Evaluation of Immobilized Modified Lipase: Aqueous Preparation and Reaction Studies in *n*-Hexane

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ABSTRACT: Lipase Saiken 100 (*Rhizopus japonicus*) and its immobilized form displayed very poor activity (hydrolysis and interesterification) in microaqueous *n*-hexane solutions. Enzyme modification by the addition of stearic acid or sorbitan monostearate significantly improved activity. A ceramic carrier (SM-10) was used to immobilize modified lipase Saiken (stearic acid, sorbitan monostearate, and lecithin) and was found to further enhance hydrolysis and interesterification rates in *n*-hexane. In addition, the biocatalysts were re-used for four consecutive batch reactions with no significant shortfall in activity. Reaction rates were also greatly affected by the total reaction water content. Careful control of the biocatalyst water content prior to use and additional reaction water were required to optimize activity and minimize hydrolytic diglyceride byproducts. Hydrolysis and interesterification reaction rates were favored with immobilized biocatalyst water contents of 6.25 and 0.43 wt% with additional reaction water contents of 600 and 20 mg/L, respectively.

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KEY WORDS: Fats and oils, *n*-hexane, hydrolysis, immobilized lipases, interesterification, modification, stearic acid.

Recent attention has focused on using lipases for the conversion of fats and oils to produce high-value products (1,2). Lipases typically show little or no activity in organic solvents, hence, methods to improve both activity and stability are of particular importance (3,4). Enzyme coating by oil-soluble surfactants and lipids has greatly enhanced activity in organic media (5–9). However, surfactant or lipid modification also improves biocatalyst dispersibility in *n*-hexane, making it increasingly difficult to separate products from the enzyme and modifying agent. In addition, the cost of producing lipases necessary to catalyze such reactions as hydrolysis and interesterification is often prohibitive (10). The immobilization of low-cost modified lipases on cheap supports offers attractive possibilities for easy biocatalyst separation and re-use, and thus for extending their practical use.

The role water plays in enzyme organic catalysis has also been the topic of much interest in recent years. Pioneering works by Zaks and Klibanov established that enzymes active in organic media require a certain amount of water to confer the conformational flexibility necessary for catalysis (11–13). Other studies have substantiated the critical role of water in determining lipase activity (14–18) and the effect of solvent type on biocatalysis (19–24).

This paper evaluated preparation conditions for immobilized modified lipase (IML), where stearic acid (SA) and a ceramic carrier (SM-10) were used for modification and immobilization, with regard to hydrolysis and interesterification activity in microaqueous *n*-hexane solutions. Activity was compared to crude lipase, modified lipase (ML) and immobilized lipase (IL). Enzyme modification using sorbitan monostearate (SM) and soybean lecithin (LEC) was also discussed together with IML performance during repeated re-use over consecutive batch reactions. Reaction water content was also investigated with studies on the effect of adding water to the reaction system and the role biocatalyst water content had on enzyme activity.

MATERIALS AND METHODS

All chemicals were supplied by Wako Pure Chemicals Ltd. (Tokyo, Japan) except for the following: crude lipase Saiken 100 (*Rhizopus japonicus*) was obtained from Nagase Biochemicals (Kyoto, Japan). The enzyme contained 11.2 wt% protein, 75 wt% lactose as a stabilizer, and the rest was non protein nitrogenous compounds. All mono-, di-, and triglycerides were purchased from Sigma (St. Louis, MO). Sorbitan monostearate was obtained from Kao Chemicals (Tokyo, Japan), and SM-10 (particle diameter 160 μm) was kindly donated by NGK (Nihon Gaishi) (Nagoya, Japan). SM-10 is a porous ceramic comprising SiO_2 (66%), MgO (30%), Al_2O_3 (2%), and CaO (1%). Before use *n*-hexane was dried over molecular sieves (200 g molecular sieves 4 A/(3 L *n*-hexane) to give a water concentration of 10 mg/L.

Analytical methods. The course of hydrolysis and interesterification reactions was followed by determining the concentration of substrates and products with time. Samples (0.4 mL) were removed from the reaction media, filtered (0.5 μm, Millipore, Milford, MA) and analyzed by thin-layer chromatography with a flame-ionization detector (TLC/FID) and gas chromatography (GC). The hydrolysis of tripalmitin (PPP) yields diglycerides, monoglycerides, and free fatty acids (FFA), which were detected using TLC/FID (Iatroscan MK-5, Iatron Laboratories, (Tokyo,

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Japan). Chromarod S III quartz rods used were coated with silica and impregnated with 3% boric acid (25). Media reaction samples (1 μL) were applied to the rods and developed in chloroform/NH_4OH/methanol with a volume ratio of 20:79.83:0.17. After approximately 40 min the rods were dried and scanned at 25 cm/min. Interesterification (acidolysis) involves the reaction between PPP and SA to form triglycerides and FFA, which were detected by GC analysis as previously described (5). A sample volume of 200 μL was added to an equal volume of chloroform, and the solvent was evaporated using nitrogen which was blown over the sample while it was held at 60°C.

