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Asymmetric organic carbonate synthesis catalyzed by an enzyme with dimethyl carbonate: a fruitful sustainable alliance†

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We have successfully developed an easy and efficient bioprocess for asymmetric organic carbonate synthesis by performing Novozym 435 mediated esterification of DMC and alcohols in this work. Under the optimized conditions (60 °C, molar ratio of alcohol to DMC 1 : 12), the highest yield of carbonate can reach 95.6%. An additional advantage of the new process is the fact that 90% of the original activity of the enzyme is retained after being recycled nine times. Consequently it has potential as a useful enzyme-catalyzed process for the industrial production of asymmetric organic carbonates.

1. Introduction

Because of concerns regarding global sustainability, environmentally benign chemicals and synthetic methods have become urgently needed. Asymmetric and symmetric organic carbonates,[1] as essential intermediates,[2] play an indispensable role in the lithium battery industry and chemical manufacturing. For instance, asymmetric organic carbonates, well-known solvents with relatively high oxidation potentials, are actually a more important component in lithium-ion battery electrolytes than symmetric carbonates.[3] Furthermore, organic carbonates can be used as plasticizers, lubricants, protecting groups[4] of alcohols and phenols, and mild carbonylation or methylation agents in place of poisonous phosgene and dimethyl sulfate. With the dramatically expanding applications of lithium batteries, the importance of aryl and alkyl carbonates is undoubted as reported by many patents[5] and research articles,[6] however, few carbonates are commercially available except for propylene carbonate (PC), diethyl carbonate (DEC), ethylene carbonate (EC) and dimethyl carbonate (DMC). Several methods for the synthesis of alkyl carbonates from alcohols have thus been exploited and reactants such as urea or its carbamate derivatives, carbon monoxide or supercritical CO_2, lead to low yields. Moreover, the synthesis of these compounds still uses highly toxic and harmful reagents,[1,7] such as phosgene ($COCl_2$), dimethyl sulfate, pyridine and carbon monoxide.

The new pathways involve carbonated building blocks like DMC, which has been shown to be an efficient methoxy carbonylating agent and can be formed by a clean synthetic process. The synthesis of asymmetric organic carbonates through the transesterification of DMC with alcohols has been achieved by the use of solid base catalysts, for instance, MCM-41-TBD (TBD, 157-triazabicyclo[4.4.0]dec-5-ene),[8] Mg/La metal oxide,[9] CsF/α-Al_2O_3,[10] (n-Bu_2SnO)$_6$ and nano-crystalline MgO.[11] These heterogeneous catalytic systems are complicated, requiring sophisticated and tedious procedures, high temperatures (>100 °C) and rigorous pressure conditions. Recently Matthieu Bandres *et al.*[12] showed the efficiency of K_2CO_3, NaOH or MeONa in such reactions with a high temperature of 90 °C (reflux) which is still slightly high for DMC, but the result could not be duplicated in our lab. Even the only successful TBD catalysis published in 2012 still has some drawbacks, such as causticity and inflammability.[13]

Nowadays, as awareness of environmental protection is gradually aroused, eliminating metal component involvement in catalyst design and abating volatile organic solvent usage in chemical syntheses is more promising for our future. Hence, there is the need to identify "green" catalyst systems for transesterification reactions which are "environment-friendly" and can obtain high yields at ambient conditions. One of the most promising strategies to achieve these goals is the application of enzymes which exhibit a number of features that make their use advantageous compared to conventional chemical catalysts. Except for a high level of catalytic efficiency, enzymes generally operate under mild temperature, pressure and pH conditions with reaction rates of the order of those achieved by chemical catalysts under more extreme conditions. Although enzymes have been used mostly for aqueous phase reactions,[14] non-aqueous enzymology has potential applications in synthetic chemistry.[15] Advantages such as high solubility, the ability to

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carry out new reactions impossible in water, greater stability, and an easier synthesis procedure, mean that lipases are the most frequently used enzymes for organic syntheses,[16] including aminolysis, alcoholysis, amidation and perhydrolysis. As a catalyst it has also been reported for the transesterification of various refined vegetable oils in many literature reports. A suitable choice for catalyzing transesterification is Candida Antarctica lipase B (CaLB),[17] while the commercially available lipase for the immobilization of CaLB is Novozym 435.[18] The enzyme is adsorbed on a polymethylmethacylate carrier, mainly due to its tolerance to organic solvents and reasonable thermal stability, which has been shown to be a highly efficient biocatalyst.

