichment medium and cultivated for 24 h in 28°C at a stirring speed of 120 rpm. Then 0.5 mL culture solution was taken to daub the preliminary screening medium (the composition is the same as the solid slant medium) and cultivated at 30°C for 72 h.
- (3) The larger colonies were selected into peptone slant medium, cultivated for 72 h at 30°C, and then they were inoculated to the duplicate screening medium and cultivated for 24 h at 30°C with a stirring speed of 120 rpm.
- (4) 0.5 mL culture cultivated in the duplicate screening medium was inoculated into the fermentation medium and fermented at 30°C for 72 h at a stirring speed of 120 rpm, and used to determine the lipase activity.

### Identification of lipase

We compared the 16 s rDNA of this strain with *E. agglomerans*, and found that 99.1% of them is homologous. Moreover, the characteristics of this strain are consistent with *E. agglomerans* too. Therefore, it can be confirmed to belong to *E. agglomerans*.

### The catalysis reaction

3.11 mL (0.01 mol) raw oils (the average molecular weight of tea oil, sesame oil and rapeseed oil was 280, and the density, 0.913 g/mL) were added to the fermentation medium of *E. agglomerans*; the oil-methanol molar rate is 1: 3, and 0.405 mL methanol (the density is 0.913 g/mL) was added to the medium for three times, and the reaction time was 48 h.

### Gas chromatography analysis

Products from methanolysis or methylated fatty acids were re-dissolved in 2.0 ml ether. The sample was then injected to a gas chromatograph (SP-6890) coupled with a glass column (chromatograph column DB-23 for the chromatograph workstation of N3000) and a flame ionized detector (FID). The column, injection port, and oven temperatures were 180, 220 and 230°C, respectively. Methyl heptadecanoate purchased from Sigma Chemical Co. was used as an internal standard.

## RESULTS AND DISCUSSION

### Effect of carbon source

The enzyme production usually depends greatly on the composition of the medium (Pimentel et al., 1996). In this

**Table 1.** Effect of different carbon sources on the production of *Enterobacter agglomerans*<sup>a</sup>.

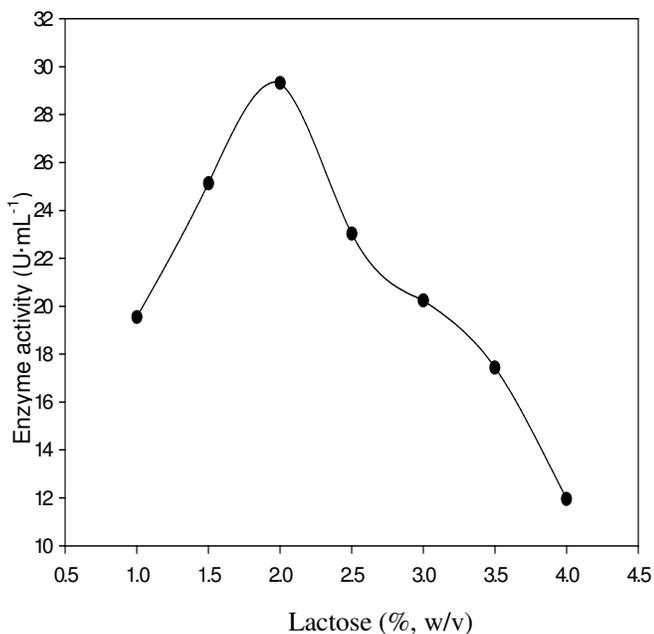
Carbon source (2.0%, w/v)	Glucose	Maltose	Fructose	Sucrose	Mannose	NaHCO <sub>3</sub>	Xylose	Lactose
Enzyme activity (U·mL <sup>-1</sup> )	9.77	6.98	11.87	9.77	12.56	11.17	13.96	29.32

<sup>a</sup>the experiments were performed for 48 h at 30 °C, initial pH 7.0, with a stirring speed of 120 rpm.

