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Immobilized Pseudornonas cepacia lipase for biodiesel fuel production from soybean oil,

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Abstract

Enzymatic transesterification of soybean oil with methanol and ethanol was studied. *0f* the nine lipases that were tested in the initial screening. lipase PS from *Pseudornonas cepacia* resulted in the highest yield of alkyl esters. Lipase from *Pseudornonas cepacia* was further investigated in immobilized from with in chemically inert, hydrophobic sol-gel support. The gel-entrapped lipase was prepared by polycondenzation of hydrolyzed tetramethoxysilane and iso-butyltrirnethoxysilene Using the immobilized lipase PS, the effects of water and :alcohol concentration. enzyme loading, enzyme thermal stability, and temperature in the transesterification
reaction were investigated. The optimal conditions for processing log of soybean oil were: 35'C. 1:7.5 oil methanol molar ratio, 0.5g water and 475mg lipase with the reactions with methanol, and 35"C, 1:15.2 oil ethanol molar ratio 0.3s water, 4751ng lipase for the reactions with ethanol. Subject to the optimal conditions, methyl and ethyl esters formation of 67 and 65mol'Z8 in I h of
reaction were obtained for the immobilized enz yme reactions. Upon the reaction with the immobilized lipase. the triglycerides reached negligible levels after the first 30min of the reaction and the immobilized lipase was consistently more active than the free enzyme. The immobilized lipase also proved to be table and lost little activity when was subjected to repeated uses.

Keywords:

Transesterification; Biodiesel immobilization; Entrapment: Lipase; Sol-gel: Soybean oil: Pseudornonas cepacia

Introduction

Vegetable oils have attracted attention as a potential renewable source for the production of an alternative for petroleum based diesel fuel due to the diminishing petroleum reserves and environmental consequences of exhaust gases from diesel engines. Methyl and ethyl esters of fatty acids, better known as biodiesel, are nontoxic, biodegradable, and an excellent replacement for petroleum diesel. Biodiesel cetane number, energy content, viscosity and phase changes are also similar to those of petroleum-based diesel fuel (Mittelback and Tritthart, 19SSi. Moreover, biodiesel is essentially sulfur free and the engines fueled by biodiesel emit significantly fewer particulates, hydrocarbons, and less carbon monoxide than those operating on conventional diesel fuel. missions of KO,, however, are slightly higher than those of diesel engines operating on conventional diesel fuels (Schumacher et al., 1996; Ali et al., 1995). Transesterification of vegetable oils and animal fats for the production of fatty acid alkyl esters in a well established industrial process. The conventional biodiesel technology involves the use of an inorganic base or acid catalyst a t or near the boiling temperatures of the triglyceride alcohol mixture. The removal of catalyst is through neutralization and eventual separation of salt from the product esters, which is difficult to achieve. Moreover. the physiochemical synthesis schemes often result in poor reaction selectivity and may lead to undesirable side reactions. Enzymatic conversion of triglycerides has been suggested as a realistic alternative to the conventional physiochemical methods. Enzymatic transesterification of triglycerides offers an environmentally more attractive option to the conventional process.

