Wax Ester Synthesis in a Membrane Reactor with Lipase–Surfactant Complex in Hexane

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ABSTRACT: A lipase–surfactant complex (LSC) was prepared by mixing an aqueous solution of lipase (E.C. 3.1.1.3) derived from *Pseudomonas* sp. with an alcoholic solution of sorbitan monostearate. This LSC was applied to the catalysis of esterification of cetyl alcohol and palmitic acid to synthesize wax ester in hexane. A membrane reactor system was investigated for the repeated batch synthesis of wax ester with LSC. High conversions were maintained during repeated experiments; however, the flux decreased during the run. Sponge washing of the membrane restored the flux to 70% of its original value.
JAOCS 72, 887–890 (1995).

KEY WORDS: Bioreactor, esterification, flux, lipase–surfactant complex, membrane, wax ester.

Wax esters, consisting of higher alcohols and higher fatty acids, may be obtained from such natural sources as jojoba seed (*Simmondsia chinensis*), carnauba (*Copernicia cerifera*), and sperm whale. These wax esters have potential applications as premium lubricants, parting agents, and antifoaming agents, as well as in cosmetics (1). Currently, wax esters are manufactured by reacting an alcohol and a fatty acid at high temperature, up to 250°C, in the presence of tin, titanium, or sulfuric acid catalysts for up to 20 h. This process has some disadvantages in that the high temperature and long reaction time can lead to degradation of the ester, and it also has a high energy cost (2). By contrast, enzymatic reaction processes have both economic and ecological advantages because of moderate temperature, pH, and other mild conditions. Enzymes have been used mainly in aqueous systems; however, various enzymes have shown catalytic activity in organic media. Under high water concentration in the reaction mixture, the enzymatic reaction equilibrium shifts toward hydrolysis. Enzymatic reaction in organic solvent that contains a small amount of water can shift the equilibrium toward synthesis. Enzyme reaction in organic media is recognized as an effective system for ester synthesis (3–5). Several attempts to prepare substitutes for premium wax esters by use of enzymes have been reported (6–8). However, there are few examples of industrial applications involving ester synthesis in organic media, probably because of low enzyme activity.

Enzyme activity is highly dependent on the organic solvent, and in many cases the activity decreases radically in organic solvents (9). Moreover, it may be necessary to improve the solubility or affinity of an enzyme for organic solvent, because the enzyme surface is usually hydrophilic. To solve these problems, several approaches have been studied (10–14). Okahata and Ijiro (15,16) have reported a lipase surfactant complex (LSC). The LSC was prepared with crude enzyme and synthetic surfactant, and the enzyme surface was modified to obtain hydrophobicity. Consequently, the LSC exhibited good solubility, dispersibility, and activity in organic media. We already have reported an improved method for making the LSC (17) and the availability of the LSC for wax ester synthesis in hexane (18). Cetyl palmitate, which is known as a principal ingredient of whale oil (1), was the object wax ester in this investigation. As the supply of natural whale oil has been insecure, a substitute of this wax ester has been desired.

Although many experiments have been done with membrane bioreactors, there is limited information available on membrane enzyme reactors for organic reaction systems (19,20). The aims of the present study were to determine LSC stability in repeated batch reactions in organic solvent and the feasibility of the membrane reactor system for the enzymatic synthesis of wax ester in organic solvent.

MATERIALS AND METHODS

Enzyme and chemicals. Lipase MF-30 from *Pseudomonas* sp. was provided by Amano Pharmaceutical Co., Ltd. (Nagoya, Japan) and used without further purification. The surfactant sorbitan monostearate (Nonion SP-60R) was kindly supplied by NOF Corporation (Tokyo, Japan). Cetyl alcohol, palmitic acid, and hexane were purchased as reagent grades from Wako Pure Chemical Co., Ltd. (Osaka, Japan). All other chemicals used were of reagent grade.

Preparation of LSC. A method for preparing modified LSC was reported in our previous paper (17). An aqueous solution (1000 mL, 0.05 N acetic buffer, pH 5.6) of lipase (1 g) and ethanol solution (2 mL) of surfactant (500 mg) were mixed and stirred with a magnetic stirrer (500 rpm) at 5°C for

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lyophilized. The LSC white powder was stored under refrigeration. Protein content of the LSC was determined with an automatic total nitrogen analyzer (Model FP-428; LECO Corp., St. Joseph, MI).

Membrane reactor system. Polyimide complex flat sheet membrane (NTU-4208, molecular weight cut-off 8000; Nitto Denko Corp., Osaka, Japan) was used. The membrane was held in a stirred cell (UHP-62K, filter size 62 mm, total membrane area 27 cm^2, total volume capacity 200 cm^3; Advantec Toyo, Tokyo, Japan). A schematic illustration of the system is shown in Figure 1. The membrane was washed sequentially with water, ethanol, and hexane before each experiment.