**Table 2.** Effect of nitrogen source on the production of *Enterobacter agglomerans*<sup>a</sup>.

Nitrogen source (2.0%,w/v)	Yeast extract	peptone	CO(NH <sub>2</sub> ) <sub>2</sub>	NH <sub>4</sub> Cl	Beef extract	KNO <sub>3</sub>
Enzyme activity (U·mL <sup>-1</sup> )	16.75	8.38	8.38	16.75	18.15	6.98

<sup>a</sup>The experiments were performed for 48 h at 30 °C, initial pH 7.0, with a stirring speed of 120 rpm.



**Figure 1.** Effect of lactose concentration on the production of *Enterobacter agglomerans*. The culture conditions are: peptone 2.0% (w/v), (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 0.5% (w/v), K<sub>2</sub>HPO<sub>4</sub> 0.1% (w/v), MgSO<sub>4</sub>·7H<sub>2</sub>O 0.1% (w/v), KH<sub>2</sub>PO<sub>4</sub> 0.3% (w/v), initial pH 7.0, 30 °C for 48 h.

study, we tested the effect of different carbon sources with a total concentration of 2.0% (w/v). In order to determine the optimum medium, different kinds of carbohydrates were tested, including glucose, maltose, fructose, xylose, sucrose, mannose, NaHCO<sub>3</sub>, and lactose. The results presented in Table 1 reveal that among the carbohydrates, lactose has maximal enzyme activity, followed by xylose, while maltose has the poorest enzyme activity. Then we investigated the effect of lactose concentration. The results are shown in Figure 1. It can be seen that the glucose concentration of 2.0% (w/v) is better than others, and the corresponding enzyme activity can reach 29.32 U·mL<sup>-1</sup>.

### Effect of nitrogen source

The effect of nitrogen sources was investigated at 30 °C and a stirring speed of 120 rpm after 48 h of cultivation, with 2.0% (w/v) lactose as carbon source. With the total nitrogen source concentration fixed at 2.5% (w/v), yeast extract, peptone, beef extract, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, CO(NH<sub>2</sub>)<sub>2</sub>, and NH<sub>4</sub>Cl were investigated. Table 2 presents the variations in the enzyme activity. The maximum enzyme activity is 18.15 U·mL<sup>-1</sup>, obtained by the beef extract-supplemented culture, followed by the NH<sub>4</sub>Cl (16.75 U·mL<sup>-1</sup>), yeast extract (16.75 U·mL<sup>-1</sup>), and NH<sub>4</sub>NO<sub>3</sub> (15.36 U·mL<sup>-1</sup>) cultures, while the use of KNO<sub>3</sub> and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> could result in a lower enzyme activity.

Then we studied the effect of compound nitrogen source on the production of lipase of *E. agglomerans*. It was observed that the liquid culture has deposits when we used 0.5% (w/v) NH<sub>4</sub>Cl with 2.0% (w/v) beef extract or 2.0% (w/v) yeast extract as nitrogen source. So we cannot accept these nitrogen sources although the production of lipase of *E. agglomerans* increased obviously. 0.5% (w/v) NH<sub>4</sub>NO<sub>3</sub> with 2.0% (w/v) beef extracts or 2.0% (w/v) yeast extract as nitrogen source also failed to achieve good results. Finally we preferred beef extract and yeast extract as nitrogen source. As shown in Table 3, when 1.0% (w/v) yeast extract and 1.5% (w/v) beef extract were used as nitrogen source, the best results were obtained.