However, the high cost of the enzymes often makes the enzymatic processes economically unattractive. The key step in enzymatic processes lies in successful immobilization of the enzyme. which will allow for its recovery and reuse (Balcao et al.. 1996). Immobilization is the most widely used method for achieving stability in lipases and to make them more attractive for industrial use (Cowan, 1996; Clark, 1994) Common immobilization techniques include physical adsorption onto a solid support (e.g. Bosley and Pielow. 1997), covalent bonding to a solid support (e.g. Walt and Agayn. 1994) and physical entrapment within a polymer matrix support (e.g. Pizarro et al., 1997). Entrapment of lipase entails capture of the lipase within a matrix of a polymer (Hartmeier, 1985). 'The immobilized lipase by entrapment is much more stable than physically adsorbed lipase and unlike the covalent bonding method, this method uses a relatively simple procedure and at the same time the immobilized lipase maintains its activity and stability (Kennedy and Melo, 1990). A variety of methods have been used for trapping lipases in a polymer matrix (Bickerstaff, l9Y71. Entrapment of enzymes in an inorganic polymer matrix is one method that has received a considerable attention in recent years. This method which was pioneered by Avnir et al. (1994) is based on sol-gel process. The application of the sol-gel material in the immobilization of lipases is well documented (e.g. Reetr, 1997). 4 substantial collection of research on the enzymatic transesterification of triglycerides has focused on free enzyme reactions with and without organic solvents. Nelson et al. (1996) studied the lipase-catalyzed transesterification of triglycerides in hexane, using deferent lipases and a variety of triglycerides and alcohols. The lipase from Mucoi. miehi (Lipozyme IM-20) was found to be themost effective in converting tallow to their respective alkyl esters with primary alcohols, whereas the lipase from Candida antactica as found to be most suitable for reacting with secondary alcohols, giving branched alkyl esters. Wu et al. (1999) employed response surface methodology (RSM) to optimize reaction parameters such as temperature, time, level of lipase, and molar ratio of reactants in the Pseudomonas cepacia lipase and Candida antactica lipase catalyzed transesterification reaction of restaurant grease to ethyl esters using 9Y.6 ethanol. Results showed a synergistic effect when the two lipases were used in sequence which surpassed the RSM predictions. Abigor et al. (2000) studied the lipase- catalyzed transestcrification or two Nigerian lauric oils: palm kernel oil and coconut oil. by transesterification of oils with different alcohols using PS-30 lipase as catalyst. In the conversion of palm kernel to alkyl esters, ethanol resulted in the highest conversion of 7204,. Other alcohols tested and their corresponding transesterification conversions included: I-butanol 62%, I-butanol -12%, n-propanol42%, and iso-propanol 24%, while only 15") metlrylesters was observed using methanol. Kaieda et al. (2001) studied the erect of methanol and water concentrations n the methanolysis of soybean nil using different lipases in a solvent Free system. Lipase from pseudomonas cepacea showed the greatest methanol resistance among the tested lipases. This lipase also exhibited the highest catalytic activity toward the transesterification reaction, even in the absence of water, methyl ester content reached *32""* after 50h of reaction. Cao et al. (1'192) reported the etherification and transesterification reactions catalyzed by Porcine pancreatic lipase adsorbed on glass, acetone precipitated on porous glass. kisselghur, aluminum oxide and afar beads in organic solvents. Results showed the lipase adsorbed on kieselgulir and agar beads with the highest activity. Shimada et al. (1999) and Watanabe et al. (2000) used immobilized Candida antactica (Novozyme 435) for the conversion of vegetable oil to biodiesel. Results showed incomplete methanolysis or vegetable oil which was attributed to the inactivation of the enzyme. Stepwise addition of methanol prevented this inactivation and conversions in excess of Yes% were obtained. Samoa et al. (2000) investigated the effect of the preoccupation of immobilized Candida Antarctica lipase (Novoz)me 435) in methyloleate and soybean oil prior to the biodiesel production. Results indicated a much i s t e r rate nf of methanolysis for the preiniubated lipase. Hsu et al. (20011 developed a novel

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procedure Tor the immobilization of lipase from Pseudornonas cepacia within a phyllosilicate sol-gel matrix. In this process phyllosilicate clay saturated with sodium ions was suspended in water and then exchanged with alkyl ammonium ions by the addition of cetyltrimethyl ammonium chloride. This mixture was then used in the entrapment of Pseudomonas cepacia with tetramethoxysilane (TMOS) as the polymerization precursor. The immobilized enzyme so prepared was then used in the transesterification of tallow and grease where conversions in excess of 95% were reached. In the present study, lipase PS from Pseudomonas cepacia was entrapped within a sol-gel polymer matrix. prepared by polycondenzation of hydrolyzed TMOS and iso-buryltrimethoxysihne (iso-BTXLS). The immobilized enzyme was used in the transesterification of soybean oil with methanol and ethanol.

2. Methods

2.1 Materials

All lipases were donated by Amano Enzyme (Nagoyri. .lapan). Soybean oil was donated by Archer Daniel Midland Co. (Lincoln. NE). Methanol and ethanol were purchased from Fisher Scientific (Pittsbureh, PA). Bis-(trimethylsilyl) trifluroacetamide IBSTF.A, derivative grade). 1.2.3-tridecanoylglycerol jtricaprine: g%), pyridins. and di'ltomaceous earth were purchased from Sigma Chemical Co. (St Louis, MO). Hexane (GC erade) was purchased from EM Science (Gibbstown, N . Tetramsthoxysilane (TMOS, 95%), iso-butyltrimethoxysilane (iso-BTMS, 97'%), and sodium fluoride (NaF) were purchased from the Aldrich Chemical Company (Milwaukee, WI).

2.2 Lipase immobilization.

A specified amount of lipase PS (optimally l) was measured into a Rask and lOml of water was added. The mixture was stirred at li0rpm using a magnetic stirrer for about 5min To this mixture, l ml of a 1 M NaF solution and the silica precursors were added. Upon the addition of the precursors, the reaction occurred almost immediately and the gel was formed in 2min. The flask was removed from the stirrer and left sealed at room temperature [or 24h. The flask was then uncapped and was incubated in a water bath at 33°C for about 24h. The ceramicpolymer was then broken up and grounded in a mortar. The powder was washed with lO0ml of distilled water in a 250ml Rask for l h at a mixing speed of OO rpm. The mixture was then filtered. About 90ml of supernatant was collected. This supernatant was further estimated for its residual activity (see the section on the Degree of immobilization tests). The net paste was dried again at 33 "C for 24 h. The immobilized lipase was then crushed in a morlar to yield the final product. The Fine powder was stored at 4 ° C until use. About 6g of the sol-gel material was resulted in this procedure. Based on the degree of immobilization tests, about 95% of the enzyme was immobilized in this procedure. The actual enzyme loading was determined : t 475mg of lipase PS per 3 g of gel.

