ISSN : 0974 - 7435 Volume 11 Issue 8

BioTechnology
An Indian Journal

FULL PAPER

BTAIJ, 11(8), 2015 [286-290]

Comparison of prepared magnetic nanolipase and novozym 435 for enzymatic ring-open polymerization of ε-caprolactone

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ABSTRACT

Immobilized magnetic nanolipase (MNL) prepared in our lab was employed to catalyze ring-open polymerization of ε-caprolactone. The commercial immobilized lipase, Novozym435, was used as biocatalyst as comparison for the same reaction. Effects of solvent, substrate concentration, temperature, reaction time were investigated. The results showed the conversion of ε-caprolactone catalyzed by MNL was similar to that of Novozym435. And both immobilized lipase had similar thermal stability. The pH stability of MNL was much higher than that of Novozym435. These results suggest that MNL could be good candidate for enzymatic polymerization in the industry.

KEYWORDS

Lipase;
Magnetic nanoparticles;
Ring-open polymerization;
Novozym 435;
Enzymatic synthesis.

INTRODUCTION

Free enzymes normally are produced from bacteria, fungi, plant or aminmal, and exist as liquid solution. It is not economic to employ free enzymes for biocatalytic reactions because they have bad stability and can not be reused. In order to improve the stability and reusability of enzymes, immobilization is the first option because stable biocatalyst can be achieved by the technique.

Lipase (EC 3.1.1.3), known as triacylglycerol ester hydrolase, has many industrial applications such as resolution of racemic mixtures[1,2], synthesis of drugs and pharmaceutical intermediates[3–5], production of biodiesel[6], hydrolysis of lipid[7]. Because of the versatile properties, lipase has been widely used in detergent, pharmaceutic, leather, textile, cosmetic, and paper industries[8]. Among these applications, the most interesting one is the enzymatic polymerization because lipases are powerful catalysts for the preparation of polyesters, polycarbonates, and even polythioesters and polyamides[9]. Same as the other enzymatic catalysis, it is environmentally benign, and lipase-catalyzed polymerization also has the advantages including stereochemistry, regioselectivity, and chemoselectivity[10]. The bottleneck of enzymatic process is the high cost of enzyme. So in order to lower the application cost of lipase, immobilization has been studied in many research groups to obtain stable immobilized biocatalyst in the past few years. In our previous work, magnetic nanopliase was prepared by immobilization onto Fe_3O_4@SiO_2 nanoparticles, and stable MNL was obtained[11].

In the present research, we employed magnetic

nanolipase (MNL) prepared with the previous method to catalyzed ring-open polymerization of ε-caprolactone to explore the possible industrial applications of the nanopliase. For comparison, the commercial immobilized lipase, Novozym 435, was employed to catalyze the reactions at the same time.

EXPERIMENTAL

Chemicals

Free enzyme was purchased from Imperial Jade Bio-Technology Co. Ltd (Lanzhou, China). Novozym 435 was obtained from Novo Nordisk (Guangzhou, China). ε-caprolactone was from Aladdin-reagent (Shanghai, China) and p-Nitrophenyl palmitate was obtained from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were from Sinopharm Chemical Reagent Co. Ltd (Shanghai, China) and were analytical or biological grade.

Enzyme and analysis

Immobilized MNL was prepared according to the previous report with some modifications[11]. Typically, the aldehyde-functionalized Fe_3O_4@SiO_2 nanoparticles were suspended in 0.3 ml of the enzyme solution (0.39 mg ml^{-1}) and mixed well at 10°C, pH 6.0. After 24 h of incubation, the immobilized MNL was then filtered and washed 5 times with the pH 7.0 50 mmol l-1 PBS.

Lipase activity was determined as the previous report[12] with slight modification. Dissolve 0.5 g of p-Nitrophenyl palmitate (pNPP) in 100 ml of ethanol as the substrate to determine lipase activity. During the reaction, the release of p-Nitrophenol leads to the increase in absorbance at 410 nm which could be measured spectrophotometrically. About 20 mg of immobilized lipase was added to a mixture of 0.04 ml of 5 mmol l-1 pNPP solutions and 0.4 ml of 0.05 m phosphate buffer (PBS, pH 9.0) and incubated for 5 min at 45°C. The mixture was centrifuged for 10 min (10,000 rpm) to terminate the reaction. Diluted 0.5 ml of the supernatant 10-folds with distilled water, and measured at 410 nm in a spectrophotometer (UV-2450/2550, Shimadzu, Japan). One unit (U) of enzyme activity was defined as the amount of enzyme which catalyzed the production of 1 mmol p-Nitrophenol per minute under the experimental conditions.

