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A promising alternate lipase for biodiesel fuel production

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Abstract By screening samples from different sources, a high-yield yet low-cost lipase of *Enterobacter aerogenes* was found and used in biodiesel production. The enzyme activity of *Enterobacter aerogenes* reached $34.16 \text{ U} \cdot \text{mL}^{-1}$ under the optimum conditions of initial pH 9.0, agitating at 30°C for 40 h, using NaHCO_3 and glucose as carbon sources and peptone as nitrogen source, with 0.1% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. When adding 1.0% (w/v) olive oil to the culture, the enzyme activity reached $66.31 \text{ U} \cdot \text{mL}^{-1}$, which increased by 56.5% compared to that obtained by using 2.0% (w/v) olive oil as the sole carbon source. The lipase of *Enterobacter aerogenes* was immobilized by diatomite and used to catalyze biodiesel with rapeseed oil as raw materials, the transesterification rate was 92.91% under the optimum reaction conditions, which were using 1000 U immobilized lipase and 5 mL n-hexane, with ethanol as acyl acceptors, at the molar ratio of oil/ethanol of 1:4, and adding ethanol twice, at 35°C for 48 h. Therefore, the *Enterobacter aerogenes* will potentially serve as a promising alternative lipase for biodiesel production.

Keywords *Enterobacter aerogenes*, enzyme activity, biodiesel, transesterification rate

1 Introduction

Lipases used to catalyze biodiesel fuel attracted more and more attention for the by-product (glycerol), with easier recovery and more simple purification of biodiesel fuel (Taichi et al., 2000) and simultaneously without special requirements for raw materials (Watanabe et al., 2002). Furthermore, biodegradable lubricants can also be obtained in the process as by-products (Funda et al., 2007). The high cost of lipase has restricted its commercialization, although an immobilized lipase

method can be used to reduce the cost of lipase (Mitsuhiro et al., 2005; Shweta et al., 2008), most of the commercial lipases are still much expensive, making the industrialization of lipase catalysis biodiesel fuel very difficult. Some researchers are interested in screening high-yield lipases at a low cost, and the lipase production of a mutant of *Candida* sp. 99–125 has reached $8060 \text{ U} \cdot \text{mL}^{-1}$ in a 1500 L fermenter (Tan et al., 2003). *Bacillus* sp. has also resulted in the extracellular and intracellular lipase activities of $15 \text{ U} \cdot \text{mL}^{-1}$ and $168 \text{ U} \cdot \text{mL}^{-1}$, respectively (Sevgi et al., 2007). Moreover, there are also literatures reporting the screening of lipase used on biodiesel fuel production, but the results are not satisfying (Houria et al., 2002; Burkert et al., 2004; Lin et al., 2006).

In our study, the influences of different culture conditions on the enzyme activity of lipase *Enterobacter aerogenes* were investigated, including carbon, nitrogen, pH, and so on. The lipase was immobilized and then used in biodiesel fuel production, and the effect of immobilized lipase volume, acyl acceptors, oil/ethanol molar rate, temperature, reaction time, and n-hexane content on transesterification rate was investigated too.

2 Materials and methods

Olive oil (2.0%, w/v) was used as the sole carbon source and with respect to production of lipases, 25 positive strains of bacteria and yeasts were separated from 7 soil samples from different sources. The highest enzyme activity among the strains was $42.87 \text{ U} \cdot \text{mL}^{-1}$. In order to make the experimental results reliable and reproducible, each experiment in this study was carried out in triplicates. Peptone, K_2HPO_4 , Na_2CO_3 , $(\text{NH}_4)_2\text{SO}_4$, KCl, and other chemical compounds were purchased from Sigma Chemical Co..

