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Immobilization of Lipase from *Candida Rugosa* on Palm-Based Polyurethane Foam as a Support Material

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Abstract: Lipase from *Candida rugosa* was immobilized onto palm-based polyurethane foam (PU), which the polymer was pre-soaked in co-immobilized agent. The activities of PU-immobilized lipase were tested by the esterification reaction of oleic acid and oleyl alcohol in hexane. The PU-immobilized lipase was then characterized in term of its thermal, operational and storage stability. The optimum temperature for native and PU-immobilized lipase was at 40°C. This shows that the immobilization did not alter the general character of the lipase. The PU-immobilized lipase shows different enzymatic characteristic-incubation time, enzyme concentration, solvent and operational stability compared to Lipozyme IM. The reuse stability of PU-immobilized lipase was at least four cycles. The % conversion above 80% was still achieved for the sample stored at -5°C after 9 days storage.

Key words: *Candida rugosa*, enzyme, immobilization, polyurethane

INTRODUCTION

Immobilized enzymes are known to be used for organic synthesis. The most common immobilized enzymes are lipases used for esterification reactions in organic media[1]. Immobilized enzymes have received considerable attention because of their advantages over unimmobilized counter parts as they improve storage and operational, thermal and conformational stabilities. They are easily recovered for reuse[2]. Most carriers for immobilization have been granular particles including celite or ion exchange resins[3-4], which is one reason for the high costs. Granular immobilized enzymes can be reused by filtration, but are easily broken down by agitation in two-phase systems[5]. In this study, lipase from *Candida rugosa* is immobilized onto palm-based polyurethane foam through the physical adsorption method.

Polyurethanes are well known for their ability to entrap or otherwise immobilized biological[6,7]. They have been successfully used as immobilzation matrices for living microbial cells in the biodegradation of toxic chemicals[8]. The effectiveness of covalently attaching lipase to polyurethane foam has been reported by Vasudevan and co-workers[9]. However, this technique has the disadvantage that much of the enzymes were imbedded deeply into the foam macrostructure. The objective of this study is to analyze the behaviour of the prepared immobilized enzyme in esterification of fatty acids and monohydric alcohols.

MATERIALS AND METHODS

Materials: Lipase from *Candida rugosa* (type VII) was purchased from Sigma Chemical Co. (St. Louis, USA) while Lipozym IM (lipase from *R. meihei*) was purchased from Novozyme A/S (Bagsvaerd, Denmark). Polyurethane foam was prepared in the laboratory. Oleic acid was obtained from Cognis Oleochemical (M) Bhd. (Kuala Lumpur, Malaysia). Oleyl alcohol was from Merck (Darmstadt, Germany). All other reagents were of analytical grade and used as received.

Immobilization of lipase: The polyurethane foam was pre-soaked in two volumes of co-immobilization chemicals containing, lecithin, gelatin, polyethylene glycol and magnesium chloride. Then the mixture was dried at room temperature. The treated dry support was used for immobilized lipase. The support and lipase solution were mixed and dried overnight at room temperature.

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Lipase-catalyzed reaction: Unless stated otherwise, oleic acid (0.02 mol) and oleyl alcohol (0.04 mol) were placed in a screw-capped conical flask with 100 mg immobilized lipase. Hexane was used as a solvent. The reaction mixture was incubated at 40°C for specified time with continuous shaking speed at 150 rpm in a horizontal shaker bath. All experiments were done in triplicate. The control experiments were carried out without enzyme. The reaction mixture was terminated by dilution with 3.5 mL of ethanol/acetone mixture (1:1, vol/vol) and the remaining oleic acid in the mixture was then determined by titration with sodium hydroxide (NaOH, 0.01M) solution until pH 10. The percentage conversion was calculated based on the following equation.

Conversion (%) =[($V_{control}$ -V_{sample})/ $V_{control}$] X 100%

Where $V_{control}$ = average volume of NaOH used for blank, while V_{sample} = average volume of NaOH used for samples.