The lipase-catalyzed synthesis of glycerol carbonate from glycerol (a renewable and cheap raw chemical) with dimethyl carbonate has received increasing attention.[19] However, these catalytic processes require relatively harsh reaction conditions, organic solvents and other additives like molecular sieves, then moderate yields of the targeted product are obtained and yet another intricate purification step by distillation is necessary. In this paper, we extended the scope of the catalyst to the synthesis of asymmetric organic carbonates. The main advantage of the developed synthesis method is that the strategy follows an eco-friendly and non-toxic route. The process efficiently synthesizes asymmetric organic carbonates, with alcohols and DMC, and is totally green since the reaction is catalyzed by Novozym 435, and no other additives are involved except for an acyl donor and solvent. This enzyme-catalyzed process has the potential for the industrial production of asymmetric organic carbonates for lithium-ion battery electrolytes. To the best of our knowledge, it is the first enzymatic example to synthesize asymmetric organic carbonates.

2. Materials and methods

2.1. Enzyme

Crude lipase from *Penicillium expansum* (PEL) (5000 U mg^{-1} solid), *Penicillium neutral expansum* (PNEL) (10 000 U mg^{-1} solid), *Aspergillus niger* (ANL) (120 000 U mg^{-1} solid), *Rhizopus chinensis satio* (RCSL) (10 000 U mg^{-1} solid), *Porcine pancreatic* (PPL) (10 000 U mg^{-1} solid), neutral proteolytic enzyme (20 000 U mg^{-1} solid), acidic proteolytic enzyme (20 000 U mg^{-1} solid), alkaline proteolytic enzyme (20 000 U mg^{-1} solid) was kindly donated by Shenzhen Leveking Bio-engineering Co. Ltd., China. These enzymes were produced by spraying the concentrated supernatant from fermentation with the addition of a certain amount of starch as a thickening agent. Immobilized (Novozyme 435) *C. antarctica* lipase B (EC 3.1.1.3) was donated by Novozymes (China) Investment Co. Ltd.

2.2. Chemicals

All chemicals were purchased from Aladdin reagent company, which were of analytical grade and used without further purification.

2.3. Experimental procedure

The enzymatic esterification was performed in a 50 mL flask on a rotary shaker at 180 rpm. The entire reactor assembly was immersed in a thermostatic water bath, which was maintained at a desired temperature with an accuracy of ± 1 °C.

A typical reaction mixture consisted of 0.0113 mol alcohol and 0.136 mol DMC without solvent. The immobilized enzyme was added to initiate the reaction. The mass ratio of enzyme/DMC is 1/100. The reaction mixture was agitated at 60 °C at a speed of 180 rpm. Liquid samples were withdrawn periodically from the reaction mixture and analyzed on a gas chromatograph.

2.4. Analysis

The analysis of the reaction mixture was carried out on a gas chromatograph (sp-6890) equipped with a flame ionization detector (FID) and a capillary column (SE-54, 30 m $\times$ 0.25 mm $\times$ 0.25 μm). The column temperature was kept at 100 °C for 1 min and then raised to 150 °C for 10 min at a rate of 10 °C min^{-1}. The temperatures of the injector and detector were maintained at 320 and 320 °C, respectively. The products were further identified using ^{1}H NMR (Nuclear Magnetic Resonance).

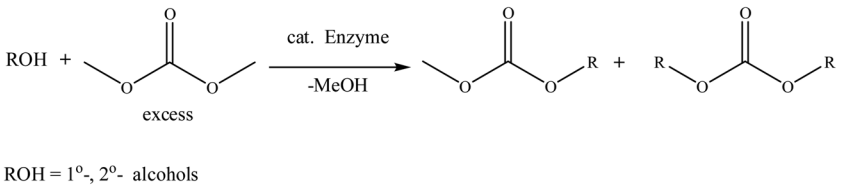
The catalyst enzyme was recovered by centrifugation of the resulting suspension and then washed using acetone. The residue obtained was dried at 45 °C under reduced pressure overnight (at 1 Torr for 24 h) and was then used for the next generation. The conversion of alcohol, the yield of alcohol and the selectivity for asymmetric carbonate were calculated using eqn (S1)–(S3),† where the number of moles was determined by the Internal Standard Method from the chromatographic analysis.