The media were prepared as follows: the solid flat medium (w/v) of 1.0% olive oil, 0.5% $(\text{NH}_4)_2\text{SO}_4$, 2.0% peptone, 0.5% K_2HPO_4 , 0.1% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 1.5% agar, at pH 7.0 adjusted by using $0.1 \text{ mol} \cdot \text{L}^{-1} \text{ Na}_2\text{CO}_3$, which was cooled to 60°C after sterilization, added with $4.0 \text{ mg} \cdot 100 \text{ mL}^{-1}$ Victoria blue solution and emulsified for

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1 min in the asepsis condition; the enrichment medium (w/v) of 1.5% olive oil, 1.0% peptone, 0.1% K_2HPO_4 , 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, and 0.3% KH_2PO_4 , the slant peptone medium (w/v) of 0.5% peptone, 0.5% yeast extract, 0.3% NaCl, 0.1% K_2HPO_4 , and 1.5% agar; the duplicate screening medium (w/v) of 1.0% olive oil, 1.0% peptone, 0.1% K_2HPO_4 , 0.05% $MgSO_4 \cdot 7H_2O$, 0.05% KCl, and 0.3% KH_2PO_4 ; and the fermentation medium (w/v) of 2.0% olive oil, 2.0% peptone, 0.1% K_2HPO_4 , 0.1% $MgSO_4 \cdot 7H_2O$, 0.3% KH_2PO_4 , and 0.5% $(NH_4)_2SO_4$. The final pH was adjusted to 7.0 using $0.1 \text{ mol} \cdot \text{L}^{-1} \text{ Na}_2\text{CO}_3$ or $0.1 \text{ mol} \cdot \text{L}^{-1} \text{ HCl}$.

2.1 Assay of enzyme activity

Enzyme activity was determined by the olive oil-PVA emulsion method previously described (Marija et al., 1999). The reaction mixture consisted of 4.0 mL olive oil emulsified in PVA, 5.0 mL $0.05 \text{ mol} \cdot \text{L}^{-1}$ glycine-NaOH buffer (pH 10.30) and 1.0 mL medium of the strain. Incubation was performed at 30°C for 15 min. The reaction was terminated by adding 15 mL ethanol. The liberated free fatty acid was titrated by $0.1 \text{ mol} \cdot \text{L}^{-1}$ sodium hydroxide solution with phenolphthalein as an indicator. One unit of lipase activity (U) was defined as the amount of enzyme that liberated $1.0 \mu\text{mol}$ fatty acid per minute at 30°C .

2.2 Screening of strains

All the strains used in this study were isolated from soil samples abundant in lipids. The screening process was as the following stages: (1) 1.0 g sample was taken and diluted to $10^{-4} \text{ g} \cdot \text{L}^{-1}$, followed by daubing 0.5 mL dilute solution on 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 at 28°C for 24 h at a stirring speed of $120 \text{ r} \cdot \text{min}^{-1}$, and thereafter, 0.5 mL culture solution was daubed on the preliminary screening medium (the composition is the same as that of the solid slant medium) and cultivated at 30°C for 72 h, (3) the larger colonies were laid on the peptone slant medium, cultivated at 30°C for 72 h, and then inoculated to the duplicate screening medium and recultivated at 30°C for 24 h at a stirring speed of $120 \text{ r} \cdot \text{min}^{-1}$, (4) 0.5 mL culture cultivated in the duplicate screening medium was inoculated to the fermentation medium and fermented at 30°C for 72 h at a stirring speed of $120 \text{ r} \cdot \text{min}^{-1}$, which was used to determine the enzyme activity.

2.3 Identification of lipase

We compared the 16s rDNA of this strain with *Enterobacter aerogenes* and found that they had 100% homology; furthermore, the characteristics of this strain

were consistent with those of *Enterobacter aerogenes* too. Therefore, we confirmed that it belongs to *Enterobacter aerogenes*. In addition, the lipase of *Enterobacter aerogenes* is an extracellular enzyme.

2.4 Gas chromatography

Products from methanolysis or methylated fatty acids were redissolved in 2.0 mL aether, which was 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 temperature of column, injection port, and oven was 180°C , 220°C , and 230°C , with the retention time of 1, 5, and 1 min, respectively. Methyl heptadecanoate served as the internal standard for gas chromatograph GC analysis to determine the fatty acid methyl ester (FAME) content. For the time course studies, an aliquot of 100 mL reaction medium was taken at various time intervals and was diluted in n-heptane for GC analysis.

