Lipase-catalyzed Continuous Ring-opening Polymerization of ε-Caprolactone in a Packed-bed Reactor

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A packed-bed reactor (PBR) system was developed for continuous lipase-catalyzed ring-opening polymerization of ε-caprolactone (ε-CL). The parameters affecting the poly(ε-caprolactone) (PCL) production in the PBR such as residence time, height to diameter (*H*/*D*) ratio, and monomer feed concentration were investigated. Monomer conversion of ε-CL increased with the increase of residence time, reached the highest (over 98 %) at the residence time of 12 min. And under this residence time it would almost not change even if the *H*/*D* ratio varied from 4 to 20 or the monomer concentration varied from 0.2 to 2.25 mol L^{-1}. The number-average molecular mass (Mn) of PCL did not change in agreement with the monomer conversion, and approached the maximal values of 15600 g mol^{-1} with polydispersity index (PDI) of 2.1 under 20 min residence time, 12.0 *H*/*D* ratio and 1.75 mol L^{-1} monomer concentration. The Mn of PCL maintained around 15600 g mol^{-1} without appreciable loss during the long-term operation of 460 h with the PCL productivity of 19.15 g g^{-1}_{enzyme} d^{-1}).

Key words:
Packed-bed reactor (PBR), lipase, ring-opening polymerization, poly(ε-caprolactone)

Introduction

Biodegradable polyesters are very important materials formed with repeated ester bond. Due to awareness of the pollution problem and the huge environmental accumulation, biodegradable polyesters have aroused great interest recently. Poly(ε-caprolactone) (PCL) is one of the most important biodegradable polyesters, with the advantages of biocompatibility, biodegradability, permeability and miscibility with other polymers.[1] PCL is thus widely employed as a long-term item, fibers containing herbicides to control aquatic weeds, seedling containers and slow release systems for drugs.[2] Consequently, researches on production of PCL have received increasing attention.

Different processes are currently available to achieve ring-opening polymerization for the production of PCL, which include chemical and enzymatic catalysis. Chemical processes give high molecular mass of polyester in anhydrous conditions with metallic catalysts, which are difficult to remove from the product and may be toxic in nature.[3] However, the enzymatic polymerization processes have offered a more attractive option, because of the advantages of forming polymers with defined structures and mild reaction conditions, such as temperature, pressure and pH, etc. Besides, the enzymes are derived from renewable resources. Therefore, enzymatic polymerization processes can be regarded as an environment-friendly synthetic process for polymeric materials production, giving it a "green polymer chemistry" appeal.[4]

A rapidly increasing number of publications have appeared on various aspects of *in vitro* lipase catalyzed lactones ring-opening polymerizations. Novozym-435 (immobilized lipase B from *Candida antarctica*) has been proved to be the most active lipase for the ring-opening polymerization of ε-CL.[5,6] Kobayashi *et al*. reported that Novozym-435 could be reused for the ring-opening polymerization of ε-CL within 5 cycles with the product molecular mass of 3600 g mol^{-1}.[5] Many researchers have investigated various factors on the lipase-catalyzed ring-opening polymerization in order to increase the monomer conversion and the molecular mass, such as lipase concentration,[7] organic medium, reaction temperature,[8] relationships between water contents and temperature.[9] They have made significant progress in that the monomer conversion and the number-average molecular mass (Mn) reached 85–97 % and 11970–17800 g mol^{-1}, respectively. However, these reactions were all carried out in vials or sealed tubes with shaken equipment. There were some drawbacks of ring-opening polymerization by lipase in a batch reactor, such as large

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amount of enzymes with low operational stability, low productivity within a practical time scale and complex operation process. These were recognized as major factors limiting the lipase used in the large scale ring-opening polymerization.[10,11]

Continuous ring-opening polymerization in an enzyme packed-bed reactor (PBR) may be a way to solve the problems mentioned above. The PBR has been extensively investigated because it offered low cost, high efficiency, high stability of enzyme, automatic separation of product from enzyme and ease of operation.[12,13] It may provide many opportunities for transferring the protocols of *in vitro* enzyme-catalyzed polyester synthesis from the laboratory to an industrial scale. Recently, Wosnick and Faucher reported the PBR for continuous enzymatic ring-opening polymerization of lactones in AICHE conference proceedings in 2009, and protected this work by a patent.[14] However, a very expensive lipase (Novozym 435) was used as the biocatalyst in their work, and thus lacked obvious economic attractiveness.