3. Results and discussion

The effects of various parameters on the conversion and rate of reaction were studied systematically. The reaction is shown by Scheme 1.

3.1. The efficacy of various catalysts

Because it is cheap and renewable, fusel oil production is a rapidly growing biomass industry, isoamyl alcohol, which is distilled off fermented alcohol for use in beverages or biofuels, has been investigated less compared to other alcohols, from C2 to C5, especially for carbonates. For this reason, isoamyl alcohol was selected as a model substrate to carry out our elementary experiments in the search for a highly efficient catalyst that follows the principles of green chemistry, and the results are listed in Table 1. As shown in Table 1, different lipases or chemical catalysts were used to evaluate their efficacy under similar conditions. Novozym 435 showed the highest catalytic performance for the production of asymmetric organic carbonates, while other lipases exhibited little activity and almost no asymmetric organic carbonates could be found. NaOH, MeONa and $CaCO_3$ didn't show high activity as reported.[12] Based on these results, we selected Novozym 435 as the desirable catalyst for the synthesis of asymmetric carbonates.



Scheme 1 The enzyme-mediated esterification of alcohols with DMC.

Table 1 Screening the lipase sources for asymmetric organic carbonate synthesis[a]

Entry	Cat.	Conv. (%)
1	No catalyst	0
2	Novozym 435	89
3	*Parcine pancreas*	10
4	*Penicillium expansum*	2
5	*Penicillium neutral expannsum*	2
6	*Aspergillus niger*	0
7	*Rhizopus chinensis satio*	0
8	Neutral proteolytic enzyme	0
9	Acidic proteolytic enzyme	0
10	Alkaline proteolytic enzyme	0
11	NaOH	20
12	MeONa	38
13	$CaCO_3$	52

[a] *Conditions*: DMC : isoamyl alcohol = 12 : 1; 1% (w/w) catalyst; temperature = 55 °C; 48 h incubation time. For NaOH, MeONa, $CaCO_3$: temperature = 90 °C.

3.2. The effect of Novozym 435 loading

In the reaction process, the amount of lipase not only plays a key role in the catalytic performance, but also demonstrates a crucial economical factor for successful industrial application. The effect of catalyst loading was studied from 5 to 180 mg under similar conditions.

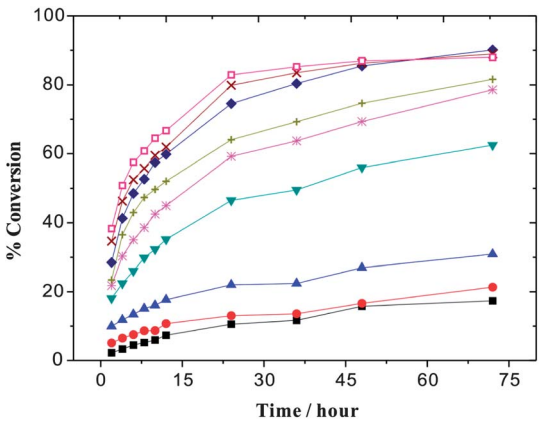


Fig. 1 Effect of catalyst loading. *Reaction conditions* – isoamyl alcohol: 11.3 mmol, DMC: 0.136 mol, speed of agitation: 180 rpm, temperature: 55 °C, catalyst: 5–180 mg, time: 72 h, (■) 5 mg; (●) 10 mg; (▲) 20 mg; (▼) 40 mg; (*) 80 mg; (+) 100 mg; (◆) 120 mg; (×) 160 mg; (□) 180 mg.

As shown in Fig. 1, the initial reaction rate increased with an increase in enzyme loading, which is in contrast to the data at 2 h when the substrate concentration was much higher than the enzyme concentration (as will be mentioned in Section 3.4). Based on the Michaelis–Menten equation $V = V_{max}[S]/(K_m + [S])$ (where [S] is the substrate concentration, V is the reaction rate, V_{max} is the maximum rate achieved by the system at maximum (saturating) subtrate concentrations and K_m is the Michaelis constant *i.e.* the substrate concentration at which the reaction rate is half of V_{max}), when the substrate concentration is high enough, *i.e.*, $[S] \gg K_m$, then $V \approx V_{max}$. At this time, if we increase the concentration of the enzyme, the enzymatic reaction rate will become proportional to the change in enzyme concentration, *i.e.*, $V = k_3[ES]$ (ref. 20) (where [ES] is the enzyme concentration). Because the dosage of 5–40 mg enzyme was small and therefore the reaction was slow, we can regard the data (2.3%, 5.1%, 10.0% and 18.1%) at the time of 2 hours as proof of the existence of k_3.