2.5 Immobilization of lipase from *Enterobacter aerogenes* by diatomite

The fermentation broth was centrifuged at $5000 \text{ r} \cdot \text{min}^{-1}$ for 20 min. After the sediment was removed, $6 \text{ g} \cdot 100 \text{ mL}^{-1}$ ammonium sulfate was added to the supernatant at 4°C , salted out for 3–4 h, and centrifuged at $7000 \text{ r} \cdot \text{min}^{-1}$ for 20 min, with the collected sediment dissolved in sterile water to desalinate and freeze-dried to obtain dry enzyme powder. Thereafter, 1 g dry enzyme powder was dissolved in 5 mL distilled water, added with 10 g diatomite, and stirred for 1.5 h at 20°C . Finally, the dry immobilized lipase of *Enterobacter aerogenes* was obtained by rinsing the mixture with acetone three times, which was centrifuged at $5000 \text{ r} \cdot \text{min}^{-1}$ for 2 min with the sediment collected and freeze-dried.

2.6 The catalyze reaction

The transesterification reactions were carried out in a 100-mL screw-capped vessel and heated to 30°C in a shaking incubator at $180 \text{ r} \cdot \text{min}^{-1}$. The composition of the reaction mixtures contained 15.47 mL crude rapeseed oil, 5 mL n-hexane, and 500 U immobilized lipase, with three-step adding methanol by 2.05 mL in each step (a 1:3 molar ratio of oil/methanol). After 48 h reaction, 100 mL sample was taken from the reaction mixture and centrifuged to obtain the upper layer. Then, it was determined by GC.

3 Results

3.1 Effects of carbon sources

In general, the enzyme production depends greatly on the

composition of the medium (Pimentel et al., 1996). We tested the effects of the carbon source in the fermentation medium by replacing olive oil with selected carbon sources, including glucose, maltose, fructose, xylose, sucrose, mannose, NaHCO_3 , and lactose, while simultaneously maintaining the total carbon source concentration at 2.0% (w/v) and keeping all the other conditions constant. The results revealed that among the carbohydrates, glucose had the maximal enzyme activity, followed by NaHCO_3 and fructose, while sucrose had the poorest enzyme activity (Table 1). Furthermore, we investigated the effects of glucose concentration. In Fig. 1, it can be seen that the 2.5% glucose concentration (w/v) is better, and the corresponding enzyme activity can reach $16.21 \text{ U} \cdot \text{mL}^{-1}$. When the glucose was mixed with NaHCO_3 or fructose as a carbon source, at the total concentration of 2.5% (w/v), we discovered that the mixture of 1.5% (w/v) NaHCO_3 and 1.0% (w/v) glucose had the best performance with $22.1 \text{ U} \cdot \text{mL}^{-1}$ enzyme activity, and the NaHCO_3 was much cheaper than mere glucose.

3.2 Effects of nitrogen sources

The effects of nitrogen sources were investigated with 1.5% (w/v) NaHCO_3 and 1.0% (w/v) glucose as carbon sources, with all the other conditions constant. The total nitrogen sources concentration was fixed at 2.5% (w/v) and different nitrogen sources including yeast extract, peptone, beef extract, $(\text{NH}_4)_2\text{SO}_4$, $\text{CO}(\text{NH}_2)_2$, and NH_4Cl were investigated. Table 2 presents the variation in the enzyme activity. The maximum enzyme activity is $24.78 \text{ U} \cdot \text{mL}^{-1}$, obtained by the peptone-supplemented culture, followed by the $(\text{NH}_4)_2\text{SO}_4$ ($8.04 \text{ U} \cdot \text{mL}^{-1}$), beef extract ($7.03 \text{ U} \cdot \text{mL}^{-1}$), and NH_4Cl ($5.36 \text{ U} \cdot \text{mL}^{-1}$) cultures. The results indicated that the $\text{CO}(\text{NH}_2)_2$ and yeast extract could result in a lower enzyme activity. We also investigated the effects of different peptone concentration on the enzyme activity of lipase *Enterobacter aerogenes* (Fig. 2). The maximum enzyme activity was observed at 1.5% peptone concentration (w/v).