Protein contents were determined using the Lowry *et al.* assay (26). Insoluble protein was determined using the modified Bradford assay (27).

Water content assay. Water content in the reaction system was measured with a Karl Fischer titrator 684 KF (Coulometer, Metrohm, Herisau, Switzerland).

Biocatalyst preparation. For preparation A (Table 1), lipase was added to Tris buffer (pH 5) and mixed for 5 min, followed by the dropwise addition of lipid dissolved in 0.6 mL ethanol (40°C). For preparation B, lipase was added to SM-10 in water (pH 7). For preparation C1, lipase was added to water, followed by the addition of lipid and SM-10 while the solution was continually stirred. For preparation C2, SA was added to *n*-hexane at 40°C, followed by the addition of SM-10 and lipase. After stirring overnight, the solution was allowed to stand for 30 min and *n*-hexane was decanted off. The precipitate was dried at 35°C for 4 h and recovered. For preparation C3, SA was added to 1 g SM-10 in water and stirred for 12 h at 5°C. The precipitate was recovered and re-wetted in 3 mL of water and 90 mg lipase was added. All preparations were sonicated for 15 min after the final addition and stirred (600 rpm) for 12 h at 5°C. With the exception of C2, the precipitates were recovered by centrifugation ($7000 \times g$; 10 min), dried under vacuum for 8 h, and stored overnight over silica gel at room temperature. The preparations were kept at 5°C until used. All immobilized precipitates (300 mg) were washed with 10 mL of distilled water (pH 7) for 3 h to determine the amount of unbound enzyme trapped between or precipitated onto SM-10 particles after recovery by centrifugation. Lipase Saiken was previously shown to have 1,3-positional specificity (5).

Reaction conditions. The hydrolysis reaction was between 121 mg PPP (6 mmol/L) and additional water volume of 10 μL water (400 mg/L). Interesterification was carried out between 250 mg PPP (12.4 mmol/L) and 250 mg SA (35 mmol/L) with 0.5 μL additional water (20 mg/L). The reactions were initiated by the addition of 300 mg immobilized biocatalyst or 20 mg crude lipase to each flask containing 25 mL dry *n*-hexane, substrates, and additional water. The flasks were held in a water bath at 40°C and magnetically stirred at 600 rpm. Hydrolysis and interesterification activities are expressed as mmol FFA and 1,2-dipalmitoyl-3-stearoyl glycerol (PPS) per g initial protein h.

RESULTS AND DISCUSSION

Reaction kinetics. Figure 1 shows the hydrolysis reaction of PPP using IML (preparation C1) in *n*-hexane. A 10 μL (400 mg/L) volume of distilled water (pH 7) was added to dry *n*-hexane (water content, 10 mg/L) at the reaction start. After 2 h or so there was little further depletion of PPP and the diglyceride concentration had peaked. After 7 h, 62% of the PPP had been hydrolyzed. The FFA concentration increased for about 7 h and remained constant thereafter. Small traces of monoglycerides were also monitored after 24 h (not shown). After an initial increase (after PPP addition), the *n*-hexane water content decreased from around 20 mg/L (after 30 min) to 11.5 mg/L (7 h). Activity of 10.8 mmol FFA/(g · h) was recorded after 4 h. Figure 2 depicts the interesterification reaction between PPP and SA producing PPS and SPS (1,3-stearoyl-2-palmitoyl glycerol). A water volume of 0.5 μL (20 mg/L) was added to dry *n*-hexane at the reaction start. The reaction had reached equilibrium after 10 h. Activity rate of 13.6 mmol PPS/(g · h) was determined after 4 h. The total diglyceride concentration did not exceed 6 wt% of the initial PPP concentration. The water content during the reaction remained more or less constant at around 15 mg/L with a slight reduction to 12 mg/L after 7 h.

Comparison of immobilization preparation methods on activity. Crude lipase Saiken 100 has very poor hydrolysis and no interesterification activity in *n*-hexane (Table 2). Addition of SM or LEC to *n*-hexane at the start of the reactions aided visible enzyme dispersbility but had no improved effect on activity (results not shown). Lipase immobilization by SM-

TABLE 1
Lipase Modification and Immobilization

	Modified lipase (ML)	Immobilized Lipase (IL)	Immobilized modified lipase (IML)		
Preparation	A	B	C1	C2	C3
Lipase[a] (mg)	90	90	90	90	90
Modifying lipid (50 mg)	SA/SM	—	SA/SM/LEC	SA	SA
SM-10 (g)	—	1	1	1	1
Liquid phase	Buffer	Water	Water	*n*-Hexane	Water
volume (mL)	30	3	3	3	3
Water content dried Ppt (wt%)	5	4	6	0.1	6

[a]Lipase (Saiken 100), *n*-hexane (10 mg water/L), buffer (5 mmol/L tris (hydroxymethyl) aminomethane, pH 5), water (distilled, pH 7). Abbreviations: stearic acid, SA; sorbitan monostearate, SM; lecithin, LEC; precipitate, Ppt.