In this spirit, we developed a continuous ring-opening polymerization process for PCL production in a PBR using a home-made cheap immobilized lipase. The reaction conditions were investigated in order to achieve the optimal continuous ring-opening polymerization.

Materials and methods

Materials

The aminated carrier (LH-HA) was provided by Shanghai Bairui Biotech Co., Ltd. (Shanghai, China). It was mixed with 0.1 mol L^{-1} pH 8.0 phosphate buffer (1:4, w/v) containing φ = 2 % glutaraldehyde, and stirred at 25 °C and 200 rpm for 1 h. After filtration and extensive washing with deionized water, the resulting activated carrier was stored at 4 °C before use. The monomer ε-caprolactone, purchased from Sigma Aldrich, was dried over CaH_2 and then vacuum-distilled in nitrogen atmosphere prior to use. All other chemicals and reagents were obtained commercially and were of analytical grade, and dried over a molecular sieve 4 Å for several days before use.

Preparation of the immobilized lipase

Lipase from *Candida sp.* was obtained by fermentation in our laboratory in the form of crude enzyme powder with a specific activity of 8200 U g^{-1}. 10 g of crude enzyme was dissolved in 1 L of 0.1 mol L^{-1} phosphate buffer (pH 8.0), then centrifuged to remove insoluble impurities. 100 g of wet activated LH-HA was immersed in the supernatant enzyme solution and stirred at 200 rpm and 25 °C for 24 h. The immobilized *Candida sp.* lipase was filtered and washed three times with the deionized water. The resulting immobilized lipase (named LH-HA 703) was dried over P_2O_5 in a vacuum desiccator (1 mmHg, 25 °C, 24 h) before use.

Experimental apparatus

Fig. 1 shows the experimental apparatus setup of the packed bed reactor. A (1.0 cm × 20 cm) column with a water jacket (1) was packed with LH-HA 703 beads as the lipase packed-bed reactor. The upper and lower end of the column was layered with glass wool. The ε-CL and the organic solvent was mixed by a stirrer (3) in a reservoir (5.0 cm × 15 cm) (2) and preheated by a constant temperature bath (4). Then the substrate mixture was fed upwards through the column by a peristaltic pump (6). The system temperature was maintained by a circulating water bath with a proportional-integral-derivative (PID) controller (5).

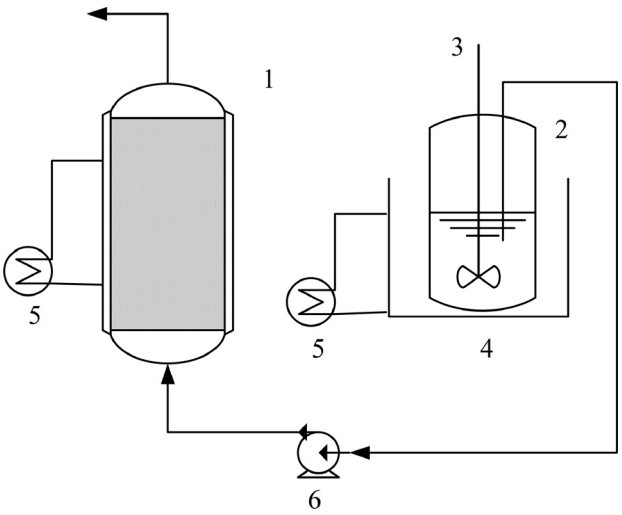

Fig. 1 – *Schematic diagram of packed-bed reactor (PBR) system: (1) lipase packed-bed reactor with a water jacket; (2) substrate reservoir; (3) stirrer; (4) constant temperature bath; (5) PID reactor temperature controller; (6) peristaltic pump*