3.3 Effects of initial pH

Using 1.5% NaHCO_3 (w/v) and 1.0% glucose (w/v) as

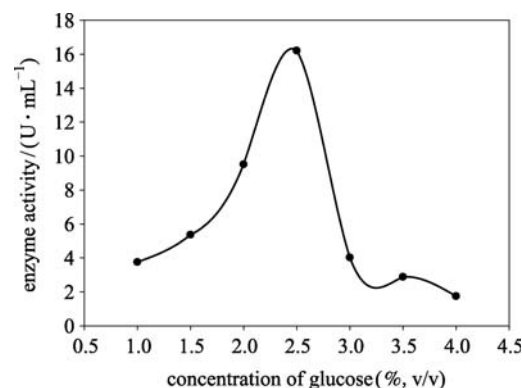


Fig. 1 Effects of glucose concentration on the production of lipase of *Enterobacter aerogenes*

carbon sources and 1.5% peptone (w/v) as a nitrogen source, with all the other conditions constant, we investigated the effect of initial pH in the pH range of 4.0–10.0. The pH of the medium was adjusted to the desired value with $0.1 \text{ mol} \cdot \text{L}^{-1}$ HCl or $0.1 \text{ mol} \cdot \text{L}^{-1}$ NaOH. Figure 3 shows that the maximum enzyme activity appears at the initial pH 9.0. The results demonstrated that pH is a critical factor and the relatively small variation in pH can affect the enzyme activity of the system significantly. Although most bacteria prefer the pH value around 7.0 for the best lipase production, there are also literatures reporting the maximum activity at higher pH values (Camargo de Morais et al., 1998; Rohit et al., 2001).

3.4 Effects of metal ions

Based on the results above, we used metal ions as additives to evaluate their effects on the enzyme activity of lipase *Enterobacter aerogenes*. We used 0.1% metal ion concentration (w/v) in place of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. Table 3 indicates that the highest enzyme activity appears when Mg^{2+} is present in the medium and Na^+ and Mn^{2+} also have a positive effect, while K^+ almost has no effect. In contrast, Ca^{2+} , Cu^{2+} , Zn^{2+} , and Fe^{2+} have an inhibitory effect. Moreover, some authors have reported the stimulatory effect of Na^+ , Mg^{2+} (Gulati et al., 2000), and manganese ions. Furthermore, we found that the best $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ concentration was 0.1% (w/v). The final improved medium contained the following composition (w/v): 1.0% NaHCO_3 and 1.0% glucose as carbon sources,

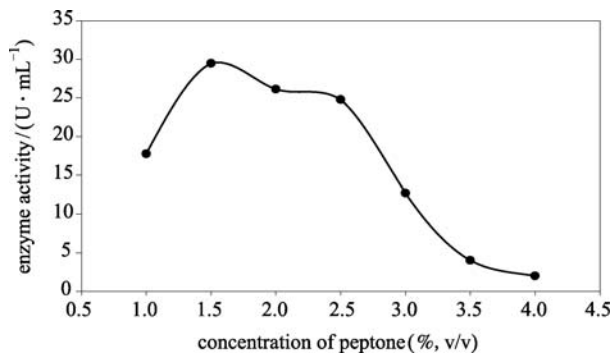
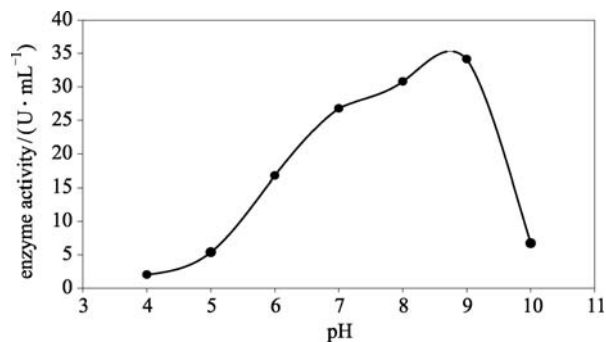
Table 1 Effects of different carbon sources on the production of lipase of *Enterobacter aerogenes*^a

carbon source (2.0%, w/v)	glucose	maltose	fructose	sucrose	mannose	NaHCO_3	xylose	lactose
enzyme activity/ ($\text{U} \cdot \text{mL}^{-1}$)	9.51	2.00	5.69	1.34	2.08	6.43	2.95	4.69

Note: ^a the experiments were performed for 72 h at 30°C, initial pH 7.0, and a stirring speed of $120 \text{ r} \cdot \text{min}^{-1}$.