Enzymatic catalyzed ring-open polymerization of ε-caprolactone

Added 2 ml ε-caprolactone into a 10 ml solvent (acetone, toluene, tetrahydrofuran, dichloromethane, chloroform, n-Hexane) in a 50 ml flask, then 50 mg immobilized lipase was added into the flask. The N_2 was injected in the reaction system to exhaust the air. Then the flask was sealed with a septum and incubated at 60°C in a water bath shaker to start the reaction. The samples were subjected to a gas chromatography (4890D, Agilent) to measure the concentration of ε-caprolactone and the conversion of the reaction could be calculated. All the experiments were repeated three times and the average conversions were obtained as the final data.

The factors including substrate concentration, temperature, reaction time and were investigated to achieved the highest conversion of ε-caprolactone. In the present work, substrate concentration was changed from 9.1-50% (v/v); and temperature ranged from 30 to 70°C; and reaction time was studied from 6 h to 48 h.

Thermal and pH stability of immobilized lipase

The thermal stability of the immobilized lipase was checked at 60°C. The immobilized lipase was incubated at 60°C and subjected for measure of relative activity at certain intervals. The pH stability was investigated similar to the thermal stability. The immobilized enzyme was incubated at different pH buffers (pH 8-12) at 60°C, and the samples were subjected to enzyme assay, then the relative activity was calculated.

RESULTS AND DISCUSSIONS

Effect of solvent on the polymerization

Although lipases from various sources have been used for ring-open polymerization, the preferred lipase is still Novozym435[13]. Thus Novo435 was used as the standard for comparison. Firstly, effect of solvent on the enzymatic polymerization was investigated. Six solvents including acetone, tetrahydrofuran, dichloromethane, chloroform, toluene and n-hexane were chosen as the solvent for enzymatic ring-open polymerization of ε-caprolactone. As shown in Figure 1, both immobilized lipase had good performance in

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toluene and n-hexane. Compared with Novozym435, the MNL prepared in our lab has good catalytic properties for polymerization. There is no much difference when the reaction was performed in the same organic solvent and the difference between the conversions was less than 10%. The high conversions could be achieved in hydrophobic solvent. On the contrary, the conversion was low in hydrophilic solvents. When Novozym435 was employed as the catalyst, n-hexane was the best solvent and the conversion reached 77.2% although there is only 3% higher than that in toluene. The highest conversion was achieved with 80.9% when MNL was used as the catalyst. It is much higher than 18% of PHB-depolymerase[14]. The previous reported that conversion of ε-caprolactone was around 80%[15]. In order to explore the possible industrial applications of MNL, toluene was chosen as the solvent for the enzymatic ring-open polymerization.