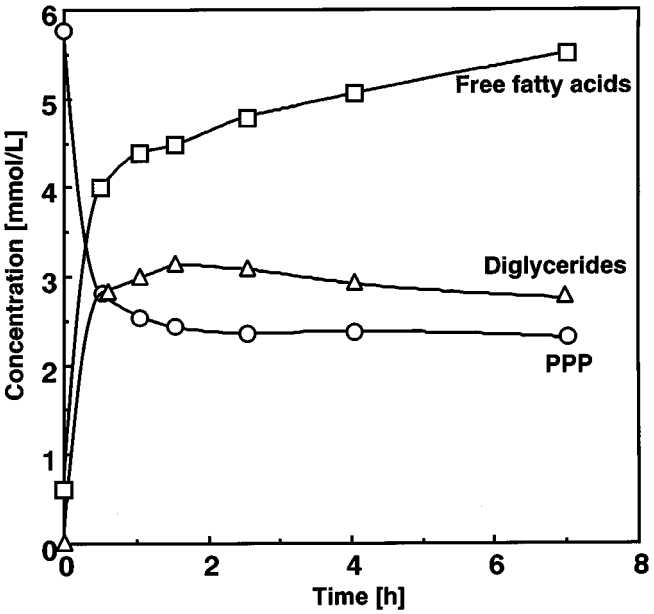

FIG. 1. Time course for the production (mmol/L) of diglycerides and free fatty acids (FFA) during the hydrolysis of 6 mmol/L tripalmitin (PPP). The reaction was carried out in 25 mL of *n*-hexane containing 10 µL (400 mg/L) water and 300 mg immobilized modified lipase (IML, preparation C1) at 40°C.

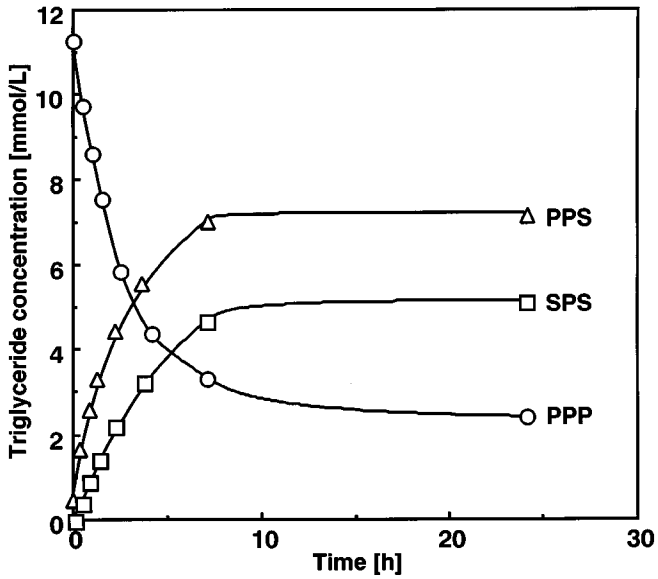

FIG. 2. Time course for the production of 1,2-dipalmitoyl-3-stearoyl glycerol (PPS) and 1,3-distearoyl-2-palmitoyl glycerol (SPS) as a result of interesterification between 12.4 mmol/L PPP and steric acid (SA) (35 mmol/L). The reaction was carried out in 25 mL *n*-hexane containing 0.5 mL (20 mg/L) water and 300 mg of IML (preparation C1) at 40°C. For other abbreviations see Figure 1.

10 (preparation B) recovered 51% of the initial protein after centrifugation. However, after washing with distilled water (pH 7), 69% of the recovered protein was lost, indicating that most of the enzyme was simply dried or precipitated onto the SM-10 particles. As a result, IL by preparation B also had very poor hydrolysis and interesterification activities (Table 2). Likewise, no improvement in hydrolysis activity was recorded by the addition of SA to the reaction mixture containing preparation B (prewashing), indicating that no enzyme modification occurred during the course of the reaction (5).