Table 2 Effects of different nitrogen source on the production of lipase of *Enterobacter aerogenes*

nitrogen source (2.0%, w/v)	yeast extract	peptone	CO(NH ₂) ₂	NH ₄ Cl	beef extract	(NH ₄) ₂ SO ₄
enzyme activity/(U·mL ⁻¹)	3.35	24.78	2.68	5.36	7.03	8.04

**Fig. 2** Effects of peptone concentration on the production of lipase of *Enterobacter aerogenes***Fig. 3** Effects of initial pH on the production of lipase *Enterobacter aerogenes*

1.5% peptone as the nitrogen source, initial pH 9.0, and 0.1% MgSO₄·7H₂O.

3.5 Effects of surfactants

The effects of different surfactants, such as, 0.1% SDS, 0.1% Triton X-100, 10⁻³ mol·L⁻¹ CTMAB, and 0.1% Tween-80 were studied. Although surfactants generally acted as inducers toward bacteria (Haba et al., 2000), we detected a significantly decreased enzyme activity when the basal medium contained CTMAB, SDS, Triton X-100, or Tween-80. Houria et al. (2002) and Castro-Ochoa et al. (2005) also reported that the enzyme activity was strongly inhibited by Triton X-100, SDS, and Tween-80.

3.6 Effects of the medium loading volume

We studied the effects of different medium loading volume in a 100 mL bottle, and the inoculation volume was fixed to

Table 3 Effects of metal ions on the production of lipase *Enterobacter aerogenes*

metal ions (0.1%, w/v)	enzyme activity/(U·mL ⁻¹)	increase rate/%
control*	18.75	—
Mg ²⁺	34.16	+ 82.14
Ca ²⁺	4.02	- 78.57
Cu ²⁺	4.69	- 75.00
Zn ²⁺	6.70	- 64.29
Fe ²⁺	5.36	- 71.43
Mn ²⁺	26.79	+ 42.86
Na ⁺	20.09	+ 7.14
K ⁺	18.75	0

Note: * refers to the basal medium in the absence of any metal ions.

be 0.5 mL. The results indicated that 50 mL was the best loading volume, which amounted to a 1.0% inoculation rate (Table 4).

3.7 Effects of temperature

The influences of the temperature on the enzyme activity of lipase *Enterobacter aerogenes* were found at a temperature range of 25–45°C with all the other conditions constant. Figure 4 displays the variation of enzyme activity at different temperatures. When the temperature was lower than 30°C, the enzyme activity increased gradually, reaching the peak at 30°C with 36.17 U·mL⁻¹ activity and then decreased quickly. Especially when the temperature was 45°C, the enzyme activity was as poor as 14.74 U·mL⁻¹. Therefore, the optimum temperature for *Enterobacter aerogenes* was 30°C, which was slightly higher than the 25°C reported by Haba et al. (2000) and consistent with the 30°C reported by Sevgi et al. (2007).

Table 4 Effects of the medium loading volume to the lipase production of *Enterobacter aerogenes*

loading volume/mL	inoculation rate/%	enzyme activity/(U·mL ⁻¹)
20	2.50	—
30	1.67	16.08
40	1.25	24.78
50	1.00	34.16
60	0.83	28.13

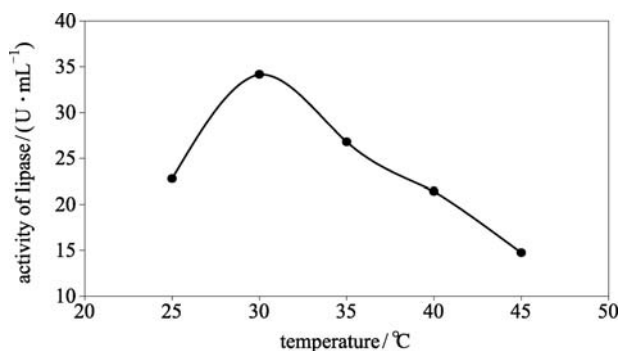


Fig. 4 Effects of temperature to the lipase production of *Enterobacter aerogenes*

3.8 Effects of time

The effects of cultivation time on the enzyme activity of lipase *Enterobacter aerogenes* were also studied, and the results showed that the enzyme activity increased sharply within the first 40 h after cultivation, reached the peak at 40 h, kept stable until 72 h, and, thereafter, declined gradually (Fig. 5). Obviously, the stable growth period was from 40 h to 72 h, which kept the steady-going of the enzyme activity of lipase *Enterobacter aerogenes*. Therefore, the lipase *Enterobacter aerogenes* should be cultivated from 40 h to 72 h for further research.