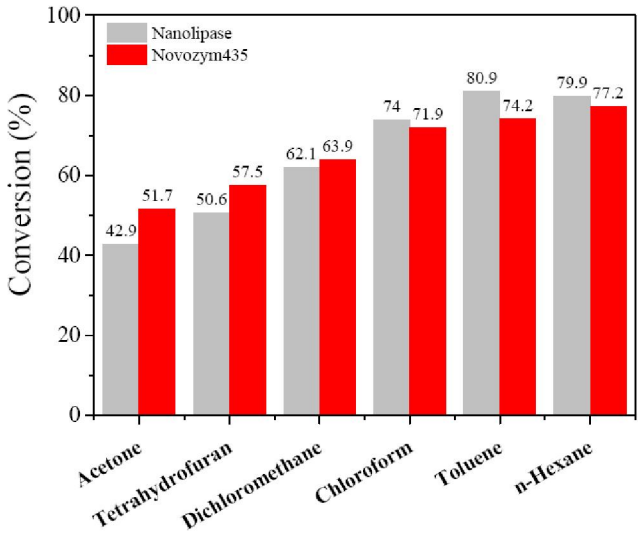

Figure 1 : Effect of solvent on the polymerization

Effect of substrate concentration on the polymerization

Substrate concentration is the essential factor influence the enzymatic process[16]. The substrate concentration of ε-caprolactone was varied from 9.1-50% as shown in Figure 2. For MNL, the highest conversion of 78% was obtained when the substrate concentration was 16.7%. It was 80% when Novozym435 was the catalyst at 28.6% substrate concentration. It could conclude that the commercial immobilized lipase has the better substrate tolerance than MNL. The reason attributed to the magnetism of the MNL because it is easy to aggregate and result substrate diffusion limitation compared to non-magnetic enzyme[17]. However, the magnetism of MNL leads to the easy recovery[18]. And there is less than 10% conversion between these two immobilized lipase.

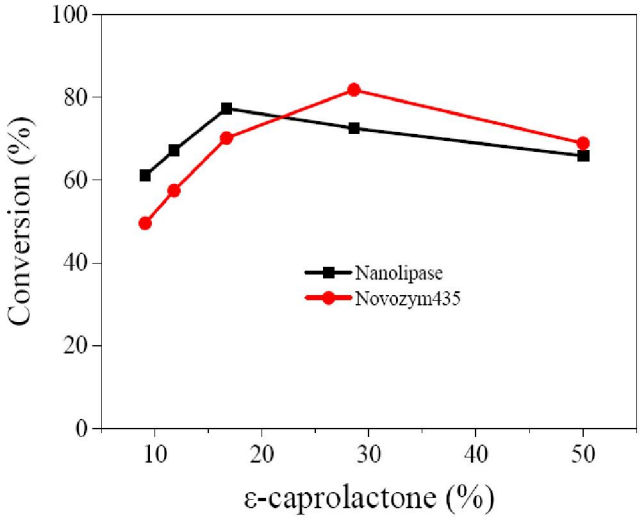

Figure 2 : Effect of substrate concentration on the polymerization

Effect of temperature on the polymerization

Optimal temperature is important for enzymatic catalysis[19]. Figure 3 shows the effect of temperature on the conversion of ε-caprolactone in the toluene. The tendency is same when Novozym435 and MNL were used for polymerization separately. The highest conversions, achieved at 60°C, were 78% and 70% for Novozym435 and MNL, respectively. When temperature was increased to 70°C, MNL showed about 8% higher conversion than that of Novozym435. It indicated that MNL has better catalytic efficiency than Novozym435 at high temperature. The results are consistent with the previous report that the polymerization temperature had little influence on the polymerisation[20].

Time course of the polymerization

The time courses of enzymatic ring-open polymerization of ε-caprolactone with MNL and Novozym435 are similar (Figure 4). The conversions reached higher than 75% after 12 h reaction, and then there were no much changes until 36 h. The results suggested that MNL has similar productivity for ring-open polymerization of ε-caprolactone, and probably could be used for other polymerization reactions.