2.3. Retraction setup and optimization:

The reactions were carried out in a constant temperature water bath, under which a Thermodyns (Dubuque, IA) Mirnk 4-p1:lce magnetic stirrer model #S73135 was placed to agitate the reaction mixture. Water was circulated into the bath from a Neslab (Portsmouth, NH) TTE-21 l BathiCirculator which, via an external probe, was able to control the temperature of the bath to

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within kO.01 'C. A standard set of conditions was used as the baseline in theo ptimization studies. These conditions were adopted from a previous study (Noureddini et al.> 20021. The initial conditions were IOg soybean oil 3g methanol (methanol to oil molar ratio of 8.2). 0.5g water, !g immobilized lipase PS, 40°C, 700rpm. And I h reaction time. In the reactions with ethanol. ()..<g of water and *5* g of ethanol (ethanol to oil molar ratio of 9.5) were used under otherwise identical conditions, In the optimization studies, only one reaction parameter was varied at a time. For example, when the effect of temperature was investigated. the rest of the reaction conditions were maintained at: IOg soybean oil, 3g methanol. 0.55 water, 3g immobilized lipase PS, 700 rpm. and I h reaction time. Lipase PS was used in all oftheoptimization stud ies.

2.4. Degree *of* immobilizationron tests

The immobilized enzyme was washed with water and after filtration, about 90 k 5ml of supernatant was collected. This supernatant may potentially contain free enzyme, partially hydrolyzed precursors, methanol, and soluble oligomers. To quantify the amount of enzyme in the supernatant, a calibration curve relating the formation or free fatty acids as a function of free Enzyme loading was constructed, which was based on the hydrolysis of soybean oil. Details about this work may be found elsewhere (Noureddini et al., 2002). By comparing the supernatant from the immobilization wash procedure with this calibration, theamount of enzyme in the supernatant and the degree of immobilization was determined. In order to mimic the exact reaction conditions. in the calibration studies, a blank gel with no enzyme was prepared. The supernatant. which was collected from washing this gel, was used as the reaction medium in the calibration experiments. Otherwise_ the e reaction procedure: for the calibration experiments was identical to the free enzyme hydrolysis reactions. The enzyme loading was varied in the range of 0.3-30mg lipase per I e of soybean oil for this calibration. The calibration curve showed a monotonically increasing activity as the enzyme loading was increased. The activity of the enzyme leveled off beyond the upper limit of 30my of enzyme per 1g of soybean oil.

2.5. Sampling Analysis:

Samples were 0.8-1.2 1111 in volume and W-ere collected in 5-ml sample vials. Samples from the reactions were initially heated to ensure enzyme denaturation freeze dried to remove excess water and alcohol. and finally derivatized with BSTFA. The silylating agent reacts with the carboxyl groups of fatty acids and results in trimethylsilyated fatty acids which are readily separated and quantified. The derivatized samples were then analyzed by a gas chromatograph (GC) to determine the concentration of fatty acid esters, free fatty acids, mono; di, and triglycerides. A Hewlett-Packard (Wilmington; DE) 6890 Series GC system was used for the chromatography work and a Hewlett-Packard Chemstation software was used for the data analysis. The GC was equipped with n Hewlett-Packard (part number 19091.1-012) HP-5 column. Sample volumes were 2pl. the carrier gas was helium, and the GC was operated in constant flow mode with a flow rate of 12.0ml/min. 4 split injector was used with a split ratio of I5:I and a temperature of 325°C. The FID detector was operated at 350zC and used a helium makeup flow to maintain a constant detector flow of 25.Ornl/min. The oven !%IS initially held at 80.OCC and was then elsateto 180°C at 15.OoC/min, to 250°C at .O0C/min, and finally to 32S°C at 8.0aC/nrin. The oven was held at this temperature for 22.95min before returning to 80.O0C. Total run rime for this method was 53.Omin. Calibration of the

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GC method was carried out by analyzing standard solutions of mixed glycerol. fatty acid esters. free fatty acids; mono-. di-_ and triglycerides. The standards were derivatired in the same fashion as the reaction samples. More details about the sample preparations and analysis procedures are explained elsewhere (Wagner, 1999).

2.6 Data Analysis :

Experimental results are presented in Fig. 1-8. In these figures. the activity of the lipases is presented by the for111iltion or esters and other reaction products in terms of molar percent of the components. The data presented in Figs. I--6 were replicated at least three times. The mean values for the replicated data are presented in the graphs. There was no error analysis for Figs. 7 and 8. In Figs. I-h, the standard deviations for fatty acids were approximately *S'!/&* of the mean values and are not shown in the figures. The data was analyzed by Microsoft Excel build-in functions.

