Study on enzymatic activity and lipase catalysis by lipase high-yield strain

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Abstract. In this paper, microorganism for high lipase productivity was screened for immobilization in biodiesel synthesis. Strains with high lipase productivity were analyzed at nucleic acid level with special universal primes. In addition, reaction course, theory of lipase adsorption, lipase specificity on substrate, kinetics, and the application of enzymatic synthesis in biodiesel were investigated. The tests of lipase productivity, lipase purification, lipase immobilization, and lipase application in biodiesel were carried out indicating that lipase from the above micro-organisms was able to catalyze the transesterification reaction of rapeseed oil with moderate oleic acid content.

Keywords: Lipase; Microorganism; screening; biodiesel.

1. Introduction
Currently, the globe has witnessed wide energy shortage, the energy conservation and emission reduction has become very urgent, hence, research and development of new and environment-friendly energy has attracted more and more attention from all over the world [1-3]. Biodiesel is a kind of oil which is made of vegetable oil and animal fat. With the development of biodiesel production technology, the quality standards of biodiesel have been established in various countries. However, the application of lipase in the catalytic synthesis of biodiesel is a new research field in recent years, which has not been widely observed [4-9]. Hence, the purpose of this project is to screen high-yield and excellent lipase producing strains before mutagenesis to get higher lipase activity so as to lay a solid foundation for further study on lipase immobilization and industrial test of lipase catalyzed synthesis of biodiesel. In this paper, the technology of enzymatic synthesis of fatty acid phthalic vinegar was studied by using crude enzyme which was extracted by our own fermentation and immobilized as biocatalyst. Effects of different enzyme dosage, substrate specificity, substrate concentration, molar ratio, water content were investigated.

The aim of this research was:
- to improve the enzyme activity highly through single factor and orthogonal experimental analysis by optimization of fermentation conditions on the mutated strain;
- to observe application of lipase in biodiesel synthesis.
2. Material and methods

2.1. Optimization of enzymatic properties
In order to make the lipase produced by isolated strain be stable and used for further research, optimum temperature, pH value, as well as thermal stability and stability were observed.

2.2. Lipase catalyzed production of biodiesel from rapeseed oil
Purified lipase powder and silica gel, as well as silica gel were added into open vial containing pH 7.0 sodium phosphate buffer before being mixed fully at 35 °C. The sample was analyzed by gas chromatography to calculate peak area of fatty acid methylacetate and its proportion according to the peak area ratio of the standard curve. Standard curve of fatty acid methylacetate was used to calculate the content of various substances and the amount of fatty acid methylacetate. The micromolecular number of fatty acid methylacetate produced in unit time is recorded as the cool exchange reaction rate. The reaction system for the preparation of biodiesel from rapeseed oil is as follows: anhydrous methanol with molar concentration ratio of 1:3 and refined rapeseed oil were dissolved in n-heptane methanol to get final concentration of 0.6 mg/L rapeseed oil. In a 100 mL triangular flask with stopper, the reaction substrate and immobilized lipase prepared from refined mixture containing methanol, triglyceride, catalyst, glycerin, fatty acid salt, free fatty acid and immobilized lipase were vibrated at 30 °C.

3. Results and discussion

3.1. Analysis of fermentation conditions for lipase production

![Figure 1. Effect of temperature, pH, liquid loading ratio, seed age on enzyme production](image)

In order to investigate the optimal growth and metabolism temperature of the experimental bacteria, culture and enzyme production tracking experiments were carried out ranging from 20 °C to 40 °C. It could be seen from Figure 4 that the suitable temperature range of bacterial fermentation is about 28 °C,
and the enzyme activity is relatively high at about 29 °C. PH value is not only one of the external environment for the survival of microorganisms, but also an important factor affecting microbial fermentation. It could be seen from Figure 1 that when the initial value is 6.0, the enzyme activity of fermentation broth is relatively high. Furthermore, enzyme activity is relatively high when liquid loading percentage is 20%. General speaking, the cultivation time of seed, or seed age is of great value when the bacterium is in logarithmic growth period because this period is beneficial to shorten the fermentation period and improve the enzyme production rate, while in the opposition period, enzyme production capacity will be reduced and the bacterium will be premature aging or even be autolysis due to insufficient activity. It could be seen enzyme activity is relatively high when almost suitable seed age is 24 hours. As surfactants could change the permeability of cell membrane and improve the oxygen transfer speed at the gas-liquid interface, some surfactants added to the culture medium could improve the enzyme production. The effects of some surfactants and their concentrations on enzyme production were investigated supposing enzyme activity of the fermentation broth without surfactant as 100%. It could be seen from Table 1 that Tween 80 and gum arabic could promote the production of lipase, while polyethylene glycol and sodium dodecyl sulfate could inhibit the production of lipase.