Product identification: The isolated product was identified by spectral studies (Fourier transform infrared, FTIR and nuclear magnetic resonance, NMR). FTIR was recorded on a Nicolet Magna IR550 (Nicolet, Madison WI) spectrophotometer. The NMR spectra were recorded on a JOEL ECA-400 spectrometer at 400 MHz. The chemical shifts are expressed in ppm with tetramethylsilane as internal standard.

Spectra data for oleyl oleate. FTIR: 3005 cm^{-1} (-C=C-), 2931 cm^{-1} (-CH_2-), 1740 cm^{-1} (-COO-); ^{1}H-NMR: 0.96 (CH_3-), 1.29-1.33 (-CH_2-), 1.96 (-CH_2-C=), 2.25 (-CH_2-COO-), 4.08 (-CH_2-O-C-), 5.43 ppm (-HC=CH-); ^{13}C-NMR: 173.2 (C=O), 131.0 (-HC=CH-), 65.9 (-CH_2-O-), 33.5 (-CH_2-COO-), 22.5-30.1 (-CH_2-), 14.5 ppm (CH_3-)

RESULTS AND DISCUSSION

Effect of temperature on the activity of immobilized and native lipase: Within the temperature range study, 40°C gave the highest conversion (Fig. 1) for immobilized and native lipase. This shows that the immobilization did not alter the general character of the lipase.

Effect of lipase concentration : Effect of PU-immobilized lipase concentration on oleyl oleate production was compared to Lipozyme IM (Fig. 2). The conversion was increased by increasing lipase concentration up to 300 mg, with which the highest conversion of 79.5% for PU-immobilized lipase and 81.8% for Lipozyme IM could be attained.

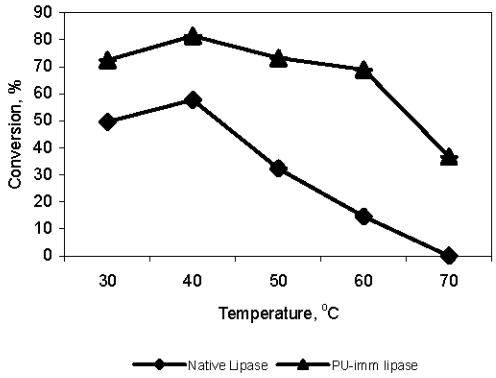

Fig. 1: Effect of temperature on the activity of native and PU-immobilized lipase. Reaction conditions: 24 h, 150 rpm, 100 mg PU-immobilized lipase or 50 mg native lipase, oleic acid/oleyl alcohol mole ratio 1:2

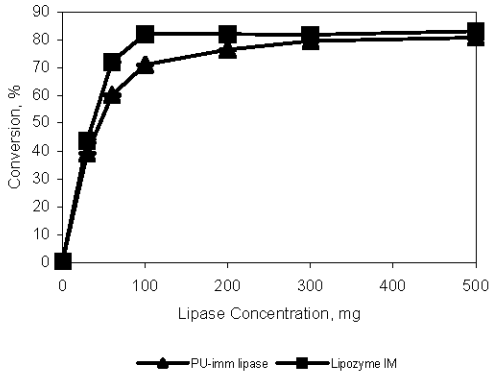

Fig. 2: Effect of lipase concentration. Reaction conditions: 40°C, 150 rpm, 24 h for PU-immobilized lipase and 3 h for Lipozyme IM, oleic acid/oleyl alcohol mole ratio 1:2