IML by preparation C1 (SA modified and immobilized together) showed good hydrolysis and excellent interesterification activity, reflected in part by the high amount of protein recovered. Preparation C2, prepared in *n*-hexane (dried over molecular sieves) instead of water (C1), displayed good hydrolysis but poor interesterification activity, perhaps owing to the low water content of the preparation solvent, which was found to be especially critical with interesterification. IML by preparation C3 (SM-10 modification followed by lipase Saiken addition) showed good overall activity. With preparation C3, any excess SA was effectively removed following precipitate recovery by centrifugation, indicating fatty acid interaction between the subsequent addition of lipase and SM-10 carrier. IML by preparation C1 displayed the best activity for hydrolysis and interesterification, and this preparation method was used to obtain IML using SA, SM, and LEC for all subsequent experiments.

Figure 3 shows the effect of SM-10 loading on protein recovery after 1 h from various lipase solutions (3 mL). Protein was recovered from lipase (90 mg) solutions of distilled water (pH 7) with and without SA (50 mg), and from 5 mmol/L Tris

TABLE 2
Protein Recovery (%), Protein Content (wt%), Precipitate Weight (Ppt wt), and Activity [mmol/(g · h)] with Crude, Immobilized Lipase (IL), and Immobilized Modified Lipase (IML) Preparations

				Hydrolysis	Interesterification	
Preparation	Protein recov (%)	Protein cont (wt%)	Ppt wt (g)	Activity mmol FFA/(g · h)	Activity mmol PPS/(g · h)	%Conv 4 (24) h
Crude Saiken	—	11.2	20 mg*	0.6	0	0 (0)
IL Preparation B[a]	51	0.50	1.01	1.1	0.18	0.5 (1.0)
IML Preparation C1[b]	71	0.68	1.05	9.2	16.7	89 (100)
IML Preparation C2[c]	—	—	1.14	11.7	0.16	0 (1.4)
IML Preparation C3[d]	52	0.54	0.96	7.2	10.1	76 (96)

[a]Preparation B, IL.
[b]Preparation C1, lipase modified and immobilized together.
[c]Preparation C2, lipase modified and immobilized in *n*-hexane.
[d]Preparation C3, lipase added to modified immobilized carrier. All modification was undertaken using 50 mg SA and immobilization with 1 g SM-10 (NGK, Nagoya, Japan). Abbreviations: FFA, free fatty acids; PPS, 1,2-dipalmitoyl-3-stearoyl glycerol; *, no ppt; % Conv. conversion percentage; 4 (2H) h, conversion at 4 and 24 h.For other abbreviations see Table 1.

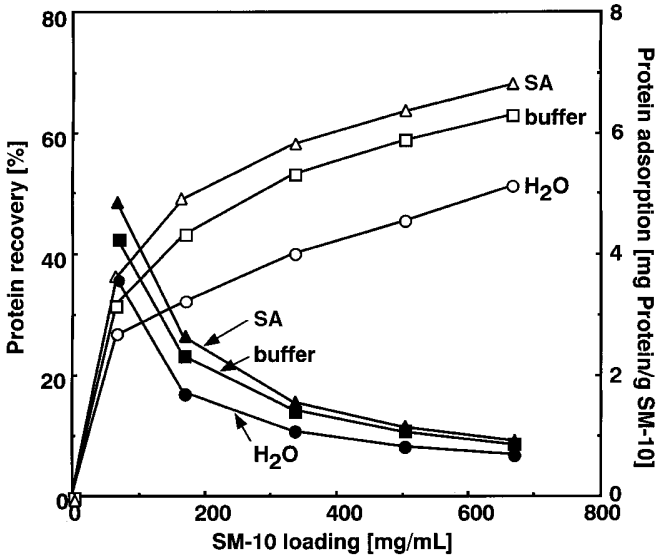

FIG. 3. The effect of SM-10 loading (mg/mL) on protein recovery (%) and protein adsorption (mg protein/g SM-10) after 1 h from lipase solutions (3 mL). Protein was recovered from lipase (90 mg) solutions of distilled water, pH 7 with 50 mg SA, and from 5 mmol/L Tris buffer, pH 5. Open and closed symbols indicate protein recovery and protein adsorption, respectively. For abbreviation and for manufacturer see Figures 1 and 2, respectively.

buffer (pH 5). Despite improved recovery with higher SM-10 loading, mg immobilized protein per g SM-10 decreased rapidly with increasing SM-10 additions (Fig. 3). The solution containing SA had the highest adsorption profile, followed by lipase buffer, which in turn was higher than the lipase water solution (circles). These data support previous studies (8,29) where protein recovery was favored with acidic Tris buffer (5 mmol/L) and by the presence of SA. SA therefore promotes the recovery of protein during ML preparation, which implies that fatty acid and ionic interactions are important for the binding of enzyme and carrier over the range of loading concentrations tested.

