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Preparation of PVA/chitosan lipase membrane reactor and its application in synthesis of monoglyceride

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Abstract

Polyvinyl alcohol (PVA)/chitosan (CS) composite lipase membrane was prepared in this paper, which was used for enzymatic processing of fats and oils. The parameters, such as concentration of lipase, pH, and cross-linking agent as well as metal ions, which influence the immobilization of lipase in membrane, were optimized. The immobilized activity of lipase was 2.64 IU/cm^2 with recovery of 24%. The membrane reactor was used in a two-phase system reaction to synthesize monoglyceride (MG) by hydrolysis of palm oil, which was reused for at least nine batches with yield of 32–50%.

Keywords: Lipase; Membrane reactor; Monoglyceride

1. Introduction

Lipase catalyzes the hydrolysis, esterification, acidolysis, alcoholysis and so on, which had found many applications in synthesis of some high value products, such as enantiomerically pure compounds and fine chemicals [1]. The microbial lipases have attracted considerable attention owing to their potentials, such as high production, good stability and many stero-specific properties [2]. The heterogeneous reaction systems, such as aqueous oil two-phase system were often used in lipase catalysis. To increase the interfacial area for contact of substrates, some surfactants or lipase–surfactant complex were used or a microemulsion system was introduced [3,4]. However, the result is far from satisfactory. In the recent decade, enzyme membrane reactor was introduced, which separates the aqueous and organic phases by an immobilized enzyme membrane, in which reaction is carried out. The advantages of membrane reactor are its integration catalytic conversion, product separation and catalyst recovery into a single operation. Synthetic hollow fiber membranes, such as polypropylene or PS hollow fiber membranes were often used in the membrane reactor and the immobilization of enzyme was obtained by an adsorption or cross-linking [5,6]. However, the capacity of enzyme-immobilized by adsorption is low, because the immobilization only takes place on the surface of the membrane.

Monoglyceride (MG) is one of the most important emulsifiers in food and pharmaceutical industries [7]. MG is produced, in the industry, by transesterification of fats and oils at high temperature with alkaline catalyst. The synthesis of MG by hydrolysis or glycerolysis of triglyceride (TG) with immobilized lipase attracted attention recently, because it has mild reaction conditions and avoids formation of side products. Silica and Celite are often used as immobilization

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carriers [8]. But the immobilized lipase particles are difficult to reuse due to adsorption of glycerol on this carriers [9]. Van der Padt et al. [10] reported esterification with cellulose hollow fiber membrane for synthesis of MG, in which the lipase was adsorbed at inner fiber side and low lipase capacity was obtained. Chitosan (CS) membranes have been used in pervaporation of ethanol due to its good mechanical property and high selectivity [11]. However, enzyme reaction by chitosan membrane has not been reported. A new membrane enzyme reactor by polyvinyl alcohol (PVA)/chitosan was prepared, in this paper, and was used for the synthesis of MG.

2. Materials and methods

2.1. Materials

Palm oil was bought from local market, provided by Malaysian Palm Oil. The lipase from *Rhizopus oryzae* (1100 IU/g) was provided by Prof. Xu Jiali, Institute of Microbiology (AS.3458), Chinese Academy of Science. Polyvinyl alcohol (1799) was produced from Beijing Chemicals Plant. Chitosan was prepared in our laboratory with 85% degree of deacetylation.

2.2. Analytical method

Lipase activity was measured by olive oil emulsion method [4]. One unit (IU) is defined as the amount of enzyme required to hydrolyze the olive oil to produce 1 μmol fatty acid at 37 °C/min.

The activity of immobilized lipase was measured as following. The lipase-immobilized membrane was cut into small pieces (about 1 mm in length and width) and the pieces were used as enzyme particle in activity measurement as before. The recovery of activity was defined as the percent of activity measured after immobilization with total lipase activity added in the immobilization.

The MG content was assayed by $NaIO_4$ titration method [12]. The yield of MG is defined as the ratio of MG produced (% (w/w)) to the maximum MG concentration (% (w/w)), if triglyceride (TG) in palm oil is converted completely to MG by sn-1,3 specific lipase-catalyzed hydrolysis, which is about 38.0% (w/w).

