바이오디젤 공정을 위한 리파아제 생산의 최적화

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Optimization of lipase production for biodiesel process

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Introduction

Biodiesel is commonly transesterificated from animal and plant oil by alkali catalyst. This chemical method has many advantages of cheap catalysts and high conversion yield in short time. But high process temperature causes high costs. In case of using waste oil, the acid-catalyzed process can not avoid the complex pretreatment because free fatty acid and water in the oil can make reaction with alkali catalyst. That makes chemical process more complex.

In contrast, lipase catalyzed transesterification has less pretreatment steps and is able to conduct that at mild condition. This lipase catalyzed process doesn't need to remove the catalyst, thus can be simple steps. Lipase catalizes only transesterification at room temperature, that assures high quality biodiesel. In case of using waste oil, free fatty acid do not give rise to any problem, so process can be more simple. Biodiesel is a renewable and domestic resource with an environmentally friendly emission profile and is readily biodegradable. But lipase producer which has high lipase activity and its stability was need for industrial application.

The purpose of this study is the optimization of lipase production for biodiesel process.

Experimental

1. Assay of lipase

Reaction mixture was consisted of 10 ㎖ of water and 10 ㎖ of isooctane contained 10%(w/v) olive oil on 100 ㎖ Elrenmeyer flask. Enzyme solution (1 ㎖) was added to reaction mixture. It was incubated in shaking water bath at 37℃, 150 rpm for 30min, and enzyme reaction was stopped by adding 6N HCl and agitated vigorously for 30 sec. Upper layer (2 ㎖) was taken, and cupric acetate-pyridine reagent 0.5㎖ was added in a test tube. Free fatty acids dissolved in isooctane were determined by UV spectrophotometer at 715 nm.

Unit (U) = liberated free fatty acid (㎛) / reaction time (min)

2. Transesterification reaction

A mixture of oilive oil and methanol (9.65/0.35, g/g=1/1, mol/mol) was prepared and used as the starting material. The reaction was conducted at 40℃with shaking at 150 oscillations/min in water bath. For the stability of lipase in batch transesterification, stepwise addition of methanol(1 ㎖) was performed every 12 h.

3. Analysis

The methyl ester in reaction mixture was quantified using a M600D gas chromatogragh (Young-lin Co., Korea) connected to a HP-INNOWAX column (19091N-133, 30m 0.25㎛ 0.25㎛). Samples (300㎕) were taken from the reaction mixture at specified times and centrifuged to obtain the upper layer.
For GC analysis, 100㎕ of the upper layer and 9.9㎖ hexane were mixed in a 20 ㎖ bottle. Then, 1.0㎕ of the treated sample was injected into the GC. The column temperature was raised to 180℃ from 150℃ by 15℃/min, and then raised to 240℃ by 5℃/min, and then maintained at this temperature for 1 min. The injector and detecter temperature were set at 260℃.

Results and Discussion

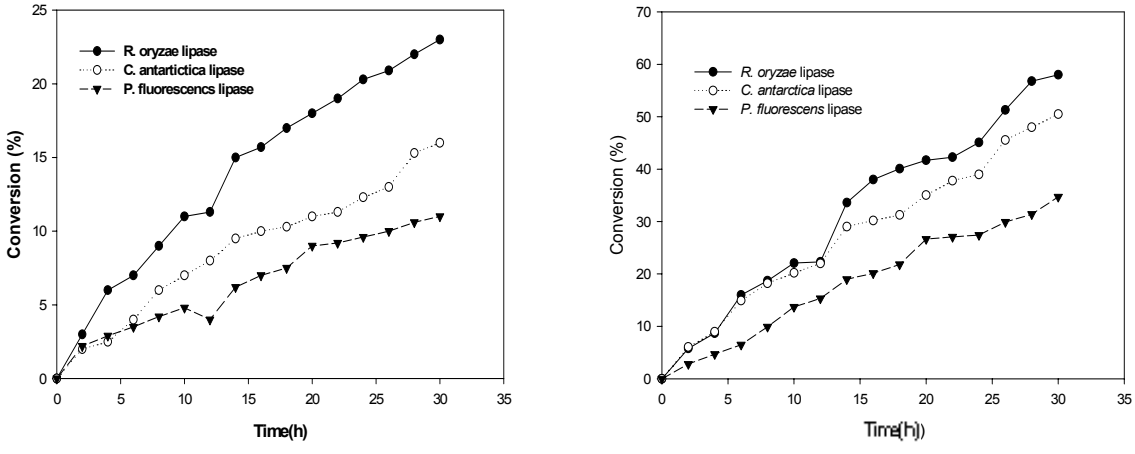
Three microorganisms, *Candida Antarctica, Rhizopus oryzae, and Pseudomonas fluorescens* were mostly possible lipase producer for biodiesel process (Table 1).

Table. 1 Lipase activities of 3 strains in the shake flask on basal medium

Strain	Lipase activity
Candida antarctica	3.2 U/ml
Rhizopus oryzae	4.5 U/ml
Pseudomonas fluorescens	1.0 U/ml

From three strains, suitable microorganism was selected by testing the reaction of tansesterification. The tested microorganisms were *Candida Antarctica, Rhizopus oryzae, and Pseudomonas fluorescens*. *Rhizopus oryzae* lipase was turned out to have the highest lipase activity compare with other microorganisms, so it was chosen for the reaction.