Esterification activity. The batch reaction was carried out in the membrane reactor with 25 mM cetyl alcohol, 25 mM palmitic acid, 40 mg of the LSC, and 25 mL hexane. The reaction mixture was stirred at 500 rpm, and the temperature was kept at 50°C by means of a coil heater. After a 30-min reaction, the solution was filtered by supplying pressure with nitrogen gas, and the permeate flux was measured. The composition of the permeate was analyzed by gas chromatography to estimate the conversion. Repeated batch reactions were performed as follows. After filtration of the reaction mixture in the first run, fresh substrate solution was added to the reactor, and the second reaction was started. The operation was repeated ten times. The permeate flux and the composition of permeate were measured for each run. The membrane surface was washed with a sponge and water after the last run. The permeate flux was determined after washing.

Gas chromatography. Concentrations of fatty acid and wax ester were measured by gas–liquid chromatography (model GC-6A; Shimadzu Corp., Kyoto, Japan) with flame-ionization detection and Silicon OV-1 (GL Sciences, Tokyo, Japan) in a 3 mm × 0.5 m glass column. The conditions were: injection temperature, 250°C; oven temperature, initial 110°C, 7°C/min up to 250°C; carrier, N_2; and flow, 5.0 mL/min.

RESULTS AND DISCUSSION

Figure 2 shows a typical time course of the esterification of cetyl alcohol and palmitic acid. Product concentration increased with the decrease of substrate. High conversion was obtained with the LSC. About 90% conversion was achieved after about 30 min, and 96% was the maximum after 60 min. The initial esterification activity was estimated to be 1875 mmol/(g LSC • min). Because the protein content of the LSC was 5.06%, the initial activity per unit of protein was 37.1 mmol/(mg protein • min). The LSC was found to have high esterification activity compared to other reports in the literature (Table 1). This result is probably due to the good dispersibility of the LSC in hexane because of the hydrophobic modification of the enzyme surface by the surfactant. Moreover, the contact probability between LSC and hydrophobic substrates (cetyl alcohol and palmitic acid) may have been improved by the modification.

In the repeated batch reaction, the conversion for each batch is shown in Figure 3. High conversions (above 80%) were maintained during the repeated operation, and the average conversion was 87.7%. The activity of the LSC was stable, and enzyme deactivation did not occur in this experimental period. Additionally, permeation of the LSC was not observed. The ultrafiltration membrane used in the present study was suitable to retain the LSC in the reaction system.

The permeate flux of the reaction mixture for each run is shown in Figure 4. The permeate flux decreased from 34 L/(m^2 • h • kg/m^2) in run 1, to 10 L/(m^2 • h • kg/m^2) in run 5. After run 5, the permeate flux remained almost steady around 10 L/(m^2 • h • kg/cm^2). A sponge washing was attempted after run 10 to test flux recovery. The washing operation gave about 70% flux restoration. This result revealed that the flux decrease was mainly caused by fouling. We also confirmed

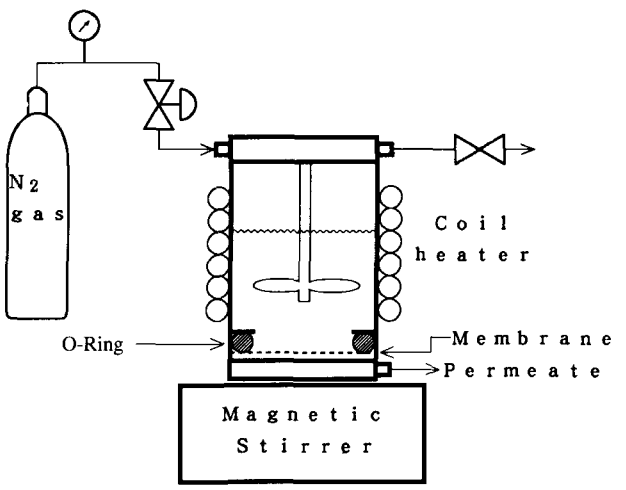

FIG. 1. A schematic illustration of the membrane reactor system for the repeated batch esterification. Membrane: NTU-4208 (polyimide, molecular weight cut off 8000; Nitto Denko Corporation, Osaka, Japan). Reactor: UHP-62K (total membrane area 27 cm^2, total volume capacity 200 cm^3; Advantec Toyo, Tokyo, Japan).