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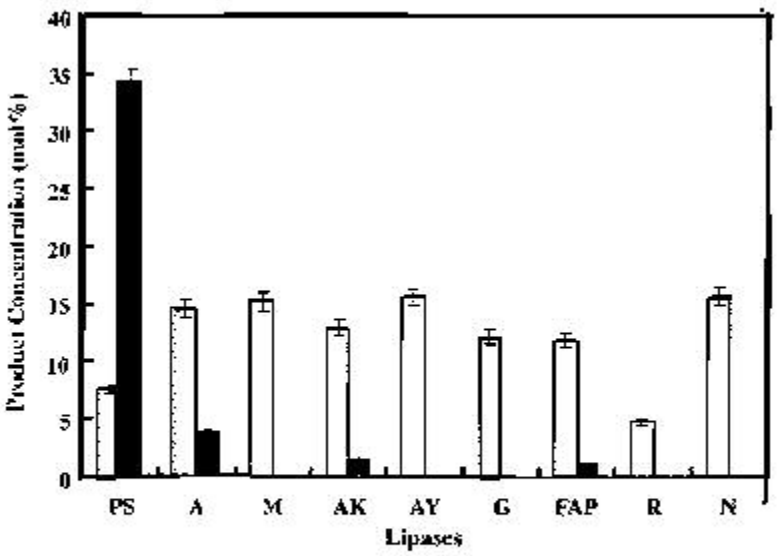

Fig. 1. Lipase screening on transesterification of soybean oil, a loading of 250 mg free lipase, 3.0 g methanol, 0.5 g water, 10 g oil, a stirring rate of 700 rpm and a 1-h reaction at 35 °C. (■) Methylesters and (□) free fatty acids.

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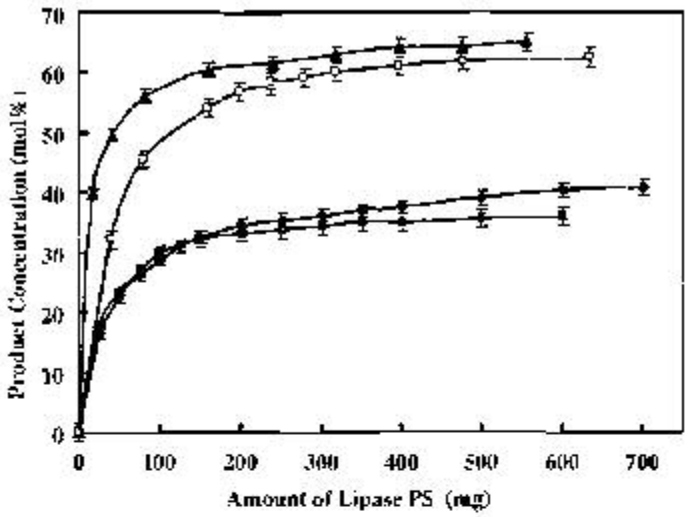

Fig. 2. Effect of enzyme loading on transesterification of soybean oil 0.5 g water, 10 g oil, a stirring rate of 700 rpm and a 1-h reaction at 35 °C. (◆) Free lipase PS, 3 g of methanol. (▲) Immobilized lipase PS, 3.0 g of methanol. (■) Free lipase PS, 5 g of ethanol reaction. (□) Immobilized lipase PS, 5 g of ethanol.

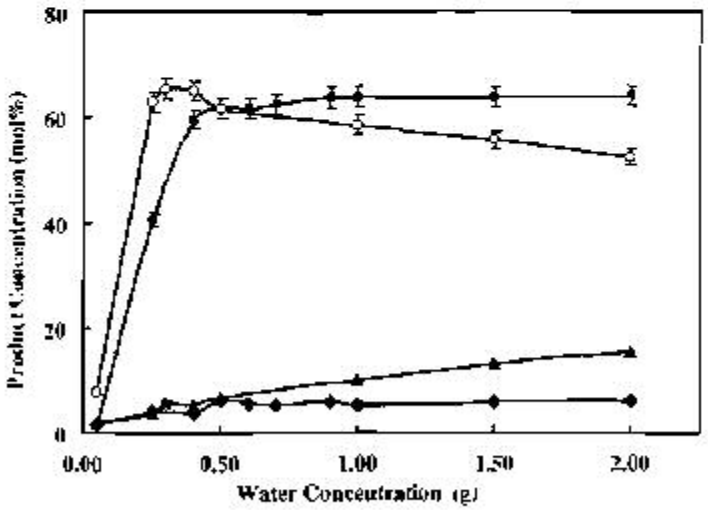

Fig. 3. Effect of water concentration on immobilized lipase catalyzed transesterification of soybean oil, subject to a loading of 3 g of gel/10 g of oil, a stirring rate of 700 rpm and a 1-h reaction at 40 °C. (■) Methylesters and (◆) free fatty acids; 3 g methanol; (○) ethylesters and (▲) free fatty acids; 8 g ethanol