2.3. Preparation of lipase immobilization membrane

2.3.1. The chitosan lipase membrane preparation

A total of 0.5 g lipase powder was dissolved in 100 ml 1% chitosan solution (1% acetic acid as solvent) with a magnetic stirrer and the solution was poured onto a self-made square mold (2 mm (*h*) × 9 cm (*w*) × 15 cm (*l*)), which was prepared by fixing four stainless steel sheets (two sheets: 2 mm (*h*) × 2 mm (*w*) × 9 cm (*l*); two sheets: 2 mm (*h*) × 2 cm (*w*) × 15 mm (*l*)) on an horizontal glass plate. The flat membrane with a thickness of 100–200 μm was formed under vacuum (0.08 MPa) at 20–25 °C for about 10 h.

2.3.2. The preparation of PVA lipase membrane

Five milliliters of glycerin, 0.45 g of lipase powder and 2.0 ml of 25% (w/v) cross-linking agent (glutaraldehyde or epichlorohydrin) were added into 100 ml 5% PVA solution (de-ionized water as solvent). The mixture was homogenized under magnetic stirring and the solution was poured onto the self-made square mold (2 mm (*h*) × 9 cm (*w*) × 15 cm (*l*)) on a horizontal glass plate. The flat membrane with a thickness of 100–200 μm was formed under vacuum (0.08 MPa) for about 10 h.

2.3.3. The preparation of PVA/CS lipase membrane

One gram of chitosan was dissolved into 100 ml of 1% acetic acid solution. Twenty milliliters of the above solution was taken and added into 100 ml 5% PVA solution with a magnetic stirrer. A total of 0.5 g lipase and 2 ml 25%(w/v) cross-linking agent (glutaraldehyde or epichlorohydrin) were dissolved into the mixture above and the solution was then poured onto the self-made square mold (2 mm (*h*) × 9 cm (*w*) × 15 cm (*l*)) on a horizontal glass plate. The flat membrane with a thickness of 100–200 μm was formed under vacuum (0.08 MPa) for about 10 h.

2.4. Synthesis of MG by enzyme membrane reactor

The hydrolysis of triglyceride in palm oil for synthesis of MG is shown in Fig. 1. One hundred milliliters of 20 mmol/l phosphate (pH 9.0) buffer was circulated with a flow rate of 40 ml/min. One hundred milliliters of palm oil was used in batch mode with a flow rate

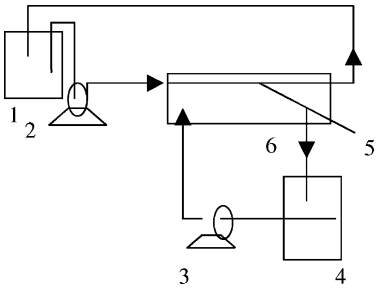

Fig. 1. Synthesis of MG with lipase membrane reactor. (1) Oil phase tank; (2 and 3) pump; (4) phosphate buffer tank; (5) membrane; (6) membrane reactor.

of 8 ml/min. The reaction was carried out at 40 °C between 2.5 and 5 h.

2.5. Immobilization of lipase by silica gel

The silica-immobilized lipase was made according to reference [9]. The crude lipase (0.5 g) was dissolved in 50 ml Tris–HCl buffer (50 mmol/l, pH 8.0). One gram of silica gel (40–60 μm) was added. The suspension was gently stirred at 25 °C for 5 h. The immobilized lipase was recovered by centrifugation at 3000 × *g* and was dried under vacuum (about 0.08 MPa) at 30–35 °C.

The free lipase reaction system and immobilized lipase reaction system consisted of 100 ml palm oil and 100 ml 20 mmol/l phosphate buffer (pH 9.0) as well as 0.5 g free lipase or immobilized lipase. The reaction mixture was stirred at 500 rpm and 40 °C for 2.5 h.

3. Results and discussion

3.1. Preparation of lipase membrane

Immobilization of lipase on the membranes by different membranes (CS, PVA, PVA/CS) was shown in Table 1. The PVA and CS membranes have lower mechanical strength compared with PVA/CS composite membrane, in which high recovery of lipase activity was also obtained. The optimum concentration of the enzyme used in formation of membrane is 0.42 g lipase in 100 ml membrane solution (Table 2). Further increasing the lipase amount does not enhance recovery of activity.

Table 1
Comparison of different membranes on immobilization

Membrane	CS	PVA	PVA/CS
Lipase-immobilized (IU/cm^2)	0.9	1.40	2.70
Recovery of activity (%)	10	12.4	24
Mechanic strength (MPa)	0.5	2.5	3.2

Twenty milliliters chitosan solution (1% w/v), 0.45 g lipase, pH 5.5, 10 ml 60 mmol/l $CaCl_2$, 5 ml 60 mmol/l $MgSO_4$.