Continuous ring-opening polymerization in PBR

The LH-HA 703 catalyzed ring-opening polymerization was carried out in PBR (Fig. 1). The LH-HA 703 (0.5 to 2.5 g) was packed to vary the height from 4 to 20 cm. The ε-CL dissolved in toluene with different concentration (0.2 to 2.25 mol L^{-1}) was stirred at 150 rpm in the substrate reservoir and introduced into the PBR with a peristaltic pump. The substrate flow rate changed in the range of 0.05 to 5 mL min^{-1}. The experiments were all carried out at atmospheric pressure and at a constant temperature of 40 °C controlled by water circulation. The reaction progress was monitored by analyzing regu-

lar samples with High Performance Liquid Chromatography (HPLC) and gel permeation chromatography (GPC) system.

Analytical methods

The monomer conversion was determined by an HPLC 1100 series instrument (Agilent Inc) equipped with an online evaporative light scattering detector (Polymerlab Inc). The polymer solution was precipitated in methanol and filtered. The filtrates were injected into a reverse phase Zorbax Eclipse XDB-C18 (Agilent Inc 250 mm × 4.6 mm, 5 μm). A gradient elution of A (acetonitrile) and B (water) was used at a flow rate of 1 mL min^{-1}, commencing with 40 % A and 60 % B, rising to 100 % A for 10 minutes, then maintaining 100 % A for 5 minutes. The column temperature was controlled at 30 °C.

The molecular mass and the polydispersity index (PDI) of the products were measured by GPC system. The GPC apparatus consisted of an HPLC 1100 series instrument (Agilent Inc), two GPC columns (Waters Inc), and an online evaporative light scattering detector (Polymerlab Inc). Styragel columns (300 × 7.5 mm) HR 4 and HR 3 with pore sizes of 10^{-6} and 10^{-7} m respectively were used in series. The sample was diluted with tetrahydrofuran (THF) and then filtrated. The columns were maintained at 50 °C to ensure uniform temperature and good separation of the peaks. THF was used as eluent at a constant flow rate of 1.0 mL min^{-1}. The molecular masses were determined based on conventional calibration curve generated by narrow molecular mass polystyrene standards obtained from Showa Denko Co.

Results and discussion

Effect of residence time on the ring-opening polymerization in PBR

The residence time of the reactant inside the reactor was one of the most important factors in the PBR, which was defined as the average time that the reactant spent in a PBR. The effect of residence time on the ring-opening polymerization in PBR with LH-HA 703 was investigated by varying substrate flow rate. As shown in Fig. 2, the conversion of ε-CL increased fast with the decrease of flow rate because of the long residence time of substrate in the PBR. Moreover, the conversion of ε-CL reached 99.8 % at the residence time of 12 minutes or more. Meanwhile, the molecular mass of PCL was also augmented along with the decrease of flow rate. The Mn value achieved the maximal value of 3700 g mol^{-1} with PDI of 2.5 at the residence time

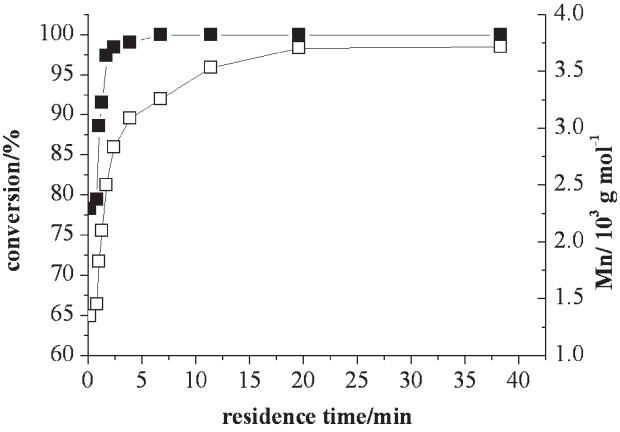

Fig. 2 – *Effect of residence time on the conversion of ε-CL (■) and the molecular mass of PCL (□). The reactor was 1.0 cm×20 cm packed with 2.5 g LH-HA 703. The substrate consisting of 0.5 mol L^{-1} ε-CL in toluene was pumped into the reactor at 40 °C.*

of 20 minutes, and maintained the maximum as the residence time increased from 20 to 38 minutes. Therefore, in order to obtain a high molecular mass polymer with high conversion of the substrate in PBR, the reaction should be carried out at the residence time of 20 minutes.