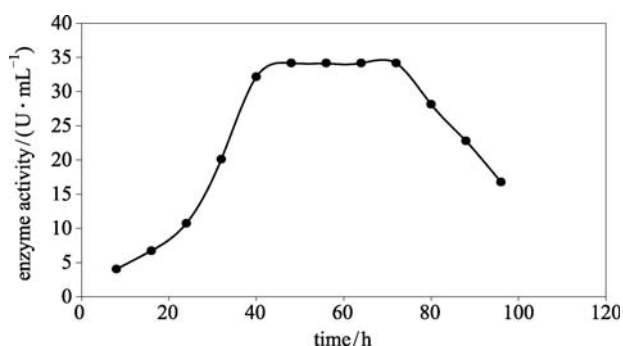


Fig. 5 Effects of time on the production of lipase *Enterobacter aerogenes*

3.9 Effects of natural oils and fatty acids

Lipidic substrates and fatty acids were generally used as inducers toward bacteria reported by Haba et al. (2000); Maia et al. (2001) and Rodriguez et al. (2006) reported that the enzyme activity could be strongly stimulated by sesame oil, corn oil, or olive oil. In our study, several natural oils and fatty acids were used to test their effect on the enzyme activity of lipase *Enterobacter aerogenes*. The results revealed that the highest enzyme activity was 66.31 U · mL⁻¹ in the olive oil, increased by 94.12% compared with that without olive oil in the medium, followed by

rapeseed oil and citric acid (36.17 U · mL⁻¹) (Table 5). In contrast, the oleic acid and lactic acid weakened the activity greatly, which was decreased by 76.47% and 47.06%, respectively, followed by tea oil (29.41%) and sesame oil (23.53%). This indicated that lipase *Enterobacter aerogenes* could be induced by natural oil or organic acid. Though the main component of olive oil is triglycerides, the same as tea oil and sesame oil, its effect on lipase *Enterobacter aerogenes* was opposite to that of the tea oil and sesame oil. Considering the fact that the oleic acid also had the negative effect, the strain may have a selective effect on the natural oils or organic acids, and the reasons why different natural oils or organic acids had different effects will be studied in the future.

Table 5 Effects of natural oils and fatty acids on the lipase production of *Enterobacter aerogenes*^a

adding materials (1.0%, w/v)	lipase activity/(U · mL ⁻¹)	increase rate/%
control ^b	34.16	—
olive oil	66.31	+ 94.12
oleic acid	8.04	- 76.47
sesame oil	26.12	- 23.53
rapeseed oil	36.17	+ 5.88
lactic acid	18.08	- 47.06
citric acid	36.17	+ 5.88
tea oil	24.11	- 29.41

Note: ^a represents the experiments were performed for 48 h at 30°C, at initial pH 9.0 at a stirring speed of 120 r · min⁻¹. ^b refers to the basal medium in the absence of any metal ions.

3.10 The catalyze reaction

3.10.1 The effects of immobilized lipase volume on transesterification rate

The effects of different volume of immobilized lipase of *Enterobacter aerogenes* on transesterification rate are presented in Fig. 6. It was found that when the immobilized

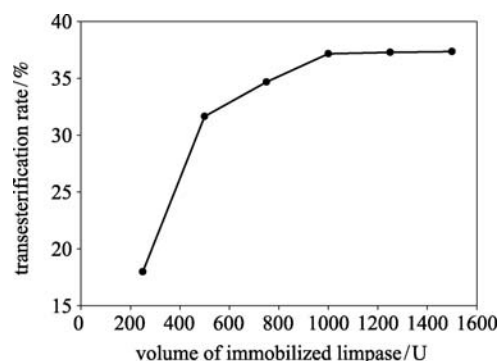


Fig. 6 The effects of different volume of lipase on transesterification rate

lipase was 1000 U, there was a better result (37.2%), and then, the transesterification rate remained stable. Therefore, the optimum volume of immobilized lipase was 1000 U in this study.