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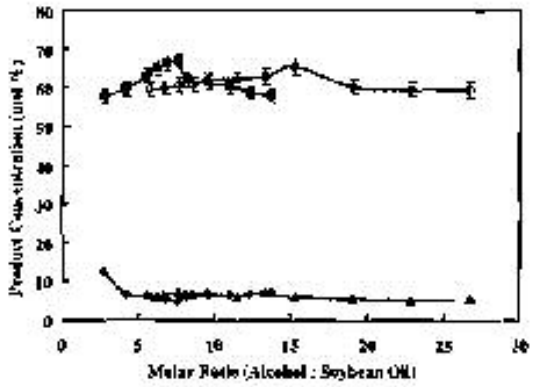

Fig. 4. Effect of alcohol concentration on immobilized lipase catalyzed transesterification of soybean oil, subject to a loading of 3 g of gel/10 g of oil, a stirring rate of 700 rpm and a 1-h reaction at 40 °C (■) Methylesters and (●) free fatty acids: 0.5 g water; (○) ethylesters and (▲) free fatty acids: 0.3 g water.

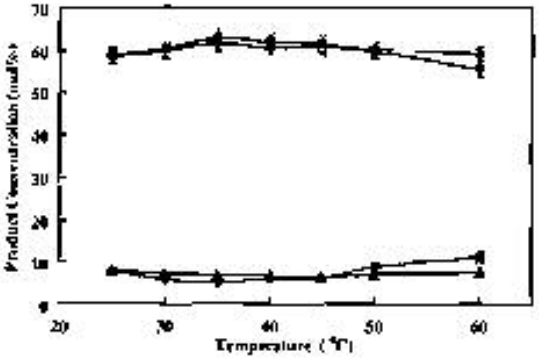

Fig. 5. Effect of temperature on immobilized lipase catalyzed transesterification of soybean oil, subject to a loading of 3 g of gel/10 g of oil, a stirring rate of 700 rpm and a 1-h reaction. (■) Methylesters and (●) free fatty acids: 3 g methanol, 0.5 g water; (○) ethylesters and (▲) free fatty acids: 5 g ethanol, 0.5 g water.

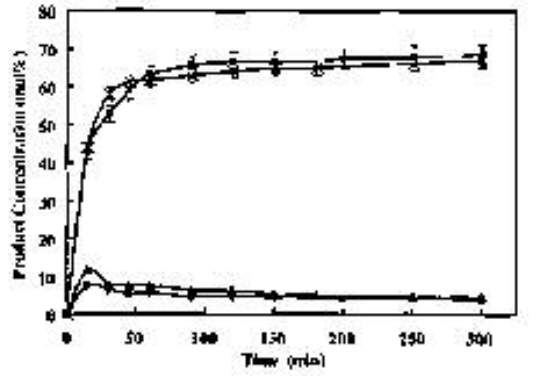

Fig. 6. Time course of the transesterification of soybean oil, subject to a loading of 3 g of gel/10 g of oil, a stirring rate of 700 rpm for at 35 °C (■) Methylesters and (●) free fatty acids: 3 g methanol, 0.5 g water; (○) ethylesters and (▲) free fatty acids: 5 g ethanol, 0.3 g water.

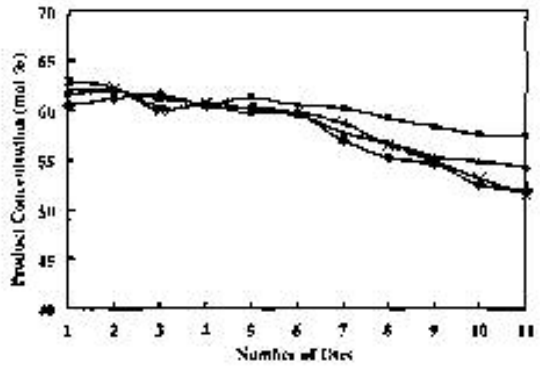

Fig. 7. Reusability of immobilized PS subject to a loading of 3 g of gel/10 g of oil, 0.3 g water, a stirring rate of 700 rpm for and a 1-h reaction at 35 °C (◆) 4 g EtOH, (■) 5 g EtOH, (▲) 6 g EtOH, (×) 7 g EtOH.

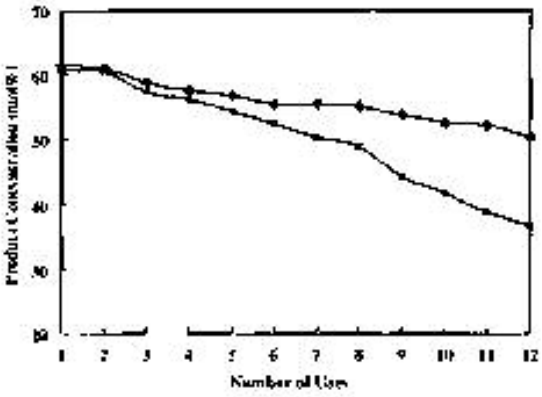

Fig. 8. Reusability of immobilized PS subject to a loading of 3 g of gel/10 g of oil, 0.5 g water, a stirring rate of 700 rpm for and a 14-h reaction at 35 °C (◆) 2.5 g MeOH, (■) 3.0 g MeOH.