The residence time determined the contact time between the substrates and the enzyme. At the short residence time, the reactant did not have enough time to react with the enzyme. Hence, the monomer conversion and molecular mass of polymer increased significantly with the increase of the residence time in the PBR. H-Kittikun *et al.* also found this phenomenon that the yield of monoacylglcerols production increased with increasing the residence time of substrates in the PBR, and concluded that the amount of substrate might exceed the catalytic activity of lipase at high substrate flow rate in PBR.[15] In previous studies, the mechanism for the lipase-catalyzed ε-CL ring-opening polymerization was proposed involving monomer activation and polymer chain propagation.[16,17] The polymer chain propagation required more residence time in the PBR than the monomer activation. Therefore, the monomer conversion and the molecular mass of polymer did not reach the maximal value at the same residence time.

Effect of the reactor's *H*/*D* ratio on the ring-opening polymerization in PBR

Another important parameter in PBR is the *H*/*D* ratio of the enzyme bed. The effect of *H*/*D* ratio on the conversion of ε-CL and the molecular mass of PCL was investigated by varying the height of LH-HA 703 bed in PBR under the same residence time. The results are shown in Fig. 3. In all experiments, the conversion of ε-CL remained

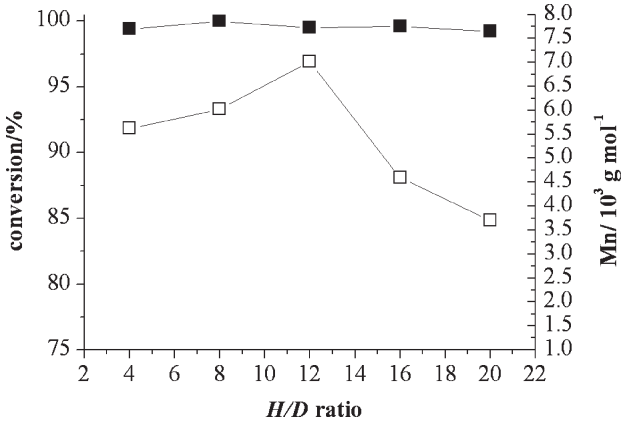

Fig. 3 – *Effect of the reactor's H/D ratio on the conversion of ε-CL (■) and the molecular mass of PCL (□). The substrate consisting of 0.5 mol L^{-1} ε-CL in toluene was pumped into the reactor at 40 °C at the residence time of 20 minutes.*

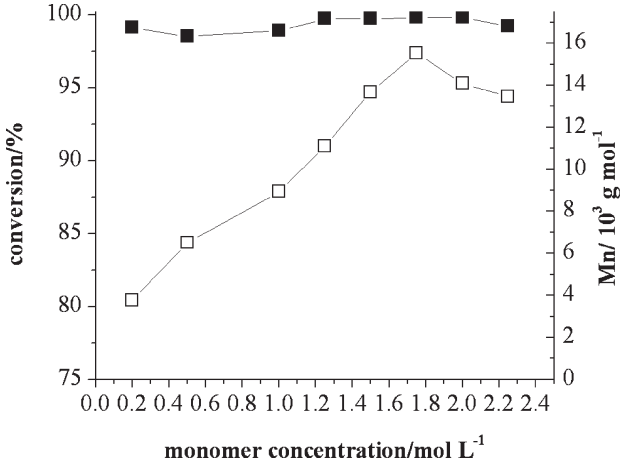

Fig. 4 – *Effect of monomer concentration on the conversion of ε-CL (■) and the molecular mass of PCL (□). The reactor was 1.0 cm × 12 cm packed with 1.5 g LH-HA 703. The substrate consisting of ε-CL in toluene was pumped into the reactor at 40 °C at the residence time of 20 minutes.*