3.10.2 The effects of acyl acceptors, molar ratios of oil/ethanol, and different ethanol-adding times on the transesterification rate

A comparative study on transesterification rate with different acyl acceptors (with the same oil/alcohol molar ratio) was conducted, and the results showed that a better result could be obtained with ethanol as acyl acceptor (Table 6).

Since the transesterification reaction is reversible, an increase in the amount of one of the reactants will cause a higher ester yield (Noureddini et al., 2005), while too much alcohol would lead to serious inactivation of lipase, so the molar ratio of alcohol to vegetable oil is one of the most important variables affecting the yield of ester (Watanabe et al., 2002). In our study, we investigated the effects of molar ratios of oil/ethanol on the transesterification rate, indicating that the highest transesterification rate of 81.16% could be obtained at the oil/methanol molar ratio of 1:4, and either higher or lower ethanol content decreased ethyl ester yield to some degree (Fig. 7). An increase in the mole of ethanol resulted in an increase in the transesterification rate. When it reached a maximum level, the further increase in the ethanol concentration resulted in a decrease in the formation of esters due to the enzyme inactivation (Freedman et al., 1984).

It is well known that excessive short-chain alcohols might inactivate lipase seriously. Thus, the reaction was conducted by adding ethanol to avoid enzyme inactivation. The number of ethanol-adding times ranged from 1 to 4. In Table 7, it can be concluded that the conversion was about 84.70% by two successive additions of methanol. Therefore, two-step methanolysis was sufficient to convert rapeseed oil at a high transesterification rate.

3.10.3 Effects of temperature on transesterification rate

The effect of the temperature on the reaction was examined within the range from 25 to 50°C (Fig. 8). As shown in Fig. 8, higher transesterification rate was achieved with the increment of the temperature when the temperature was below 35°C (92.91%). However, further increase in the temperature led to a drastic drop in transesterification rate; it might stem from the commonly observed effect of reaction rate increasing with temperature that is overcome

by its inactivating effect on the immobilized lipase (Nadir and Bülent, 2008). From the point of view of process energy saving, a low temperature (35°C) permits a good catalyst performance is an advantage, and Andrea et al. (2008) obtained the highest transesterification rate at 35°C too.

3.10.4 The effect of reaction time on transesterification rate

In order to see the effects of reaction time, transesterification experiments were performed at room temperature (35°C) for 12–56 h. As shown in Fig. 9, transesterification rate increased with time but reached 92.91% after 48 h. Therefore, all other catalyze reaction were performed for 48 h in this study.

3.10.5 The effect of n-hexane content on transesterification rate

The solvent can increase substrate molecule contact with the lipase, improve the mass transfer between substrate and products, and reduce the toxic effect of short-chain alcohols on immobilized lipase (Chen et al., 2009), wherein the effect of n-hexane content on transesterification rate was tested, and the results were shown in Fig. 10. As we can see, with the hexane content increased from 1 mL to 5 mL, the transesterification rate increased, and then, it remained stable even when the hexane content increased. Therefore, the optimum catalyze reaction conditions in this study were 5 mL n-hexane, 1000 U immobilized lipase, and 5 mL n-hexane; the molar ratios of oil/ethanol was 1:4; and ethanol was added for 2 times, 35°C for 48 h.