| Table 1. Effect of surfactants on enzyme production |
|-----------------------------------------------|
| Surfactant              | 0.05% | 0.1% | 0.15% |
|-------------------------|-------|------|-------|
| Control                 | 100   | 100  | 100   |
| Tween 80                | 506   | 301  | 356   |
| SDS                     | 29.1  | 29.1 | 68.6  |
| polyethylene glycol     | 83.5  | 60.1 | 50    |
| Gum Arabic              | 103   | 118  | 129   |

3.2. Preliminary study on enzymatic properties of lipase
The general properties of enzyme mainly include optimum temperature, value of enzyme reaction as well as thermal stability, stability, reaction rate and so on. In order to make the lipase produced by Candida sp. be stable and used for further research, enzymatic properties of lipase were studied from these above aspects. Lipase activity of the same enzyme solution was measured ranging from 25 °C to 50 °C at intervals using olive oil as the substrate to obtain enzyme activity while under different reaction temperatures, the results of which were illustrated in Figure 2 which shows 75% optimum temperature for enzyme action kept around 37 °C or so. Beyond this temperature range, the enzyme activity drops rapidly.

![Figure 2. Relationship between temperature and enzyme activity](image)
According to the method of enzyme activity measurement, enzyme activity of the same enzyme solution was measured at 37 °C under different pH conditions. It could be seen from Figure 3 that the optimal action value of this lipase is 7.0, but could still remain high enzyme activity above 8.0 in pH value.

![Figure 3. Relationship between pH value and enzyme activity](image_url)

In order to observe thermostability of enzyme activity, 40 °C, 50 °C and 60 °C water bath for 1h were designed. Samples were taken at 10 min intervals for lipase enzyme assay which was converted into percentage in comparison with untreated samples. It could be seen from Figure 4 that lipase is basically not inactivated if it was kept at 40 °C for 1h while remaining 50.7% enzyme activity if kept at 50 °C. Experiment showed lipase is basically inactivated when it is kept at 60 °C for 1h.

The properties of lipase mainly include optimum temperature and value, temperature and stability, the specificity of hydrolyzed oil and fatty acid species. On the basis of the experiment, it was found that the optimal temperature of the catalytic reaction was 37 °C, and the thermal stability and stability were good.
3.3. Synthesis of biodiesel catalyzed by immobilized lipase

According to the experimental method, curve of conversion rate with time dynamics was obtained with the ration 8.0 between methanol glycerin and triglyceride around 333K. It could be seen from Figure 5 that the catalysis reaction shows a linear relationship which demonstrates that under a certain amount of lipase catalyst, the reaction for the synthesis of biodiesel is approximately a first-order reaction.

**Figure 5.** Kinetic relationship between conversion rate and time in enzymatic reaction

**Figure 6.** Time kinetics of esterase activity

**Figure 7.** Time kinetics of hydrolase activity
By comparing the activity of microbial lipase in aqueous phase with that in organic phase in n-heptane, it is proved that the activity of lipase in organic phase is not highly correlated with that of hydrolase. It could be seen from the results of Figur 6 and Figure 7 that the reaction rate of lipase catalyzing rapeseed oil to biodiesel is not highly correlated with hydrolase activity, and moreover, the lipase catalyzing vinegar exchange reaction facilitated by high organic phase catalyzing enzyme activity has a good correlation with organic phase catalyzing enzyme activity. Compared with hydrolase, lipase activity in organic phase could better reflect the ability of lipase to catalyze vinegar exchange reaction in organic phase.

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References
[1] Elibol, M., Ozer, D. (2000) Influence of oxygen transfer on lipase production by Rhizopus arrhizus. Process Biochemistry, 36, 325-329.
[2] Rohit, S., Uttam, C. B. (2001). Production, purification, characterization, and applications of lipases. Biotechnology Advances, 19, 627-662.
[3] Gordillo, M. A., Obradors, N., Montesinos, J. L. et al. (1995). Stability studies and effect of the initial oleic acid concentration on lipase production by coulddida rugosa. Applied Microbiology and Biotechnology, 43(1), 38-41.
[4] Dalmaue, Montesiosjl, Lotti. et al. (2000). Effect of different carbon sources on lipase production by coulddida rogosa. Enzyme and microbial technology, 26(10), 657-663.
[5] Tsuchiya, K., Tada, S., Gomi, K. et al. (1992). High level expression of the synthetic human lysozyme gene in Aspergillus oryzae. Applied Microbiology and Biotechnology, 38, 109-114.
[6] Liebeton, K., Zonta, A., Schmossek, K. et al. (2000). Directed evoluction of an enantioselective lipase. Chemistry and Biology, 7, 709-718.
[7] Gridhar, M., Chandana, K., Rajnish, K. (2004). Synthesis of biodiesel in supercritical fluids. Fuel, 83(14-15), 2029-2033.
[8] Yuichiro, W., Dadan, K., Shiro, S. (2004). Reactivity of triglycerides and fatty acids of rapeseed oil in supercritical alcohols. Bioresource Technology, 91(3), 283-287.
[9] Lee, D. W., You, S. K. (1999). Isolation and characterization of a thermophilic lipase from Bacillus thermoleovorans ID-1. FEMS Microbiology Letters, 179, 393-340.