The pH of the membrane solution also influenced the immobilization of lipase in membrane. When the pH was 5.6, which is near to p*K* of chitosan (p*K* is about 5.3), the high recovery for immobilization was obtained (Fig. 2).

The glutaraldehyde and epichlorohydrin are often used as cross-linking agents to eliminate leaking of enzyme incorporated. The highest lipase activity was obtained when the concentration of cross-linking agents was at 2.0% (w/v). Further increasing the concentration of glutaradehyde, lipase-immobilized would be reduced considerably. While for epichlorohydrin, the influence was not so significant (Fig. 3). The most likely reasons for these results are that the cross-linking by gluaradehyde takes place between the NH_2 groups of molecules of chitosan and lipase, which will denature the enzyme. While for epichlorohyrin, the –OH groups of molecules in chitosan or PVA were cross-linked, which could maintain high activity of the lipase.

Some metal ions, such as Ca^{2+} or Mg^{2+} were found to have the ability to stabilize lipase (Tables 2 and 3). Ten milliliters of 60 mmol/l $CaCl_2$ and 5 ml of 60 mmol/l $MgSO_4$ were used in 120 ml membrane solution till the high recovery of lipase activity reached.

3.2. Synthesis of MG by PVA/CS membrane reactor

Hydrolysis of triglyceride with PVA/CS membrane reactor depends on the pH and temperature.

Table 2
Effect of lipase concentration on immobilization on CS/PVA membrane

Lipase concentration (g/100 ml)	0.20	0.42	0.66
Lipase-immobilized (IU/cm^2)	1.16	2.24	2.01
Recovery of activity (%)	29.0	20.4	18.1

Twenty milliliters chitosan solution (1% w/v), pH 5.3, 10 ml 60 mmol/l $CaCl_2$, 5 ml 60 mmol/l $MgSO_4$.

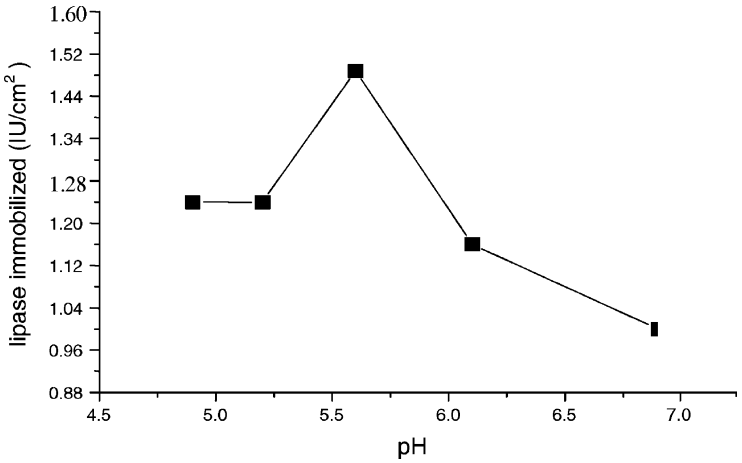

Fig. 2. Effect of pH on lipase immobilization on CS/PVA membrane; 20 ml chitosan solution (1% w/v), 0.40 g lipase, 2 ml 25% (w/v) epichlorohydrin.

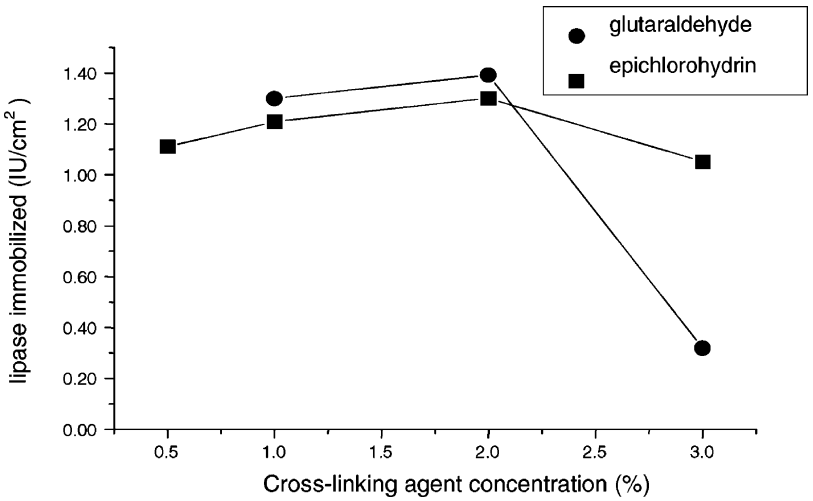

Fig. 3. Effect of cross-linking agent on lipase immobilization on CS/PVA membrane; 20 ml chitosan solution (1% w/v), 0.40 g lipase, pH 5.0.