4 Conclusion

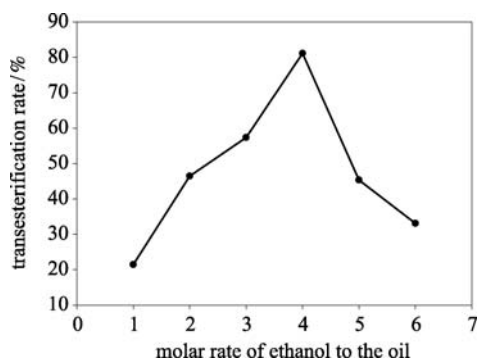
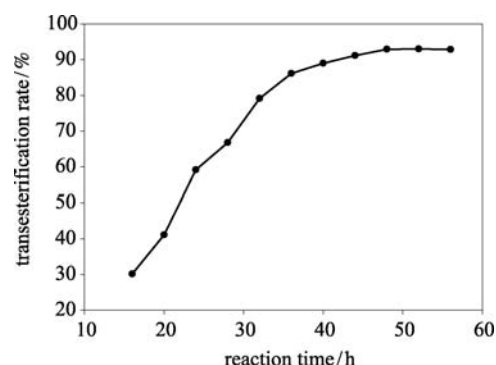
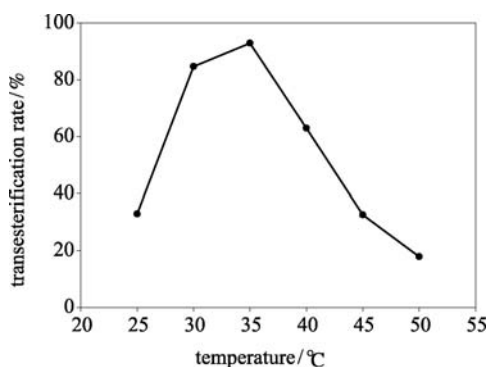
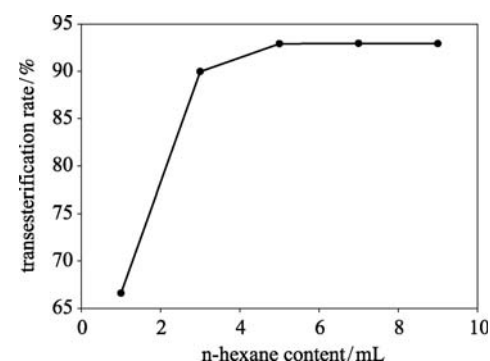
The enzyme activity of *Enterobacter aerogenes* can reach $34.16 \text{ U} \cdot \text{mL}^{-1}$ under the optimum conditions: initial pH 9.0, agitating at 30°C for 40 h, using NaHCO_3 and glucose as carbon sources and peptone as nitrogen source, with 0.1% (w/v) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. When adding 1.0% (w/v) olive oil to the culture, the enzyme activity can reach $66.31 \text{ U} \cdot \text{mL}^{-1}$, which has increased by 56.5% compared to that obtained by using 2.0% (w/v) olive oil as the sole carbon source. The immobilized lipase of *Enterobacter aerogenes* can be used to catalyze biodiesel reaction, and the transesterification rate was 92.91% under optimum reaction conditions, and the conditions were 5 mL n-hexane, 1000 U immobilized lipase, 5 mL n-hexane, the molar ratios of oil/ethanol was 1:4, ethanol was added two times, 35°C for 48 h.

Table 6 The effects of different acyl acceptors on transesterification rate

alcohol	methanol	ethanol	propanol	isopropanol	n-butanol	tert-butyl alcohol	pentanol	isoamyl alcohol
transesterification rate/%	37.2	57.37	42.0	7.34	40.86	45.04	34.54	27.98

Table 7 The effect of different ethanol-adding times on transesterification rate

ethanol-adding times	1	2	3	4
transesterification rate/%	29.65	84.70	81.16	30.25

**Fig. 7** The effects of different molar rate of ethanol to the oil on transesterification rate**Fig. 9** The effects of reaction time on transesterification rate**Fig. 8** The effects of temperature on transesterification rate**Fig. 10** The effects of n-hexane content on transesterification rate

5 Discussion

The purpose of the present study is to screen for high-lipase-production microorganisms from various soil samples and determine the optimum conditions for lipase production. In this study, we got a satisfied enzyme activity of lipase *Enterobacter aerogenes* ($66.31 \text{ U} \cdot \text{mL}^{-1}$), its cultivate time is only 48 h, which is more effective to produce enough lipase used on biodiesel. Moreover, as a raw lipase, future research, such as mutant, purified, and DNA recombinant technique will be useful to improve its enzyme activity. In addition, the lipase of *Enterobacter aerogenes* immobilized by diatomite can be used to catalyze biodiesel reaction with a satisfied transesterification rate of 92.91%. Diatomite is very cheap too, so the cost of immobilized lipase of *Enterobacter aerogenes* is cheaper, and then, it is useful to reduce the cost of enzyme catalyzed biodiesel. It may serve as a promising alternative lipase for biodiesel production.

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