Table 1
Lipases tested in the transesterification screening

Lipase[a]	Source organism
R	*Penicillium roqueforti*
AK	*Pseudomonas* sp.
PS	*Pseudomonas* sp.
M	*Mucor* sp.
A	*Aspergillus niger*
FAP	*Rhizopus oryzae*
G	*Penicillium camembertii*
N	*Rhizopus niveus*
AY	*Candida rugosa*

[a] All lipases were donated by Amano Enzyme (Nagoya, Japan).

3. Results and discussion

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Although lipases generally catalyze the hydrolysis of carboxylic esters; they hring about a range of bioconversion reactions such as etherification, transesterification; acidolysis, and aminolysis. Lipase screening was performed to find the lipase with the hest catalytic activity in the transesterification of soybean an oil. The most active lipase was then used in the immobilization studies. Nine lipases, as listed in Table I , were screened for their transesterification activity's the screening experiments were intended for an initial valuation f the activity of the lipases, they were conducted under a preliminary set of reaction conditions which may not have been the optimum set for all the lipases. In a typical reaction, 25Omg of dry enzyme was added lo the mixture of 3 g methanol (8.2 molar ratio of methanol to soybean oil), 0 5 g of water- and 10g of soybean oil. The reactions were carried out at 35OC and according to the reaction setup which was described earlier. The screening results for the tested lipase are presented in Fig. 1. Reaction products are presented as mol% of methyl esters of fatty acids in the reaction mixture. The formation of free fatty acids is also included in this figure since the presence of water in the reaction medium naturally promotes the competing hydrolysis reaction. As this figure shows, among the tested lipases, lipase PS from *Pseudomonas cepacia* showed the highest activity toward the transesterification of soybean oil with methanol. Other lipases showed very little or no activity toward the transesterification reaction. After I h of reaction with lipase PS, the product contained 34molUh methyl esters, 91nol"'o of fatty acids, 6moM of mononoglycerides. 44molYo of diglyceride and 7 mol'/a of triglycerides.

3.2 Enzyme Loading:

Experiments were performed to determine the eKecr of enzyme loading on the extent of the transesterification reaction. Enzyme loading in the range of O- 700mg of Free enzyme and 0-3.5g of the immobilized enzyme were examined in the transesterification of soybean oil with methanol and ethanol. One yarn of immobilized enzyme corresponds to l58mg free enzyme in these reactions. Based on the concentration OS soybean oil. the alcohol concentration was at 8.2 molar equivalents for methanol and 9.5 molar equivalents for ethanol (3.0g methanol and 5.02 ethanol). Other reaction parameters nsre as was stated earlier in the reaction setup and optimization section. Reaction results for the formation of methyl and ethyl esters of fatty acids are presented in Fig. 2. For all cases studied, as the enzyme loading was increased there was a sudden surge in the formation of alkyl ssters. followed by a slower rate at higher enzyme loadings. This surge was steeper and the formation of alkyl esters was significantly higher for the immobilized enzymes compared with the flee enzymes. For the reactions with methanol, the concentration of methyl esters reached 11 and 65moM for the free and the immobilized lipase, respectively. At the end of the reaction period, the concentration of triglyceride in both system reached negligible levels, while, the formation of fatty acid, mono- and di-glyceride were 8, 9, and 42moW for the free enzyme reaction and 7, 22, and 6mol% for the immobilized enzyme. Similarly; for the reaction with ethanol the level of ethyl esters reached 36 and 63mol'h for the free and the immobilized lipase. At the end of the relation period, the concentration of triglyceride in both system reached negligible levels, whils. the formation of fatty acid, mono- and diglyceride were 9, 6, 43moP/o for the free enzyme reaction and 9. 22, and 6mol'Y" for the immobilized. This behavior of the immobilized lipase was consistent with those of other researchers (e.g. Reetz. 1997) and has been attributed to lipophilic nature of the alkyl group of the solgel. Free alkyl groups in the sol-gel create a lipophilic microenvironment that subsequently

interacts with the lipase, triggering a phenomenon similar to a classical interfacial interaction. How'ever. unlike the interfacial activation. which 1s Lln interactive process, the alkyl effect is believed to be due to a more favorable lipase conformation caused during the sol-gel process. The lipophilic environments are also bsliered to facilitate the transport of the organic substrate to the biocatalyst sites in the outer surface of the support and possibly in and our of the matrix.