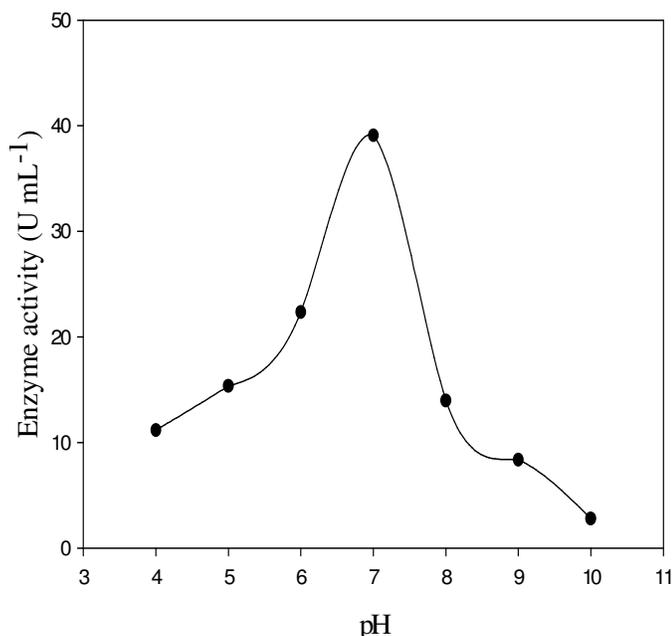
### Effect of initial pH

The effect of initial pH was investigated in the pH range 4.0–10.0 after 48 h of cultivation. The pH of the medium was adjusted to the desired value using 0.1 M HCl or 0.1 M NaOH. The results are listed in Figure 2. pH seems to be a critical factor and relatively small variations in pH can affect the enzyme activity of the system significantly. When the pH is less than 7.0, the enzyme activity increases gradually, and hits the peak at pH 7.0 with the activity of 39.09 U·mL<sup>-1</sup>, then decreases quickly. So the maximum enzyme activity is obtained with initial pH 7.0.

**Table 3.** Effect of different combination of nitrogen sources on the production of *Enterobacter agglomerans*<sup>a</sup>.

Nitrogen source (2.5%, w/v)	0.5% Yeast extract, 2% beef extract	1.0% Yeast extract, 1.5% beef extract	1.5% Yeast extract, 1.0% beef extract	2.0% Yeast extract, 0.5% beef extract
Enzyme activity (U·mL <sup>-1</sup> )	20.94	39.09	19.54	16.75

<sup>a</sup>The experiments were performed for 48 h at 30°C, initial pH 7.0, with a stirring speed of 120 rpm.



**Figure 2.** Effect of pH on the production of *Enterobacter agglomerans*. The culture conditions are: lactose 2.0% (w/v), yeast extract 1.0% (w/v), beef extract 1.5% (w/v), K<sub>2</sub>HPO<sub>4</sub> 0.1% (w/v), MgSO<sub>4</sub>·7H<sub>2</sub>O 0.1% (w/v), KH<sub>2</sub>PO<sub>4</sub> 0.3% (w/v), 30°C for 48 h.

### Effect of surfactants

Then the effect of different surfactants, such as, 0.1% (w/v) SDS, 0.1% (w/v) Triton X-100, 10<sup>-3</sup> mol·L<sup>-1</sup> CTMAB, and 0.1% (w/v) Tween-80 was studied. While we found that none of them has stimulated effect, there are also literatures (Houria et al., 2002; Lelie et al., 2005) reporting that the enzyme activity is strongly inhibited by Triton X-100, SDS and Tween-80.

### Effect of metal ions

The different metal ions 0.1% (w/v) were used as alternatives to MgSO<sub>4</sub>·7H<sub>2</sub>O to evaluate their effects on the production of lipase of *E. agglomerans*. Table 4 lists the results. The highest enzyme activity when Mg<sup>2+</sup> is present in the medium, K<sup>+</sup> and Zn<sup>2+</sup> also have positive effect, while Ca<sup>2+</sup>, Cu<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup>, K<sup>+</sup> and Na<sup>+</sup> have an inhibitory effect on this strain. Several authors have reported the stimulatory effects of Na<sup>+</sup> (En-Shyh et al., 2006; El-Gammal and Rizk, 1989), Mg<sup>2+</sup> (En-Shyh et al.,