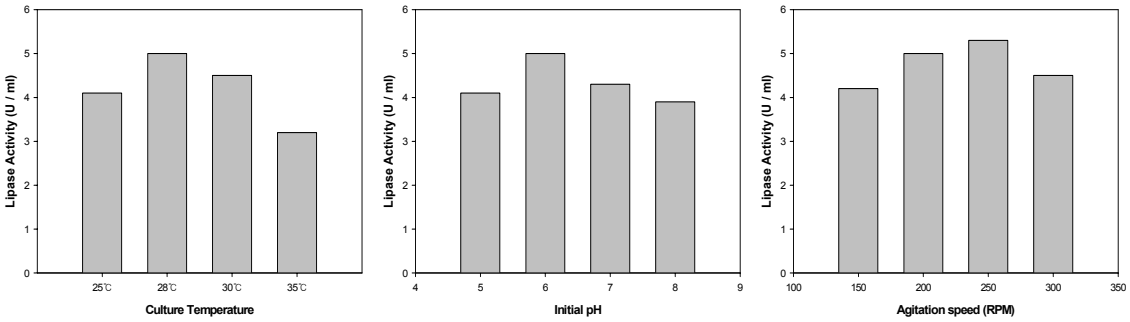
In case of using 15 U/㎖ of enzyme solution, *Rhizopus oryzae* lipase showed the highest conversion of 58% among others tested. (Fig. 1)



a b

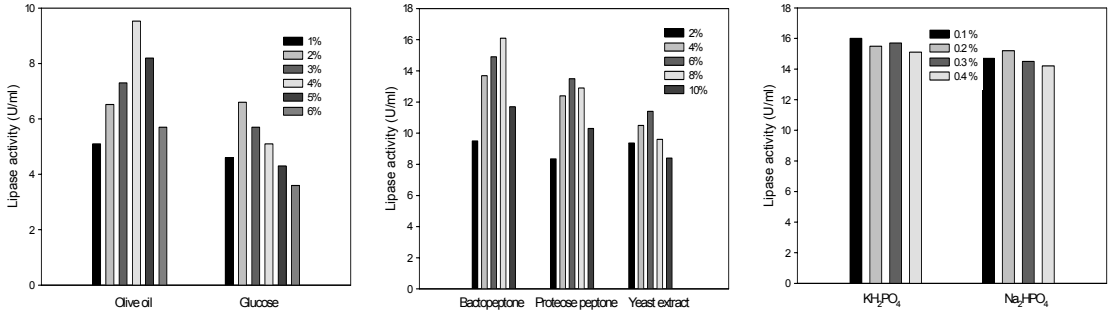
Fig. 1 Biodiesel conversion using lipase solutions of (a) 5 U/ml, (b) 15 U/ml.

For the purpose of increasing the lipase activity, the conditions of *Rhizopus oryzae* fermentation were optimized at different temperatures, initial pHs, agitation speeds, and media. The optimization was conducted by one factor at a time method.



a b c

Fig. 2 Effect of different (a) temperatures, (b) initial pHs, and (c) agitation speeds on lipase activity.

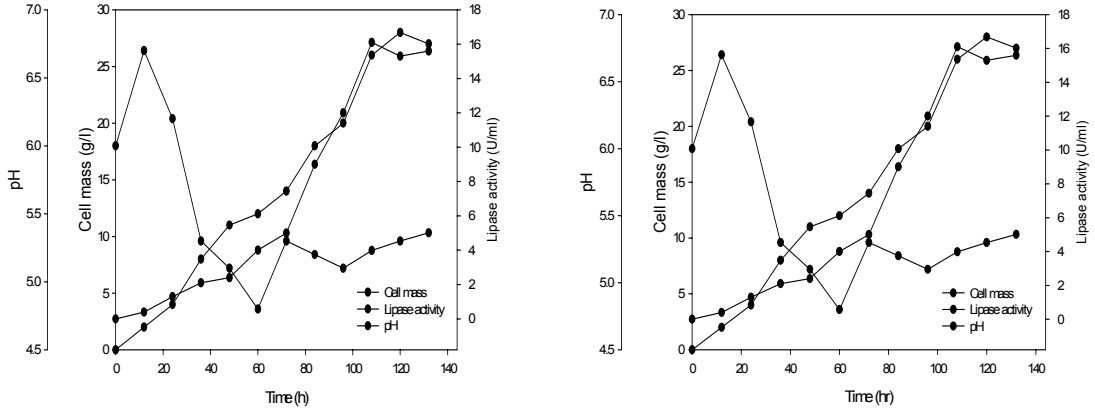


a b c

Fig. 3 Effect of different (a) carbon sources, (b) nitrogen sources, (c) KH_2PO_4 and Na_2HPO_4 on lipase activity.

The optimized conditions were found to be 28℃, pH 6.0, and 250 rpm. The optimum media for producing *Rhizopus oryzae* lipase (16.1U/ml) was 4% olive oil, 8% bacto peptone, 0.1% $NaNO_3$, 0.1% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$.

Fig. 4 shows the growth curves of R. oryzae in the shake flask with unoptimized (a), and optimized (b) conditions. As shown in Fig. 4, the activity was increased about 3.6fold after the optimization of culture conditions and media. The maximum activity obtained was 16.1 U/㎖.



a b

Fig. 4 Growth curves of *Rhizopus oryzae* in the shake flask with optimized conditions

Acknowledgement

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