3.3. Effect of water concentration:

Lipases possess the unique feature of acting at the interface between an aqueous and an organic phase. Lipases interfacial action is due to the fact that their catalytic activity generally depends on the aggregation of the substrates. Activation of the enzyme involves unnulsking and restructurin of the active site through conformational changes of the lipase molecule, which requires the presence of oil-water interface. Lipase activity generally depends on the available interfacial area. With the increased addition of water. the amount of water available for oil to form oil-water droplets increases. thereby. increasing the available interfacial area. However, since lipases usually catalyzes hydrolysis in aqueous media, excess water may 3130 stirrlulate the competing hydrolysis reaction. The optimum water content is a compromise between minimizing hydrolysis and maximizing enzyme activity for the transesterification reaction. The effect of water concentration in the range of 0.05-2.0s and at constant alcohol concentrations of 8.2 and *15.2* molar ratios of methanol and ethanol with respect to soybean oil were examined (3.9 g methanol and 8.0g ethanol). The reactions were carried out according to the reaction setup and optimization section described earlier. Results presented in Fig. 3 indicate very little enzyme activity at Low water concentrations which supports the fact that a minimum amount of water is required to activate the enzyme. With the increased addition of water there was a considerable increases iii the ester production showing the enhancement in the activity of the enzyme. In the case of methanol, the ester production reached about 62moW at O.5g water after which there was very little increase in the production of ester but also a slight increase in the formation of fatty acid. In the case of ethanol, the ester production reached a maximum of about 65molK at 0 . 3 water. At water concentrations beyond 0.3g, there
was a considerable decrease in the production of esters and accordingly an increase in the formation of fatty acid.

3.4. Effect of alcohol concentration

I11 the immobilized enzyme transesterification reaction, the reactants initially form a three-phase system (triglyceridelalcoholisuppiutT.h e reaction is diflusioncontrolled and poor diffusion between the phases exists. .As alkyl esters are formed, they act as a mutual solvent for the reactants and a two phase liquid solid system results (Noureddini and Zhu, 1997). Howc\er2 as the reaction progresses toward completion and the glycerol concentration is increased, a maiol) alcohol and glycerol phase separates from the rich alkyl ester phase and a three phase system forms again. This is more likely at lower initial alcohol concentrations and higher extent of reaction and in the i-ange of alcohol concentration that was investigated this separation did not occur. Alcohol in excess

of the stoichiometric molar ratio of 3:1, was used to ensure higher re:iztion rates as the transesterification o i triglycerides with alcohols consist of three stepwise and reversible reactions and (2) minimize the diffusion limitations. However, excess alcohol levels may inhibit the enzyme activity and thereby decrease its catalytic activity toward the transesterification reaction. Experiments were performed to optimize the amount of ester production by varying the alcohol concentration. Optimum alcohol requirements were determined for both methanol and ethanol. The amount of alcohol added was varied from 2.7 to 13.7 rnolur equivalents for methanol and from 5.7 to 26.7 molar equivalents for ethanol, based on the moles of triglycerides. Water concentration was kept constant at the optima level of 0 5 g for the methanol reactions and at 0.3g for the ethanol reactions. Results are summarized in Fig. 4. As \raj expected, an increase in the number of moles of alcohol with respect to the triglycerides resulted in an increase in the production olesters. Ultimately. the formation of esters reached n maximum level and tilrther increases in the alcohol concentrations resulted in a decrease in the formation of esters. The optimum alcohol concentration was determined at 7.5 molar ratio of methanol to soybean oil where about 6-molOh of methyl esters was formed. For the reaction of ethanol and soybean oil this ratio was at 15.25 for which about 65mo1% of ethyl esters was formed.

3.4. Effect of Temperature

Experiments were performed to examine the effect of temperature on the catalytic activity of the immobilized lipase PS in the transesterification of soybeanoil with methanol and ethanol Reactio ns \\ere carried out as was described earlier in the reaction setup and optimization section. Temperatures in the range of 2540°C at 5°C increments and at constantalcohol concentrations of 8.2 and 9.5 molar ratios of methanol and ethanol with respect to oil were examined (3.0g methanol and 5.0g ethanol). Results, presented in Fig. 5. show slight changes in the transesterifcation activity of the immobilized lipase PS with variations in temperature. Transesterification activity f lipase PS reached *3* mr -' n u µ at 35OC with methanol, where about 6311101% of methyl esters was formed. As the reaction temperature was further increased, a decrease in the ester production and an increase in the fatty acid production were observed. This behavior was consistent with our previous study (Noureddini et al., 2002) which revealed more favorable hydrolysis reactions at higher temperatures. The reactions with ethanol also showed a similar trend and an optimum temperature of 35'C at which about 62mol% of ethyl esters were formed. Experiments were also performed to examine thermal stability of the immobilized lipase PS in the transesterification of soybean oil wit11 methanol at 35'C. In these experiments, the immobilized enzyme (33) was initially incubated at 35°C in 10g of oil for up to 72h. The incubated immobilized enzyme was then used in the transesterification reaction. Results showed no change in the catalytic activity of the incubated enzyme. For the enzymes which were subjected to 24; 48, and 72h of incubation the formation of methyl esters was practically identical to the formation of methyl esters for the enzymes with no prior incubation. The observed enhancement in thermo stability of the immobilized enzymes was consistent with our previous study on the hydrolysis of soybean oil (Noureddini et al.; 2002) and also the s o r k of Kauakami and Yoshida (1996), and ma) be attributed to interaction between lipase and sol-gel support.