**Table 4.** Effect of different metal ions on the production of *Enterobacter agglomerans*<sup>a</sup>.

Metal ions (0.1%, w/v)	Enzyme activity (U·mL <sup>-1</sup> )	Increase rate (%)
Control <sup>b</sup>	18.75	-
Mg <sup>2+</sup>	39.09	±108.48
Ca <sup>2+</sup>	12.56	±33.01
Cu <sup>2+</sup>	6.98	±62.77
Zn <sup>2+</sup>	29.32	+56.37
Fe <sup>2+</sup>	9.07	-51.63
Mn <sup>2+</sup>	11.17	-40.43
Na <sup>+</sup>	8.38	-55.31
K <sup>+</sup>	16.75	-10.67

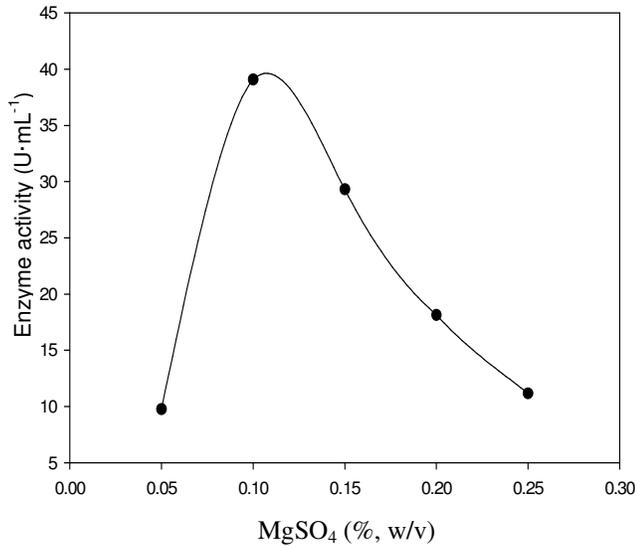
<sup>a</sup>The experiments were performed for 48 h at 30°C, initial pH 7.0, with a stirring speed of 120 rpm. <sup>b</sup>"control" refers to the basal medium in the absence of any metal ions.

2006; El-Gammal and Rizk, 1989; Gulati et al., 2000), and manganese ions. We then found that the concentration of MgSO<sub>4</sub>·7H<sub>2</sub>O 0.1% (w/v) is better than others. And we further researched the effect of the concentration of MgSO<sub>4</sub>·7H<sub>2</sub>O. The results are listed in Figure 3. We can observe that the enzyme activity increases gradually with the increase of the concentration of MgSO<sub>4</sub>·7H<sub>2</sub>O, and hits the peak at 0.1% (w/v) with the activity of 39.09 U·mL<sup>-1</sup>, then decreases quickly. So, the final improved medium had the following composition (w/v): 2.0% (w/v) lactose as carbon source, 1.5% (w/v) beef extract and 1.0% (w/v) yeast extract as nitrogen source, initial pH 7.0, 0.1% (w/v) MgSO<sub>4</sub>·7H<sub>2</sub>O.

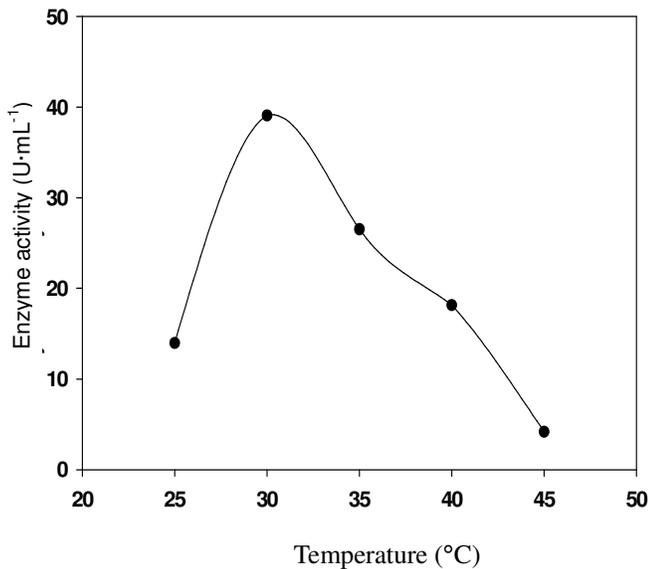
### Effect of temperature and time

A temperature range 25-45°C was screened keeping all of the other conditions constant. Figure 4 displays the variations of lipase production with different temperatures. When the temperature is less than 30°C, the enzyme activity increases gradually, and hits the peak at 30°C with the activity of 39.09 U·mL<sup>-1</sup>, then decreases quickly. At 45°C, the enzyme activity is as poor as 4.19 U·mL<sup>-1</sup>. So, the optimum temperature for *E. agglomerans* is 30°C, which is slightly higher than Haba et al. (2000) (25°C) and consistent with Sevgi et al. (2007).