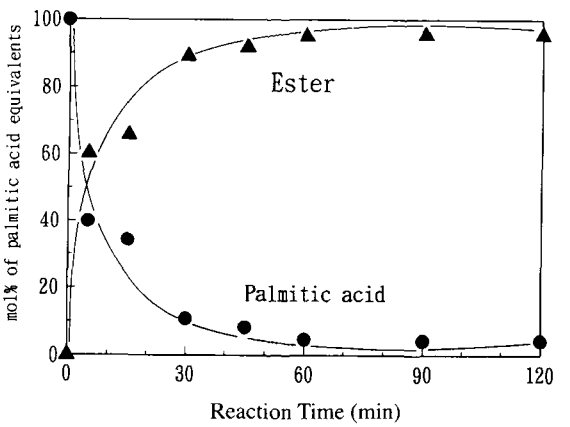

FIG. 2. Time course of the esterification of palmitic acid (●) and cetyl alcohol (▲). The reaction was carried out with 25 mM cetyl alcohol, 25 mM palmitic acid, and 40 mg of lipase–surfactant complex at 500 rpm, 50°C.

TABLE 1
Comparison of Biocatalysis Activities on Wax Esterification[a]

Reactants: alcohol + fatty acid	Biocatalyst	Solvent	Activity per biocatalyst[b]	Activity per protein[c]	Reference
8:0 + 18:1	Fungal cell	Water	5.9		2
12:0 + 12:0	PEG–lipase	TEC		28.4	7
22:1 + HEAR	IER–lipase	No solvent	90.8		8
12:0 + 16:0	PEG–lipase	Benzene		25.0	21
8:0 + 18:1	IER–lipase	No solvent	1270		22
16:0 + 16:0	LSC	Hexane	1875	37.1	This work

[a]PEG, polyethylene glycol; TCE, 1,1,1-trichloroethane; HEAR, high-erucic acid rapeseed oil; IER, ion exchange resin; LSC, lipase–surfactant complex.
[b]μmol/(min • g – biocatalyst).
[c]μmol/(min • mg – protein).

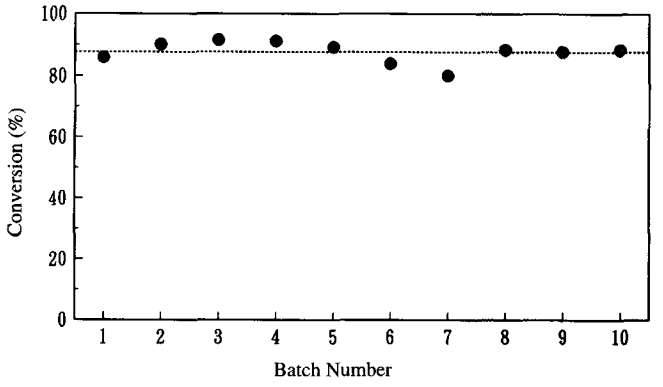

FIG. 3. Conversion in the membrane reactor of each repeated batch operation. Each run was carried out with 25 mM cetyl alcohol, 25 mM palmitic acid, and 40 mg of lipase–surfactant complex at 500 rpm, 50°C for 30 min. After the reaction, the solution was filtered by applying pressure with nitrogen gas.

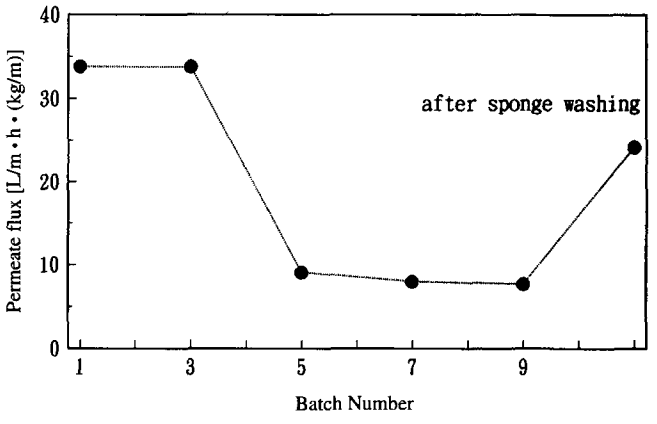

FIG. 4. The permeate flux of the reaction mixture in the repeated batch run. The membrane surface was washed with a sponge and water after the last run.

that the substrates and products were not rejected by the membrane. The LSC seems to be the main substance of fouling.

ACKNOWLEDGMENTS

The authors are grateful to Amano Pharmaceutical Co., Ltd. for providing crude lipase, to NOF Corporation for supplying surfactants, and to Nitto Denko Corporation for supplying the membrane. We also thank Dr. J.B. Snape for his valuable comments in the preparation of the manuscript. This work was partly supported by Bio-Renaissance of the Ministry of Agriculture, Forestry and Fisheries (BRP 94-I-A).

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[Received November 8, 1994; accepted April 18, 1995]