It appears that stable lipase confrontations similar to the interphone activation occur due to the interaction of enzyme with the polymer stir face. Both physical and chemical interactions such as hydrogen bonding and ionic interactions are believed to be responsible for the enhanced thermalstability of the immobilized enzyme (Reetz et al., 1996).

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3.6. Effect of Temparature:

The kinetics of the reaction plays an important role in the process scale up and design The kinetics of the transesterification reaction was further investigated for both methanol and ethanol reactions with lipase PS. The reaction conditions for the methanol reaction were log soybean oil, 8.2 molar ratio of methanol to soybean oil 13.0g methanol), 0.5% water. 35"C, and 3g immobilized lipase PS; and the reaction setup described earlier in the reaction setup and optimization section. The conditions for the ethanol reaction were identical except for 9.5 molar ratio of ethanol to soybean oil (5.0% ethanol) and 0.3 g water. The reaction results for the formation of alkyl esters and free fatty acids for the initial 300min of the reaction are presented in Fig. 6. In the cnsr of methanol transesterifcation reaction, results showed an initial surge in the formation of methyl esters during the first 30min of the reaction which was followed by a slower rate as the reaction progresses and ultimately. A state of equilibrium was approached. More than 651nol";, of methyl esters are formed during the first 90min of the reaction. Similar trends were observed for the transesterification reaction with ethanol where 63mol% of reaction was comported over the first 90min of the reaction and only 4"/1, of additional conversion was observed over the next 21Qmin of the reaction. The formation of the free fatty acids was below jrnol'% in both cases.

3.7. Immobilized enzyme: stability and reusability.

One of the most important characteristics of an immobilized enzyme is its stability and reusability over an extended period of time. Experiments were performed to examine the recyclability and the stability of the immobilized lipase PS. After each standard I h transesterification reaction, lpase-containingg el was recovered by filtration and subsequently reused. This procedure w a repeated several times to examine the extent of the stability of the immobilized enzyme. Experiments were performed with IOg soybean oil, g immobilized lipase PS, 35'C. 0.5 and 0.32 water for methanol :and ethanol reactions, respectively. The concentration of alcohols was also v:lried to esarnine its long-term effect on the activity of the immobilized lipase. Other reaction conditions were as described earlier in the reaction setup and optimization section. To facilitate the alteration of the immobilized eel after each run, 1.0g of diatomaceous earth was mixed to the reaction medium prior to the first alteration procedural The diatomaceous earth was recycled along with the immobilized gel throughout the replications. Reactions are conducted at 4.0, 5.0, 6.9, and 7.0s ethanol and l(lg soybean oil which corresponded to about *7.6,* 9.5, 11.4, and 13.5 molar ratios of ethanol to soybean oil, respectively. Experimental results summarized in Fig. 7 show a slight decreasing trend in the activity of the immobilized lipase upon repeated uses in all cases studied. The decrease in the activity was smaller at 9.5 molar ratio of alcohol to the soybean oil where only :lhout 5mo1°h loss in the activity was observed after I1 replicates. It appears that at this level of alcohol there is a good balance between the availability of the alcohol as a reaction substrate and its inhibitory effects to the lipase. In general, the loss of activity may be attributed to the deactivation of lipase and to the gradual loss of the immobilized lipase during the processing procedures. The transesterifcation reactions with rnsthanol were conducted at 2.5 and 3.0g alcohol and log of soybean oil which corresponded to 6.9 and 8.2 molar ratios or alcohol to soybean oil, respectively. Results summarized in Fig. 8 demonstrate a similar but Ignore drastic decreasing trend in the immobilized lipase when subjected to repeated uses. This may be explained by the fact that methanol exhibits a stronger inhibition toward lipase PS than ethanol.

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4. Conclusions

Enzymatic transesterifcation of triglycerides oKsrs an environmentally more attractive option to the conventional physiochemical process. The key step in enzymatic processes lies in successful immobilization of the enzyme which will allow for its recovery and reuse. In this study the immobilized-enzyme transesterification of soybean oil with methanol and ethanol was investigated. Lipase PS from Pseudomonas cepacia was immobilized by entrapment within a sol-ye1 structure which was prepared by polycondensation of hydrolyzed TMOS and iio-BTMS. The immobilized lipase so prepared was consistently more active than the free lipase toward the transesterification of soybean oil. The immobilized lipase also proved to be stable and lost little activity when was subjected to repeated uses.

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