Then the effect of cultivation time was studied at 30°C and the results were shown in Figure 5. We can observe that the enzyme activity increases sharply in the first 48 h



**Figure 3.** Effect of different content of MgSO<sub>4</sub> on the production of *Enterobacter aerogenes*. The culture conditions are: lactose 2.0% (w/v), yeast extract 1.0% (w/v), beef extract 1.5% (w/v), K<sub>2</sub>HPO<sub>4</sub> 0.1% (w/v), KH<sub>2</sub>PO<sub>4</sub> 0.3% (w/v), 30 °C , initial pH 7.0, 48 h.

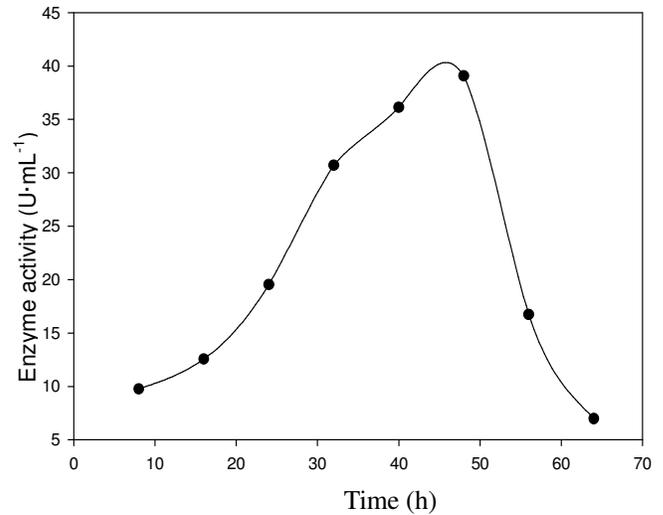


**Figure 4.** Effect of temperature on the production of *Enterobacter agglomerans*. The culture conditions are: lactose 2.0% (w/v), yeast extract 1.0% (w/v), beef extract 1.5% (w/v), K<sub>2</sub>HPO<sub>4</sub> 0.1% (w/v), MgSO<sub>4</sub>·7H<sub>2</sub>O 0.1% (w/v), KH<sub>2</sub>PO<sub>4</sub> 0.3% (w/v), initial pH 7.0, 48 h.

after cultivation, and then declines gradually.

### Effects of natural oils and organic acids

We used several natural oils and fatty acids to evaluate their effects on the yield of lipase of *E. agglomerans*. The



**Figure 5.** Effect of time on the production of *Enterobacter agglomerans*. The culture conditions are: lactose 2.0% (w/v), yeast extract 1.0% (w/v), beef extract 1.5% (w/v), K<sub>2</sub>HPO<sub>4</sub> 0.1% (w/v), MgSO<sub>4</sub>·7H<sub>2</sub>O 0.1% (w/v), KH<sub>2</sub>PO<sub>4</sub> 0.3% (w/v), 30 °C , initial pH 7.0.

results are listed in Table 5. We can observe that there have some variations of the activity, especially for citric acid (57.15%) and oleic acid (50.12%), followed by lactic acid (14.27%), this may be due to the change of final pH. While there have just slight changes of the activity for sesame oil, tea oil, rapeseed oil and olive oil, which has no change of the final pH. It seems this strain could not be induced by natural oil or organic acid, although there are several literatures reporting that the enzyme activity is strongly stimulated by sesame oil, corn oil or olive oil (Rodriguez et al., 2006; Maia et al., 2001).