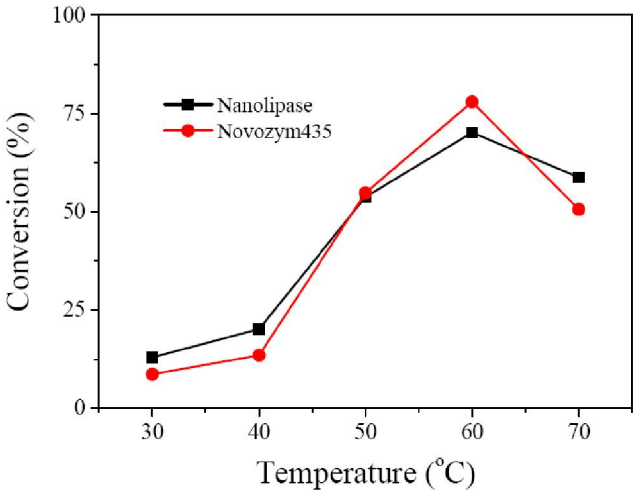

Figure 3 : Effect of temperature on the polymerization

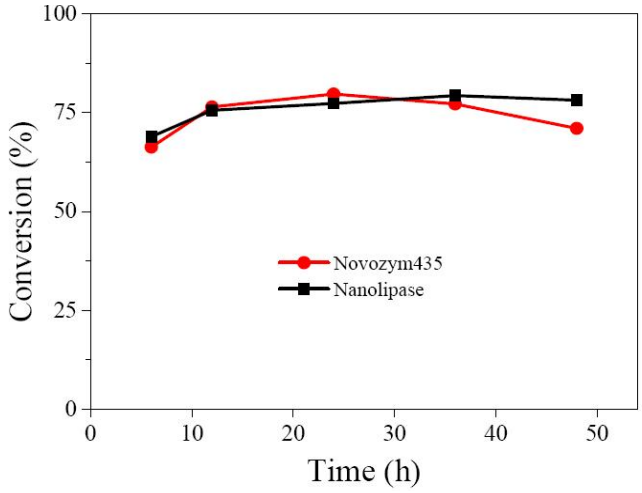

Figure 4 : Effect of reaction time on the polymerization

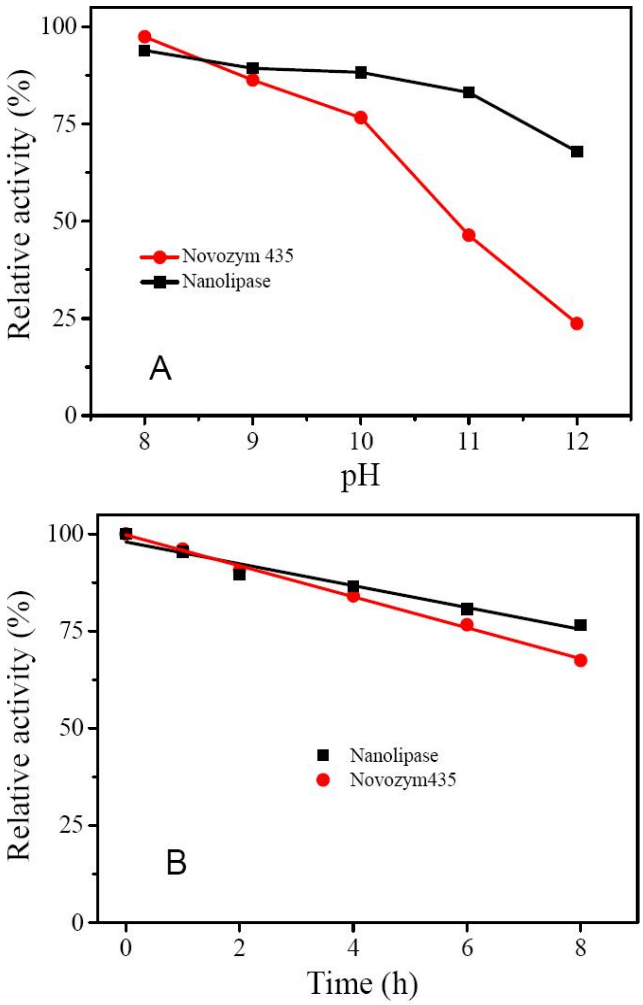

Figure 5 : Effect of pH (A) and temperature (B) on the stability of immobilized lipase

Thermal and pH stability of MNL and Novozym435

Stability of immobilized enzyme is the crucial property for industrial application[21]. In order to check the pH stability of MNL, it was incubated in different pH buffers for 3 h. It is easy to find that MNL had much higher relative activity than that of Novozym435 in Figure 5A. At pH 8-9, MNL showed similar stability to Novozym435. When pH was increased to 10, it displayed 12% relative activity higher than that of Novozym435. Especially at pH 12, the relative activity of MNL was 2-fold higher than that of Novozym435. Figure 5B showed the thermal stability at 60°C at pH 8.0. Both enzymes remained more than 80% relative activity after 4 h incubation, and remained more than 65% relative activity even after 8 h incubation. All these results indicated that MNL has better stability than the commercial Novozym435.

CONCLUSIONS

In summary, the immobilized magnetic nanolipase (MNL) was employed to catalyze ring-open polymerization of ε-caprolactone and commercial lipase (Novozym435) was used as comparison. Effects of solvent, substrate concentration, temperature, and reaction time were investigated. The results showed that MNL displayed similar catalytic performance to Novozym435. However, MNL had much higher stability than Novozym435, especially when pH is higher than 10. Thus MNL could be used as a stable biocatalyst for synthesis of polymers.

ACKNOWLEDGEMENT

We appreciated the financial support from National

Science Foundation of China (No. 21376110).

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