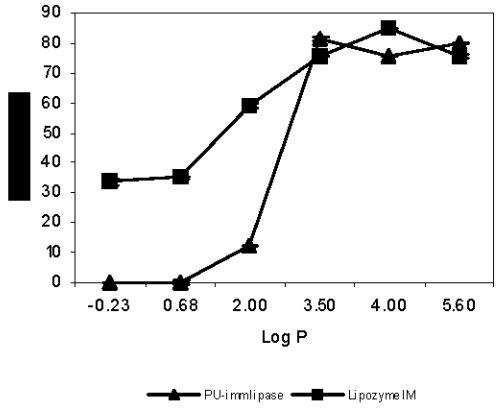

Fig. 3: Effect of organic solvent. Reaction conditions: 40°C, 150 rpm, 24 h for PU-immobilized lipase and 3 h for Lipozyme IM, oleic acid/oleyl alcohol mole ratio 1:2, 100 mg catalyst

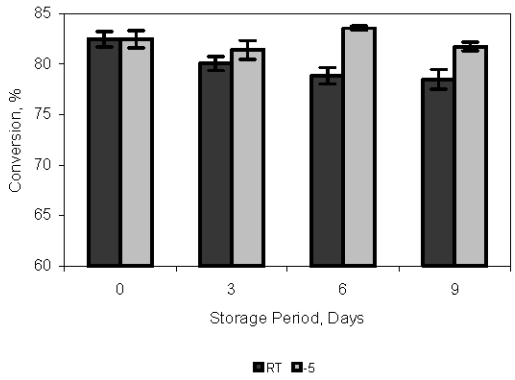

Fig. 4: Effect of storage conditions on the activity of PU-immobilized lipase. Reaction conditions: 40°C, 150 rpm, 24 h, 100 mg catalyst, oleic acid/oleyl alcohol mole ratio 1:2

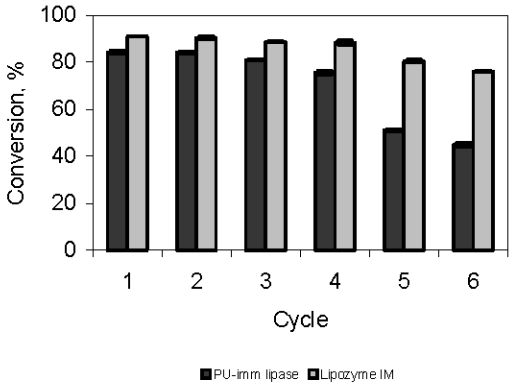

Fig. 5: Reusability test of PU-immobilized lipase. Reaction conditions: 40°C, 150 rpm, 100 mg catalyst, oleic acid/oleyl alcohol mole ratio 1:2, 24 h for PU-immobilized lipase and 3 h for Lipozyme IM

Effect of solvent: In (Fig. 3), the conversion was relatively lower with solvent having log P less than 3.0 but higher conversion was obtained in organic solvent which are having log P values greater than 3.0. Both immobilized lipases showed different maximum conversion at similar log P values. This may be due to enzymes specificity in organic solvent.

Effect of storage conditions on the activity of immobilized lipase: The ability to be stored for a period of time at a certain temperature is one of the key factors to be considered when using immobilized lipases. The storage stability of PU-immobilized lipase kept at RT and -5°C was monitored for 9 days. The percentage conversion above 80% was still achieved for the sample stored at -5°C (Fig. 4). Generally, enzymes are still active when kept at low temperature probably because lipases tend to lock its original conformation, which is catalytically active[10]. However, the percentage conversion gradually decreased from day one to day 9 for the sample stored at RT.

Repeated use of immobilized lipase: For large-scale production, the repeated use of lipase is a major issue. Long lipase lifespan in enzymatic reactions will significantly decrease the cost of the process, which will accelerate industrial applications of lipase technology. In this work the retention of lipase activity after repeated use was assessed in term of conversion at the end of each cycle. For PU-immobilized lipases, the enzyme still retained over 70% of its original activity after 4 cycles (Fig. 5). However, drastic decrease in activity for the last two cycles. This study also showed that Lipozyme IM more stable compared to PU-immobilized lipase.

CONCLUSION

Polyurethane foam showed a promising future of applying polymer as support for biocatalyst for various organic syntheses as it allows easy immobilization technique.

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