It was found that the rate of reaction and the overall conversion exhibited a similar tendency to ascend and then reach equilibrium with an increasing amount of catalyst from 5 mg to 120 mg. As the catalyst loading surpassed 120 mg the rate of reaction and conversion did not increase any more at 72 h. We believe that the reaction reached equilibrium; under these conditions it reached the highest conversion of up to 90%.

3.3. The effect of temperature

It is well known that the reaction temperature is a crucial parameter in biocatalysis. The higher the reaction temperature, the faster the reaction rate. However, the high temperature may inactivate the enzyme. The temperature also has a big effect on the thermodynamic equilibrium of a reversible reaction. Therefore, the effect of temperature was studied in the range of 50–65 °C. In Fig. 2, it is shown that the initial rate and the conversion increased when the temperature was raised from 50 to 65 °C. The final conversions after 6 h were 39.0%, 48.5%, 59.3% and 58.6% at 50, 55, 60 and 65 °C respectively. It is well known that Novozym 435 is thermally stable at 60 °C and hence there was no deactivation of the enzyme at 60 °C.[21] The asymmetric organic carbonate yield reached the highest value of 93.4% and a further increase in temperature (up to 65 °C) led to a slight decrease in the yield, which supported the previous finding that the reaction was intrinsically kinetically controlled. The rise in temperature is responsible for the activation of Novozym. Also the enthalpic contribution to the enzymatic rate enhancement suggests that there are important electrostatic and hydrogen-bonding interactions in the transition state of the

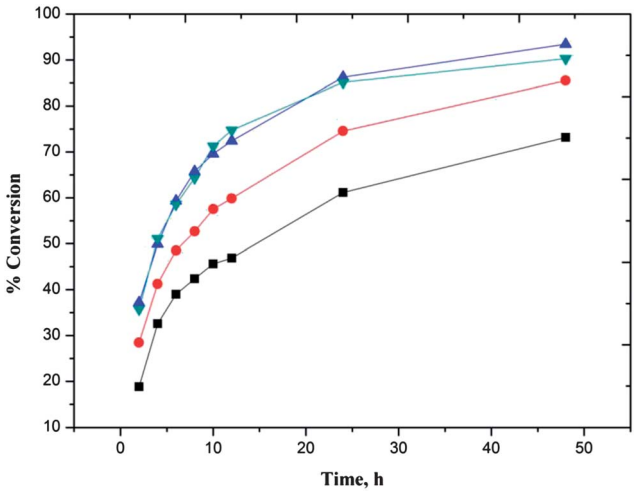


Fig. 2 Effect of temperature. *Reaction conditions* – isoamyl alcohol: 11.3 mmol, DMC: 0.136 mol, catalyst: 0.12 g, speed of agitation: 180 rpm, temperature: 50–65 °C, time: 48 h, (■) 50 °C; (●) 55 °C; (▲) 60 °C; (▼) 65 °C.

enzymatic reaction, which are responsible for the increased rate with a raised temperature.

3.4. The effect of concentration of DMC

The catalytic performance is closely associated with the amount of added reactive precursors in the reactions. In our present study, the effect of the isoamyl alcohol/DMC molar ratio was also investigated. As shown in Fig. 3, it is apparent that the catalytic performance of lipase on the transesterification reaction was significantly affected by the isoamyl alcohol/DMC molar ratio.