### The use of *E. agglomerans* on biodiesel

**Effect of the stirring speed:** We studied the effect of different stirring speeds (0, 120, 180 and 240 rpm) on the catalyze reaction, and got a better result at 180 rpm.

**Effect of reaction temperature:** In order to determine the best reaction temperature, a temperature range of 30-60 °C was screened with other conditions keep constant. The enzyme activity decreases with the increase of temperature, especially at 60 °C when there was almost no enzyme activity. So, 30 °C is the best reaction temperature.

**The catalyze reaction:** As raw materials, tea oil, olive oil and sesame oil were added to the medium cultivated with methyl alcohol under the optimum conditions, respectively. When the reaction was operated at 180 rpm, 30 °C for 48 h, we got the yield of 5.05% olive oil, 54.51% sesame oil, and 8.91% (tea oil), respectively. The main components of the biodiesel were: methyl stearate, me-

**Table 5.** Effect of natural oils and fatty acids on the production of *Enterobacter agglomerans*<sup>a</sup>

Adding materials (1.0%,w/v)	Lipase activity (U·mL <sup>-1</sup> )	Increase rate (%)	Final pH
control <sup>b</sup>	39.09	-	7.0
Olive oil	37.5	±4.07	7.0
Oleic acid	19.5	±50.12	5.4
Sesame oil	39.0	±0.23	7.0
Rapeseed oil	36.85	±5.73	7.0
Lactic acid	33.51	±14.27	8.0
Citric acid	16.75	±57.15	8.8
Tea oil	38.7	±1	7.0

<sup>a</sup>The experiments were performed for 48 h at 30°C, initial pH 7.0, with a stirring speed of 120 rpm. <sup>b</sup>“control” refers to the basal medium in the absence of any metal ions.

thyl palmitate, methyl oleate and methyl linoleate. The reason the yield is so low may be that the concentration of water is too high (92.4%), because excessive water may also stimulate the competing hydrolysis reaction (Noureddini et al., 2005). There are some literatures reporting methanolysis efficiently catalyzed in the presence of water in the starting materials (Ana and Enoch, 2003; Maia et al., 2001; Sulaiman et al., 2006). However, appropriate water content may be useful to the reaction; the lipase is more efficient in higher water content biphasic systems before the initial water content increase up to 25% (v/v) (Sulaiman et al., 2006). It was also reported an organic-solvent/water interface is more favorable for the enzyme to have an active conformation (Zuhair et al., 2003). From our investigations, the lipase of *E. agglomerans* can catalyze raw materials with a high content of water (92.4%, w/v) and the yield even reached 54.51% when sesame oil was used as raw material. So, it can be used to catalyze waste oil which is cheap and a high content of water; this can decrease the cost of biodiesel greatly. Moreover, the process reported does not need the pretreatment of the substrate contained water, which will further decrease the cost of raw materials and production, thus be effective to the industrialization of lipase catalyzed biodiesel.

## Conclusion

With olive oil as sole carbon source, the lipase of *E. agglomerans* was found in Victoria blue solution medium. Under the optimum conditions: initial pH 7.0, agitating at 30°C for 48 h, with 2.0% (w/v) lactose as carbon sources, 1.5% (w/v) beef extract and 1.0% (w/v) yeast extract as nitrogen source, 0.1% (w/v) MgSO<sub>4</sub>·7H<sub>2</sub>O, the enzyme activity of this strain can reached to 39.09 U/mL, which is a little lower than in literatures (Burkert et al., 2004; Lin et al., 2006; Houria et al., 2002). But the cultivated time of this strain is much short, and the lipase of this strain can catalyze olive oil, sesame oil and tea oil to produce biodiesel under the conditions of 30°C and 180 rpm. The

yield reached to 51.54% with sesame oil as raw material. Considering the fact that such results are achieved in the presence of 92.4% (w/v) water in the starting materials, the lipase of *E. agglomerans* can be a very promising alternative lipase for biodiesel production in the presence of water.

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