Comparison of lipase modification, before and after immobilization, on activity. Lipase Saiken 100 was modified with SM and SA previously described (preparation A). The modified lipases (ML) were then compared with their IML forms (preparation C1) for hydrolysis and interesterification activities. LEC did not form a precipitate with lipase during modification, however, the modified lipase was recovered by the subsequent addition of SM-10 carrier.

Lipase activity was greatly enhanced by enzyme modification with SM and SA. This increase may be due to improved biocatalyst dispersibility (5), water retention (8,28) and selective protein binding (29). SM- and SA-modified preparations were better for hydrolysis and interesterification, respectively, the latter activity reflecting the amount of protein recovered (Table 3). When activities of ML were compared with their IML forms it was clear that immobilization further enhanced activity in *n*-hexane, which may be attributed to improved protein recovery, water retention, and biocatalyst dispersibility. Immobilization of SM and SA ML improved hydrolysis activity by 31 and 63%, and interesterification activity by 68 and 27%, respectively (Table 2). LEC IML also displayed good activity, although SM IML showed the best overall activity (Table 2).

After immobilization, IML precipitates were washed with distilled water (pH 7) for 3 h, recovered, and activities redetermined. After washing, the immobilized preparations of SM, SA, and LEC lost 43, 34, and 48% of their protein contents, respectively. However, after washing there was very little shortfall in their respective hydrolysis or interesterification activities. In the case of SA IML, hydrolysis activity decreased from 10.1 to 9.9 mmol FFA/(g · h) while interesterification increased from 16.0 to 17.3 mmol PPS/(g · h). From these, mainly nonreactive free lipase seems to be removed by aqueous washing and there was therefore little reduction in activity. This indicates that only SM-10 bound lipase was active after lipid modification.

Biocatalyst re-use. IML stability was assessed by repeated re-use over five consecutive batch reactions. Upon completion (4 h) the reaction mixture was allowed to settle (1 h) and the majority of *n*-hexane was carefully decanted off. The precipitate was then dried under vacuum for 30 or 120 min, or by evaporation at room temperature. After each reaction, fresh media and water were added and the biocatalyst re-used for hydrolysis and interesterification reactions.

Figure 4 depicts hydrolysis and interesterification activity for SA IML over four continuous batch reactions (4 h) with the biocatalyst recovered by vacuum drying (120 min). Hydrolysis activity remained more or less constant over the first four reactions (Fig. 4). However, interesterification activity was greatly

TABLE 3
Effect of Lipase Modification and Immobilization on Protein Recovery (%), Protein Content (wt%), Precipitate Weight (Ppt wt), and Activity [mmol/(g · h)]

				Hydrolysis	Interesterification	
Preparation	Protein recov (%)	Protein cont (wt%)	Ppt wt (g)	Activity mmol FFA/(g · h)	Activity mmol PPS/(g · h)	%Conv 4 (24) h
SM ML[a]	15	4.44	0.034	5.8	6.7	61 (83)
SA ML	29	7.30	0.040	3.6	12.2	90 (100)
SM ML[b]	53	0.52	1.02	10.0	21.3	92 (100)
SA IML	58	0.57	1.03	9.5	16.7	89 (100)
LEC IML	31	0.30	1.04	9.3	19.3	89 (100)

[a]Modification (ML) was undertaken with 50 mg of SM, SA, and LEC.
[b]Immobilization (IML) was undertaken with 1 g SM-10 (preparation C1). For abbreviations and for manufacturer see Tables 1 and 2, respectively.

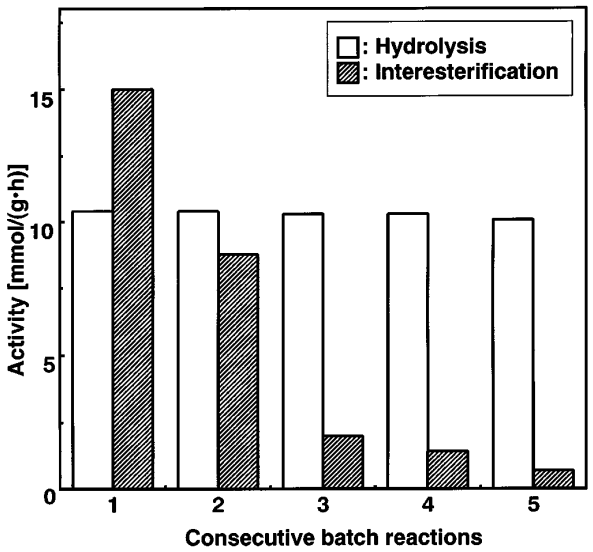

FIG. 4. Hydrolysis [light shade; mmol FFA/(g · h)] and interesterification activities (dark shade; mmol PPS/(g·h)) using SA IML after 4 h with continuous batch reactions (1–4). For reaction 5 the interesterification and hydrolysis media were switched. All precipitates were recovered by allowing reaction mixture to settle for 1 h (room temperature), decanting the solvent, and vacuum drying (120 min). PPS, 1,2-dipalmitoyl-3-stearoyl glycerol; for other abbreviations see Figures 1 and 2.

