LARGE SCALE PRODUCTION OF OCTYL-9,10-DIHYDROXYSTEARATE BY IMMOBILIZED LIPASE

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ABSTRACT

Large scale production of octyl-9,10-dihydroxystearate was performed in a 5-litre batch, stirred tank reactor with a multi-bladed propeller. The effects of reaction temperature, reaction time, enzyme dosage and agitation speed were studied. The optimal conditions for the ester synthesis were reaction temperature at 50°C, reaction time of 3 hr, catalyst concentration of 10% (w/w) and agitation speed of 300 rpm. The percentage conversion was about 90% with respect to the amount of dihydroxystearic acid (DHSA) used. The operational stability of the catalyst was also evaluated.

Keywords: dihydroxystearic acid, esterification, immobilized lipase, octyl dihydroxystearate.

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INTRODUCTION

Interest in wax esters preparation arises from their industrial applications - from lubricants to cosmetics, while the possibility of preparing esters that resemble naturally occurring waxes of commercial interest is appealing (Trani *et al*., 1991). Using lipases in esterification to produce esters, such as sugar esters, wax esters, flavour esters, *etc.* have been well documented (Krishna and Karanth, 2002; Garcia *et al*., 1999). The catalytic activity of lipases toward hydroxy fatty acids is also well studied (Gosh and Bhattacharrya, 1998; Hayes, 1996; Wagner *et al*., 1992). However, most of these studies were carried out in small scale with laboratory flasks as reactors and focused on the physical properties of the products. The results have not been very consistent (Zhang *et al*., 2000).

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Our previous studies had shown that lipase-catalyzed esterification of dihydroxystearic acid (DHSA) and monohydric alcohol can produce high yields using commercial immobilized lipase (Awang *et al*., 2000; 2005). In this study, large scale production of octyl-9,10-dihydroxystearate using a 5-litre stirred batch reactor was carried out. The activity of the biocatalyst used was analysed as a function of the reaction time, temperature and enzyme dose, while the reactor system was optimized in terms of agitation speed and number of propeller blades. The operational stability of the biocatalyst was also evaluated.

MATERIALS AND METHODS

Materials

DHSA was prepared in the laboratory (Awang *et al*., 1998). The 1-Octanol (purity, 98%) was purchased from Cognis Oleochemical (M) Bhd (Kuala Lumpur, Malaysia) and Lipozyme IM from Novozym A/S (Bagsvaerd, Denmark). All the other reagents were of analytical grade and used as received.

Octyl-9,10-dihydroxystearate Synthesis

Octyl-9,10-dihydroxystearate synthesis catalyzed by immobilized enzyme was carried out in a 5-litre stirred tank reactor equipped with an agitator system, temperature control and sampling port. Unless stated otherwise, the standard reaction

mixture consisted of DHSA (one mole), 1-octanol (two moles), immobilized enzyme (15% w/w) and hexane all placed in a 5-litre stirred tank reactor. Samples were taken from the reaction mixture at specified times. The percentage conversion was determined by measuring the remaining fatty acids in the reaction mixture by titration with 0.01 M sodium hydroxide (NaOH) solution. The degree of conversion was calculated as:

$$\text{Conversion, } \% = [(M_0 - M_t)/(M_0 - M_b)] \times 100\%$$

where M_0 and M_t are the moles of NaOH consumed by titration of the mixture at the beginning (0 hr) and end of the reaction, respectively. The M_b is the moles of NaOH consumed by titration of the mixture without fatty acids.

Product Identification

The isolated product was identified by spectral studies [Fourier transform infrared (FTIR) and ^{13}C nuclear magnetic resonance (^{13}C NMR)]. The FTIR data were recorded on a Nicolet Magna-IR550 (Nicolet, Madison, WI) spectrophotometer and the ^{13}C NMR spectra on a Bruker DRX-300 (Karlsruhe, Germany) spectrometer at 300 MHz. The chemical shifts were expressed in ppm with tetramethylsilane as internal standard.

The spectral data for octyl-9,10-dihydroxystearate were, FTIR: 3440 (-OH), 2926 (-C-H), 1741 (-COO-), 1118 cm^{-1} (-C-O-C); ^{13}C NMR: 174.08 (-COO-), 74.47 (-CH-OH at C9), 74.66 (-CH-OH at C10), 64.48 (-O-CH_2-), 34.37-24.96 (-CH_2-), 26.68 (-$\underline{CH_2}CH_3$, alcohol chain), 22.65 (-$\underline{CH_2}CH_3$, acid chain), 14.12 ppm (CH_3) (Awang *et al*., 2000).

RESULTS AND DISCUSSION

Effect of Reaction Time and Temperature

Time course for formation of octyl-9,10-dihydroxystearate showed that 60 min was the optimum time for the reaction at 40°C-50°C, as longer reaction times up to 240 min did not increase the yield much beyond about 90% (*Figure 1*). At >60°C, the conversion decreased slightly, possibly due to the lipase being denatured (Yankah and Akoh, 2000; Hirata *et al*., 1990).

Effect of Enzyme Dose

The amount of enzyme plays a crucial role in any biocatalytic process, especially in large scale production. An enzyme concentration of about 10%, was found to be optimal for esterification of DHSA and 1-octanol (*Figure 2*) under the tested reaction conditions. Further increases in the enzyme concentration had little effect on the degree of esterification.