Table 3
Effect of salt on lipase immobilization in CS/PVA membrane (120 ml membrane solution)

Metal ions	Lipase-immobilized (IU/cm^2)	Recovery of activity (%)
10 ml 60 mmol/l $CaCl_2$	1.12	11.2
10 ml 60 mmol/l $CaCl_2$, 1 ml 60 mmol/l $MgSO_4$	1.24	12.8
10 ml 60 mmol/l $CaCl_2$, 5 ml 60 mmol/l $MgSO_4$	2.40	24.0
5 ml 60 mmol/l $CaCl_2$, 5 ml 60 mmol/l $MgSO_4$, 5 ml 60 mmol/l K_2HPO_4	2.20	22.0

Lipase 2.7 g, pH 5.5.

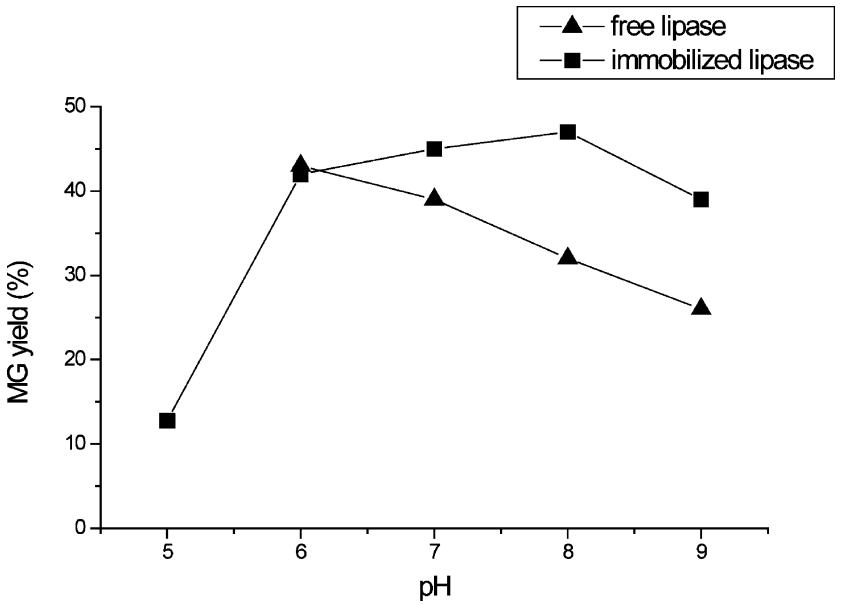

Fig. 4. Effect of pH on synthesis of MG by CS/PVA membrane; 100 ml palm oil, 100 ml phosphate (pH 9.0), reaction temperature 40 °C, reaction time 5 h.

The effect of pH in buffer for MG synthesis with free enzyme is not so considerable as that of the immobilized lipase (Fig. 4). When the pH is near the p*K* of chitosan (pH 5.4–5.6), low net charge on membrane makes the oil phase to pass through the pore of the membrane more easily, which benefits the reaction. The optimum temperature for MG synthesis was at 40 °C, which is same as that by the free enzyme.

The lipase from *R. oryzae* is sn-1,3 specific. If the hydrolysis of triglyceride is only catalyzed by the sn-1,3 specific lipase and 1 mol triglyceride is converted to 1 mol 2-MG, the maximum 2-MG concentration is about 38.0%. However, the yield of MG by the membrane reactor reached 40–52% (Fig. 4). This means that the MG concentration was during 15.2% (w/w) and 19.8% (w/w). The distributions of TG, diglyceride (DG) and MG in oil phase after reaction by HPLC are (% (w/w)): MG 15–20%; DG 15–22%; fat acids 13–18%; glycerol 6–10%; TG 23–30%. The amount of 1(3)-MG in the MG product is higher than 2-MG, indicating that the acyl migration increases conversion of DG to MG. The triglyceride is not directly converted to 2-MG by the enzymatic catalysis, in which 1,2- or 2,3-diglyceride formed by the sn-1,3 specific lipase has acyl migration to produce 1,3-diglyceride, and the 1,3-diglycerides are further hydrolyzed to MG and fatty acid as well as glycerol. The mechanism of hydrolysis can be shown in Fig. 5.