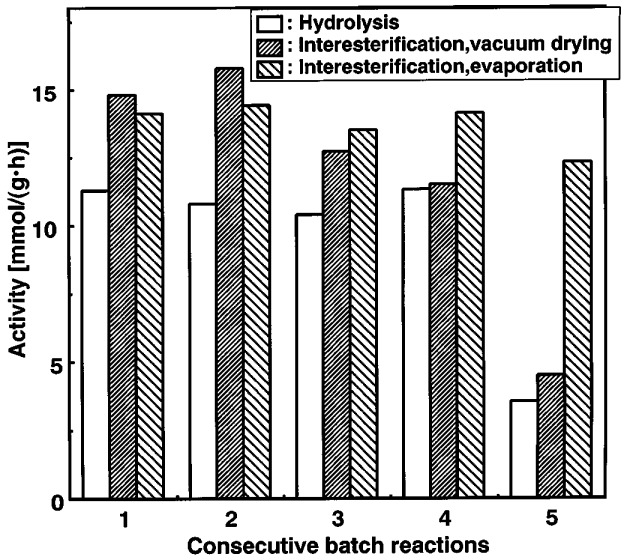

FIG. 5. Hydrolysis [mmol FFA/(g·h)] and interesterification activities [mmol PPS/(g · h)] using SA IML after 4 h with continuous batch reactions (1-4). For reaction 5 no water was added to the reaction media. The effects of vacuum drying for 30 min and evaporation at room temperature are compared. The hydrolysis data are from vacuum drying (30 min). All precipitates were recovered by allowing the reaction mixture to settle for 1 h (room temperature), decanting the solvent, and vacuum drying (120 min). For abbreviations see Figures 1, 2, and 4.

reduced after the first reaction and decreased thereafter. After the fourth reaction, the media were switched such that the IML used for hydrolysis was used for interesterification and vice-versa. The IML used for hydrolysis had very poor interesterification activity while the IML used for interesterification had good hydrolysis activity (Fig. 4).

No apparent loss of SA or lipase from the carrier was found in subsequent tests. The loss in interesterification activity may be primarily due to water stripping from the biocatalyst during vacuum drying. Evaporation at room temperature and shorter periods of vacuum drying (30 min) were subsequently investigated over four batch reactions, with interesterification activity maintained using these less "harsh" methods of drying (Fig. 5). A further fifth reaction was undertaken with no addition of water to the reaction media. For the first four reactions, interesterification activities after natural drying and vacuum drying (30 min) were similar, however, activity was more significantly reduced with vacuum drying after the fifth reaction.

Interesterification requires a low additional water content (20 mg/L), therefore, a small amount of water stripping from the biocatalyst, or more specifically the active site, will greatly affect activity. In contrast, hydrolysis favors high water contents (400 mg/L) and therefore any water stripped off by vacuum drying will have less effect on activity.

SA, SM and LEC IML retained hydrolysis and activity over four batch reactions under normal conditions, however, as exemplified by reaction five, care must be taken not to strip water off during biocatalyst recovery.

Effect of additional water. This experiment assessed the effect water had on hydrolysis and interesterification activities when added to dry *n*-hexane (10 mg/L) at the reaction start. For hydrolysis, additional water content from 0 to 1000 mg/L were tested. Activity [mmol FFA/(g · h)] increased with water additions up to 600 mg/L and then decreased (Fig. 6). This may be attributed to poor biocatalyst dispersibility as observed with the highest water addition (1000 mg/L). For interesterification, additional water contents from 0 to 200 mg/L were employed (Fig. 7). Activity [mmol PPS/(g · h)] in-