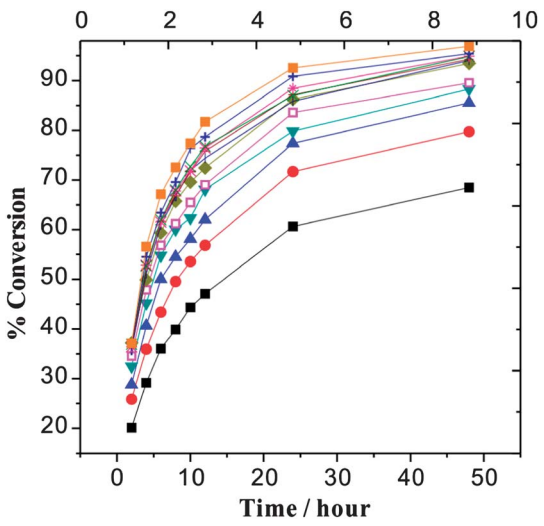


Fig. 3 Effect of concentration of DMC. *Reaction conditions* – isoamyl alcohol: 11.3 mmol, DMC: 0.011–0.283 mol, speed of agitation: 180 rpm, temperature: 60 °C, catalyst: 120 mg, time: 48 h, (■) 1 : 2; (●) 1 : 4; (▲) 1 : 6; (▼) 1 : 8; (□) 1 : 10; (◆) 1 : 12; (|) 1 : 14; (-) 1 : 16; (*) 1 : 18; (×) 1 : 20; (+) 1 : 25.

Based on the data in Fig. 3, the initial conversion rate increased along with an increase in the dosage of DMC before the ratio of DMC to isoamyl alcohol exceeded 12 : 1. When the DMC's dosage surpassed 12 : 1, the increment of its concentration wouldn't enhance the initial reaction rate, which matched the Michaelis–Menten equation $V = V_{max}[S]/(K_m + [S])$. When the substrate concentration is very low, $[S] \ll K_m$, the reaction rate is proportional to the substrate concentration; while if the substrate concentration is high enough, the reaction rate will reach the limit V_{max} and it won't change even though the substrate concentration increases.

An increase in the isoamyl alcohol : DMC molar ratio from 1 : 2 to 1 : 12 generated an increasing DMC yield with an improved product conversion from 60.6% to 93.4%. The law of chemical kinetics tells us that the reaction equilibrium is sharply impacted by the reactant concentration, and more reactant can drive the reaction to move in the opposite direction. Therefore, adding more DMC should result in a higher IMC (Isoamyl Methyl Carbonate) yield due to the reversible nature of the transesterification step. On further increasing the ratio of isoamyl alcohol to DMC from 1 : 14 to 1 : 25, a decrease in the conversion to carbonates can be observed. It may be attributed to the fact that excessive DMC decreases the valid concentration of the lipase and the enzyme activity is lowered by more and more DMC. Thus a suitable value (1 : 12) was determined and used in the subsequent investigation in consideration of energy consumption.

3.5. Reusability of the catalyst

To make the process more economical and feasible, it is necessary to study the recyclability of the immobilized lipase Novozym 435, which is regarded as the most important advantage of an immobilised enzyme compared to a free enzyme. The reusability was studied to ascertain its stability during the reaction. To further examine the potential of this bioprocess for industrial production, the catalyst was filtered after each use

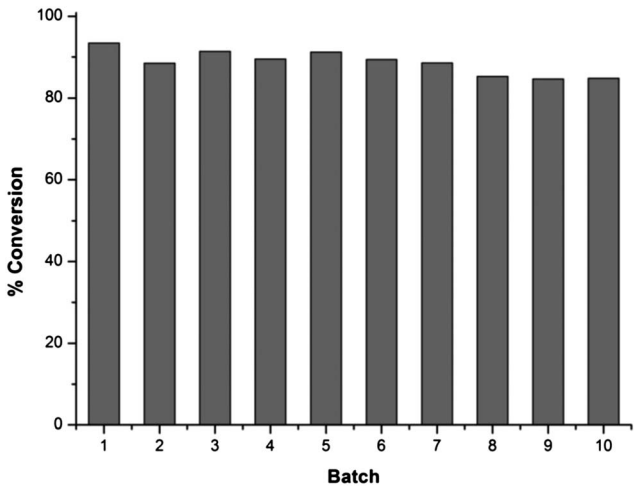


Fig. 4 Reusability of the catalyst. *Reaction conditions* – isoamyl alcohol: 11.3 mmol, DMC: 0.136 mol, speed of agitation: 180 rpm, temperature: 60 °C, catalyst: 120 mg, time: 48 h.

and washed with acetone and then dried at room temperature. As shown in Fig. 4, Novozym 435 shows good stability and retains more than 91% of its initial activity after 9 consecutive reuses. The loss of activity is believed to be due to the deactivation of lipase or the loss of catalyst during handling as the cycles are increased.