above 98 % with the *H*/*D* ratios ranging from 4.0 to 20.0. However, the molecular mass of PCL increased with the increase of the *H*/*D* ratio from 4.0 to 12.0, and then decreased with the further increase in the *H*/*D* ratio. At the *H*/*D* ratio of 12, the Mn of PCL reached 7000 g mol^{-1} with PDI of 2.3.

In PBR, the residence time governed the contact time between the enzyme and the reactants. Therefore, the conversion of ε-CL kept constant, when the residence time was controlled in a constant value. Xi *et al.* also found the same result that the initial rate of lipase-catalyzed preparation of (S)-ketoprofen remained the same with the *H*/*D* ratio changing from 2.9 to 38.6.[18] But interestingly, the molecular mass of PCL changed with the shape of the reactor, even if the residence time remained constant. This phenomenon might be caused by the difference in the rates of degradation and polymerization under different *H*/*D* ratios. Previous studies had demonstrated that degradation and polymerization occurred simultaneously in the lipase-catalyzed ring-opening polymerization.[6,16] When the *H*/*D* ratio increased from 4.0 to 12.0, the polymerization rate increased faster than the degradation rate, and the molecular mass of PCL increased. As the *H*/*D* ratio was more than 12.0, the polymerization rate increased slower than the degradation rate, and thus the molecular mass of PCL decreased. Therefore, the subsequent experiments were carried out at the *H*/*D* ratio of 12.0.

Effect of the monomer concentration on the ring-opening polymerization in PBR

The effect of monomer concentration on the conversion of ε-CL and the molecular mass of PCL was investigated by varying monomer concentration in the feed solution. Fig. 4 showed that the conversion of ε-CL remained more than 96 % with monomer concentration changing from 0.2 to 2.25 mol L^{-1}. However, the molecular mass of PCL enhanced with the increase of monomer concentration from 0.2 to 1.75 mol L^{-1}, and then decreased when monomer concentration further increased to 2.25 mol L^{-1}. The Mn and PDI of the PCL were 15600 g mol^{-1} and 2.1 respectively at the monomer concentration of 1.75 mol L^{-1}.

At lower monomer concentrations, the ring-opening polymerization of ε-CL followed the mechanism that the polymer chain propagation rate was first-order with respect to the monomer concentration.[16] The molecular mass of PCL increased with the increase of feed monomer concentration. At higher monomer concentrations, the diffusion limitation increased with the increase of the viscosity of the substrate solution. The polymer-chain propagation rate was lower due to the diffusion limitation for the same amount of enzyme-active site. This phenomenon agreed with the previous study, which investigated the model of lipase-catalyzed ring-opening polymerization of ε-CL in a batch reactor.[6] Therefore, the ring-opening polymerization reactions in PBR were carried out with monomer concentration of 1.75 mol L^{-1}.

In summary, for the ring-opening polymerization of ε-CL in the LH-HA 703 packed-bed reactor, the preferred residence time, *H*/*D* ratio, and monomer concentration were 20.0 min, 12.0, and 1.75 mol L^{-1}, respectively. These conditions resulted in the monomer conversion of 98 %, Mn of 15600 g mol^{-1} and PDI of 2.1. The ratio between the amount of LH-HA 703 and the ε-CL mass in the packed-bed reactor was very high, which was 3.8 g (LH-HA 703) g^{-1} (ε-CL). This ratio was much higher than that in a batch reactor,[6,9] which caused higher reaction performance in a PBR.