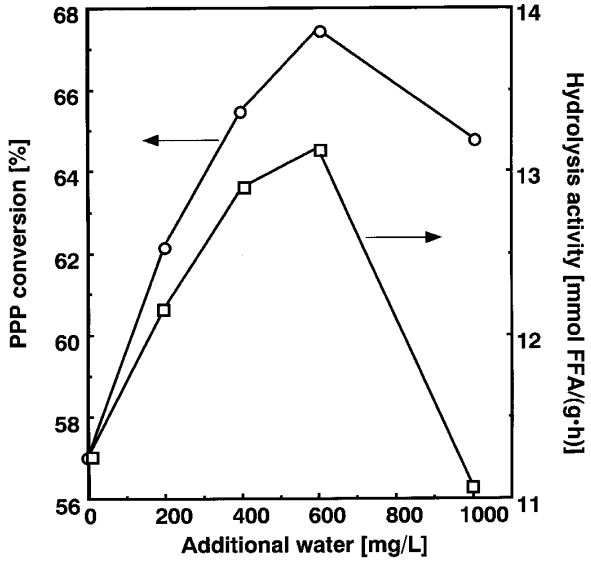

FIG. 6. Hydrolytic PPP conversion (%) and reaction rate [mmol FFA/(g · h)] after 4 h in relation to reaction water (mg/L) added to dry *n*-hexane (10 mg water/L). The reaction was carried out using 6 mmol/L PPP in 25 mL of *n*-hexane with 300 mg IML (preparation C1) at 40°C. For abbreviations see Figure 1.

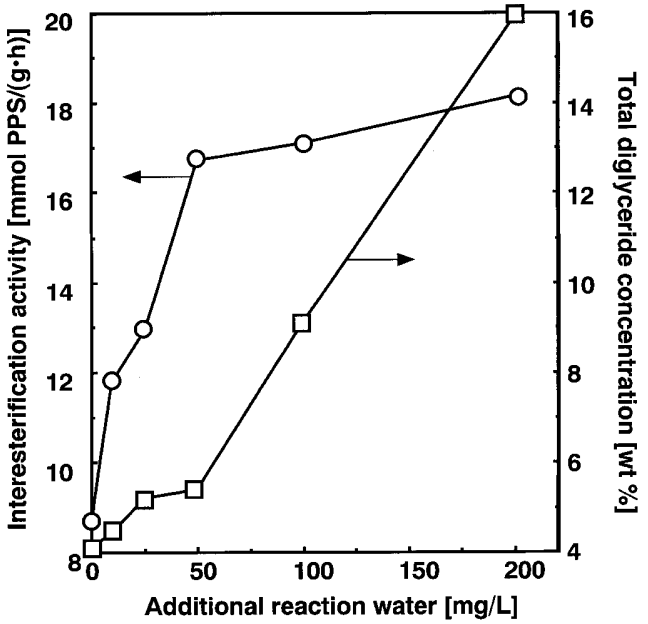

FIG. 7. Interesterification activity [mmol PPS/(g · h)] and total diglyceride concentration (wt%) after 4 h in relation to reaction water (mg/L) added to dry *n*-hexane (10 mg water/L). The reaction was carried out using 12.4 mmol/L PPP and 35 mmol/L SA in 25 mL of *n*-hexane with 300 mg IML (prep C1) at 40°C. For abbreviations see Figures 1 and 2.

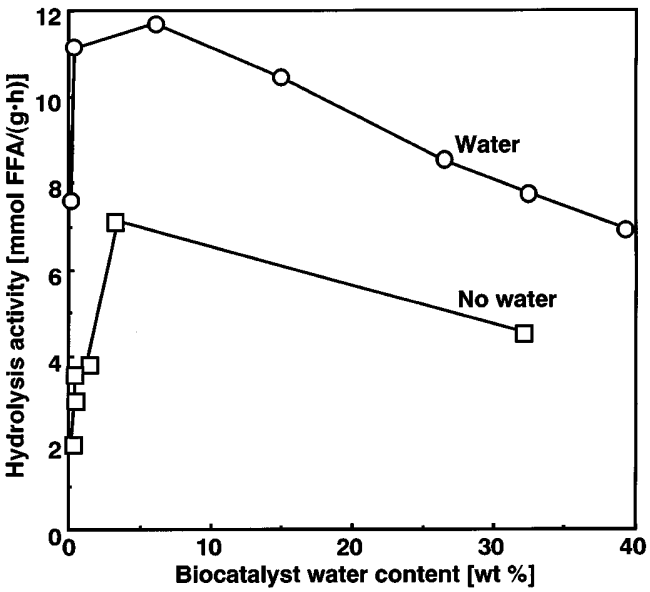

FIG. 8. Hydrolysis activity [mmol FFA/(g · h)] in relation to IML biocatalyst water content (wt%) with 400 mg/L water and without the addition of water to dry *n*-hexane (10 mg water/L). The reaction was carried out using 6 mmol/L PPP in 25 mL of *n*-hexane with 300 mg IML (preparation C1) at 40°C. For abbreviations see Figure 1.

creased sharply up to a water addition content of 50 mg/L, and increased more slowly thereafter. However, increasing water additions produced an almost linear increase in the production of undesirable hydrolytic diglycerides, water additions over 50 mg/L produced >6 wt% of diglycerides and should be avoided (Fig. 7).