Comparison of the membrane reactor with silica-immobilized lipase particles for hydrolysis of palm oil is shown in Fig. 6. Silica gel particles with many hydrophilic groups adsorbed monoglycerol, which decreased diffusion of MG through pores and resulted in denature of the immobilized lipase. The lipase membrane reactor had high yield for MG after nine batches, which reveals that the side products and product MG do not adsorb on the chitosan membrane due to cross-flow of reactant and the MG produced is easily carried into palm oil phase. Holmberg and Osterberg [13] hydrolyzed palm oil by lipase from *Rhizopus delemar* in AOT micelles system and yield of MG was about 50–60%. However, the added surfactant made it difficult for further purification. The new PVA/CS enzyme membrane has high stability and it is an effective reactor for multi-phase reaction system.

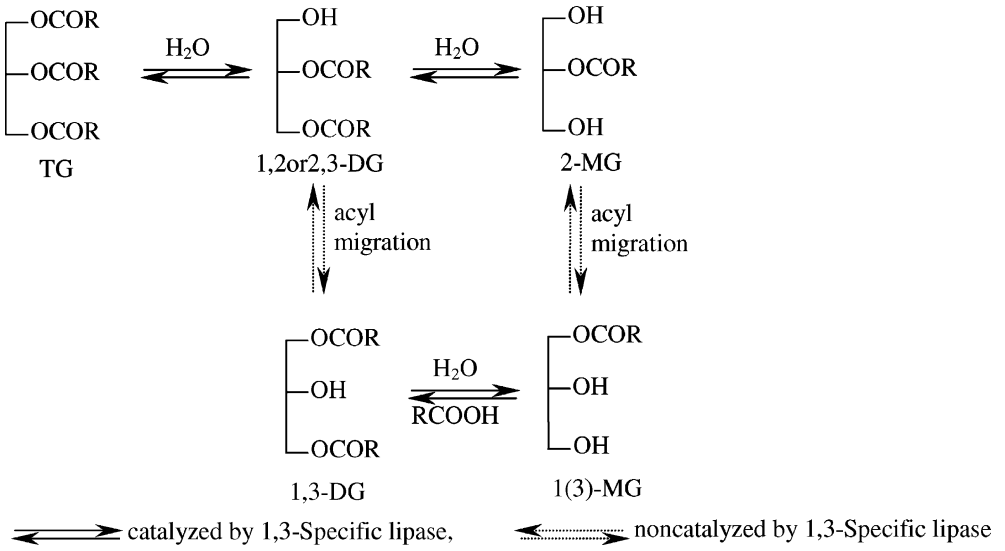

Fig. 5. The synthesis of MGs by sn-1,3 specific lipase-catalyzed hydrolysis.

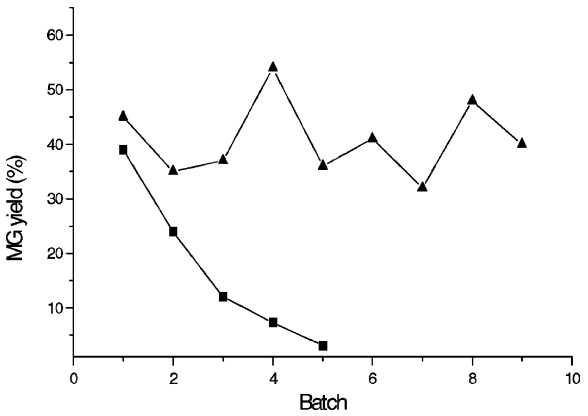

Fig. 6. Comparison of membrane reactor and silica-immobilized lipase for synthesis of MG silica-immobilized lipase (■); membrane reactor (▲); 100 ml palm oil, 100 ml phosphate (pH 9.0), reaction temperature 40 °C, for membrane reactor reaction time 5 h, for silica gel reactor reaction time 3 h.

4. Conclusion

The PVA/chitosan composite membrane is a new membrane reactor which can be used for enzymatic processing of fats and oils. The synthesis of monoglyceride with high yield by the membrane reactor reveals that the new PVA/CS enzyme membrane reactor is an effective reactor for multi-phase reaction system. When the membrane reactor was used for hydrolysis of palm oil, the yield of MG reached 35–52%.

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