3.6. Asymmetric organic carbonate synthesis

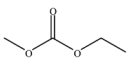
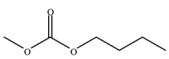
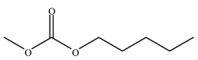
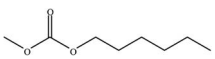
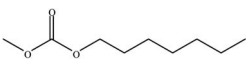
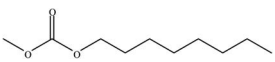
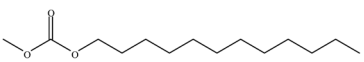
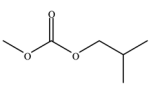
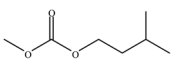
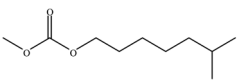
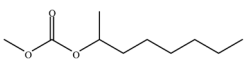
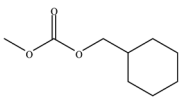
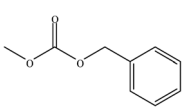
The alcohol used above was isoamyl alcohol and a number of different alcohols were chosen to evaluate the scope of the reaction. The results in Table 2 show that the esterification of DMC with different alcohols was very satisfactory.[1]

As can be seen, the length of the alkyl chains of the alcohols did not affect the conversion much. The alcohols with a branched chain, which has a huge steric hindrance, reacted with DMC and had lower conversion ratios. Among the alcohols mentioned in Table 2, cyclohexanol has the biggest strict hindrance, so it has the lowest conversion. Benzyl alcohol's hydroxide radical formed the conjugated system with the benzene ring decreasing the strict hindrance, and its methylene is mobilizable, so benzyl alcohol has a higher conversion than cyclohexanol. It can be concluded that the strict hindrance of the alkanols' hydroxide radical has an important effect on the conversion.

4. Conclusions

In our present work, we first used the enzyme to demonstrate a high-efficiency, low energy-consuming and easy-to-handle route for the synthesis of asymmetric organic carbonates. The only by-product is methyl alcohol, which indicates this process has significance in green chemistry. Besides, the catalyst Novozym 435 is industrialized and easy to obtain and recycle.

Table 2 The synthesis of various asymmetric carbonates *via* Novozym 435 catalyzed transesterification of DMC[a,b,c]

Entry	Product	Time	Conv. (%)	Sel. (%)	Yield (%)
1		24 h	94.4	95.7	90.3
2		24 h	93.9	93.8	87.3
3		36 h	94.3	>99	94.3
4		48 h	95.6	96.1	91.9
5		48 h	92.5	>99	92.5
6		48 h	91.1	95.4	86.9
7		30 h	91.3	98.8	90.2
8		24 h	85.2	>99	84.6
9		48 h	93.4	>99	93.4
10		72 h	61.7	>99	61.7
11		72 h	47.6	>99	47.5
12		72 h	35.2	>99	35.2
13		72 h	72.8	>99	72.2

[a] All reactions were carried out with 11.3 mmol alcohol, 136.1 mmol of the DMC and $w_{Enzyme}/w_{DMC} = 1.0\%$ (120 mg) of Novozym 435 (where w_{Enzyme} is the mass of the enzyme and w_{DMC} is the mass of the DMC). [b] The selectivity towards the asymmetric carbonate. [c] The conversion and selectivity were calculated for crude reaction mixtures *via* GC (Gas Chromatography) with methyl benzoate as an internal standard.

One of the products, methyl ethyl carbonate (yield 90.3%), is an excellent lithium ion battery electrolyte solvent. It has been mentioned in the literature that methyl pentyl carbonate (yield 94.3%) used for lithium ion battery electrolyte solvent can not only increase the battery's capacity density and discharge capacity, but also extend the battery's lifetime. Furthermore, it can raise the performance at low temperature, effectively overcoming the defects of conventional lithium ion battery electrolytes.[22]

Although Novozym 435 has been commercialized and is recyclable, its price is still higher than other enzymes, such as the Porcine pancreatic lipase, which showed catalytic activity in this reaction. Enzyme immobilization will be continued and delved into after this work.

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