Effect of biocatalyst water. Biocatalyst water content also has an important influence on IML activity, and this experiment aimed to determine the role biocatalyst water plays in determining hydrolysis and interesterification activities. Two experiments were conducted where the biocatalyst water content was evaluated with 400 mg/L (hydrolysis) or 20 mg/L (interesterification) water, and without water addition to dry *n*-hexane at the reaction start. Addition and nonaddition of reaction water are referred to below as being wet and dry, respectively.

With hydrolysis, activity was significantly enhanced using the wet reaction system (400 mg/L) over the range of biocatalyst water contents tested (Fig. 8). However, the activity profiles were similar for both systems. Activity with the driest preparation was very low, but a small increase in water content was sufficient to improve activity, peaking with the dry and wet systems at water contents of 3.45 and 6.05 wt%, respectively, and decreasing thereafter (Fig. 8). This decrease may be attributed to the poor dispersibility observed with the wetter biocatalysts.

A similar pattern was also observed with interesterification where activity decreased with increasing biocatalyst water content (Fig. 9). At very low biocatalyst water contents, almost no activity occurred in both systems. As with hydrolysis, the highest activity occurred with the same water contents of 3.45 and 6.05 wt% in the dry and wet systems, respectively. However, care must be taken to avoid hydrolytic diglyceride production, and with both systems very high diglyceride levels were recorded when the biocatalyst water content exceeded 3.45 wt% (Fig. 9). Care must also be taken to avoid total biocatalyst dehydration and resulting loss in activity. The biocatalyst with 0.43 wt% water displayed good interesterification activity but produced only 1.6 wt% diglycerides with the wet reaction system (Fig. 9).

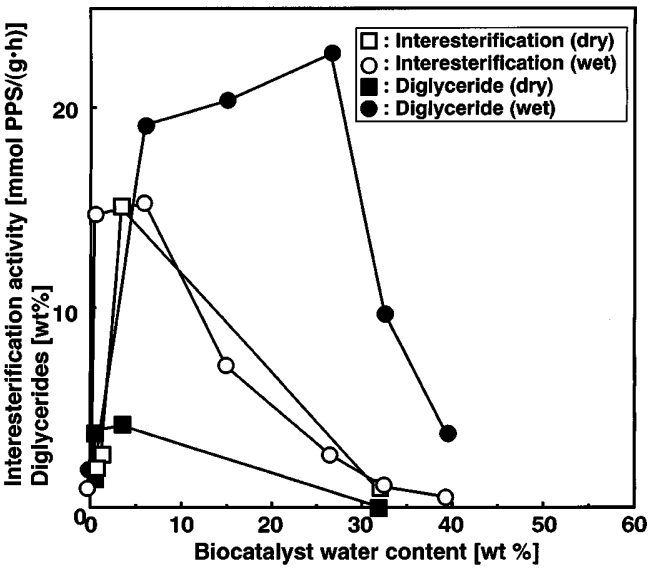

FIG. 9. Interesterification activity [mmol PPS/(g · h)] and diglyceride production (wt%) after 4 h in relation to IML biocatalyst water content (wt%) with 400 mg/L water and without the addition of water to dry *n*-hexane (10 mg/L water). The reaction was carried out using 12.4 mmol/L PPP and 35 mmol/L SA in 25 mL of *n*-hexane with 300 mg IML (preparation C1) at 40°C. For abbreviations see Figures 1 and 2.

One interesting observation is the shortfall in activity experienced with all biocatalysts when no reaction water was added. This was expected with very dry biocatalysts when water becomes the limiting factor, but it is difficult to explain why there is such difference with the wetter biocatalysts. IML can adsorb up to 40 wt% water, however, it is difficult to ascertain what proportion of this water is tightly bound or available for reaction. The addition of water to the reaction system may allow for more water to be available for reaction or to promote interfacial activation (10), hence the difference recorded between the wet and dry reaction systems.

Another experiment was conducted to assess whether a dried biocatalyst could be rehydrated and activity restored. An IML preparation was dried for 7 h at 35°C and the water content determined gravimetrically to be 0.20 wt%. Hydrolysis and interesterification activities were found to be 11.2 mmol FFA/(g · h) and 0.75 mmol PPS/(g · h), respectively. After rehydration in distilled water (pH 7) for 2 h, the biocatalyst was recovered and dried for 30 min at 35°C to give a water content of around 5 wt%. Hydrolysis activity was redetermined and was reduced to 5.9 mmol FFA/(g · h), while interesterification activity remained the same. This result suggests that IML dehydration resulting from mild drying (35°C for 7 h) permanently reduced catalytic activity with subsequent rehydration in water having no improved effect. It is clear that dehydration has a severe effect upon enzyme activity (11,30) with observed morphological changes (31). Commercial immobilized lipase preparations of Novozym 435™ and Lipozyme™ (both Novo Nordisk A/S) are supplied with water contents of between 1 and 3 wt%.

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