Long-term PCL production in PBR

The long-term operational stability of the enzyme is an important characteristic of the PBR. For the ring-opening polymerization of ε-CL in the PBR, the long-term operational stability was examined under operational parameters (20.0 min residence time, 12.0 *H*/*D* ratio, and 1.75 mol L^{-1} monomer concentration). As shown in Fig. 5, the operational stability of LH-HA 703 was longer than 460 hours, and the monomer conversion maintained above 98 % during this reaction time. The molecular mass of PCL remained around 15600 g mol^{-1} without appreciable loss with PDI of 2.1. The specific productivity of PCL in PBR was 19.15 g/g^{-1}_{enzyme} d^{-1}.

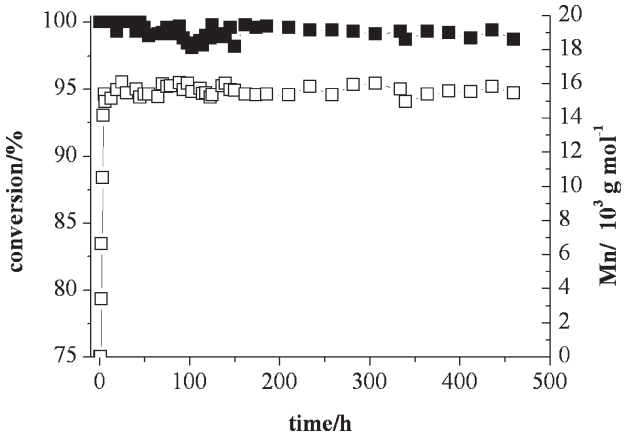

Fig. 5 – *Long-term continuous PCL production by LH-HA 703 in PBR. The reactor was 1.0 cm×12 cm packed with 1.5 g LH-HA 703. The substrate consisting of 1.75 mol L^{-1} ε-CL in toluene was pumped into the reactor at 40 °C at the residence time of 20 minutes. (■) conversion of ε-CL and (□) molecular mass of PCL.*

Gross *et al.* and Kobayashi *et al.* both indicated that the cost of enzyme necessary for polymer production would play a decisive role in determining whether the process could be considered by the industry.[10,11] And for a certain amount of lipase, the cost could be reduced by improving its operational stability. In a traditional batch reactor, Kobayashi *et al.* reported that Novozyme-435 catalyzed polymerization of ε-CL within only 5 cycles for 24 h.[5] However, in this work the LH-HA 703 showed a very high operational stability in the PBR for the ring-opening polymerization of ε-CL. Furthermore, the LH-HA 703 was home-made with a relatively low cost (about 120 US dollars kg^{-1}). Hence, the ring-opening polymerization of ε-CL continuously catalyzed by LH-HA 703 in a PBR possesses the potential for application in large-scale production of PCL.

Conclusions

The benefits of continuous processing include low cost, great process control, high productivity and ease of operation. In this work, a PBR system using LH-HA 703 for ring-opening polymerization of ε-CL was investigated. Over 98 % conversion of ε-CL was achieved when the residence time was 20 minutes or more, although the *H*/*D* ratio and monomer concentration had been varied. The molecular mass of PCL reached the maximum value of 15600 mol L^{-1} with PDI of 2.1 under the conditions of 20.0 minutes residence time, 12.0 *H*/*D* ratio, and 1.75 mol L^{-1} monomer concentration. The monomer conversion of ε-CL and the molecular mass of PCL maintained maximum value without loss during 460 hours of operation. The productivity of PCL in the PBR was 19.15 g g^{-1}_{enzyme} d^{-1}. These results suggest that polymerization in the PBR with LH-HA 703 is a promising method for industrial continuous PCL production.

ACKNOWLEDGMENTS

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List of symbols and abbreviations

PBR – Packed-bed Reactor
ε-CL – ε-Caprolactone
PCL – Poly (ε-Caprolactone)
Mn – Molecular mass
PDI – Polydispersity Index
AICHE – American Institute of Chemical Engineers
LH-HA – An aminated carrier
LH-HA 703 – Immobilized *candida sp* lipase
HPLC – High Performance Liquid Chromatography
GPC – Gel Permeation Chromatography
φ – Volume fraction, %

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