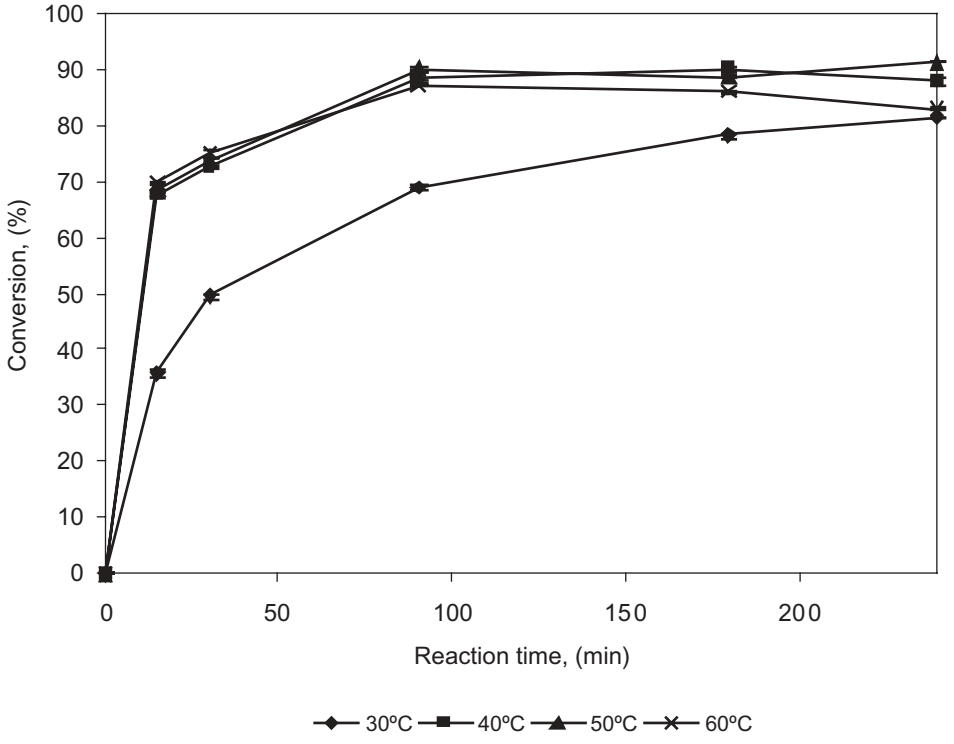

Figure 1. Time course of formation of octyl-9,10-dihydroxystearate by esterification of DHSA and 1-octanol at various reaction temperatures. Reaction conditions: enzyme concentration, 15% (w/w); agitation speed, 300 rpm.

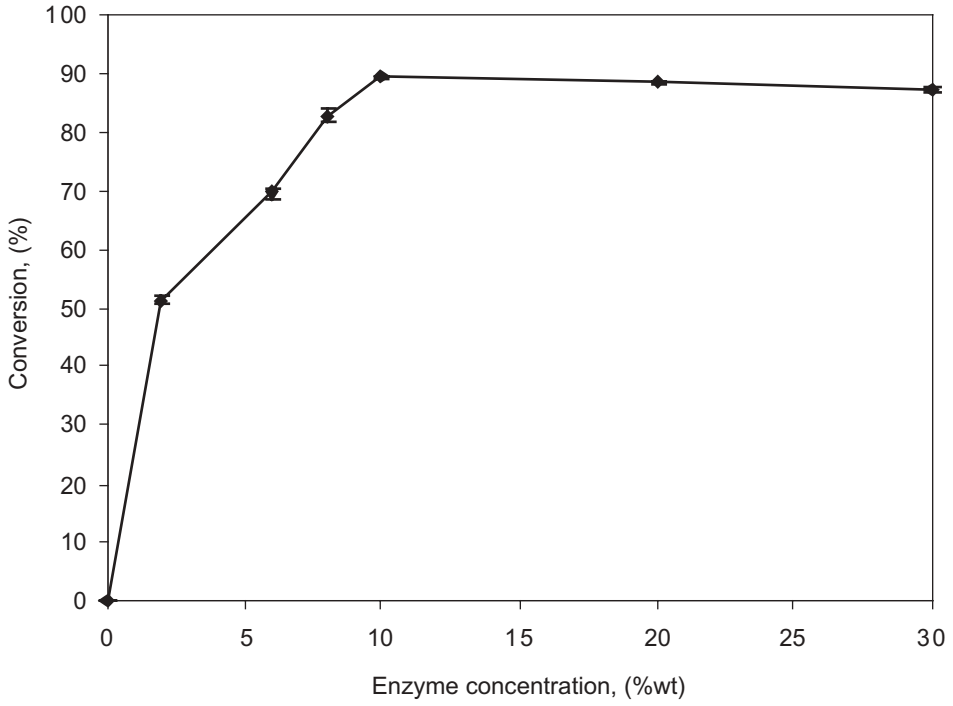

Figure 2. Effect of enzyme concentration on the esterification of DHSA and 1-octanol. Reaction conditions: reaction time, 3 hr; temperature, 50°C; agitation speed, 300 rpm.

Effect of Agitation Rate

The percentage conversion increased with the agitation speed from 150 rpm to 300 rpm, and peaked at about 400 rpm (*Figure 3*). Agitation facilitates interaction of the enzyme and substrates in a heterogeneous reaction. Moreover, it also increases the external mass transfer rates between the bulk phase of the reaction mixture and the surface of the enzyme (Akkara *et al*., 1999).

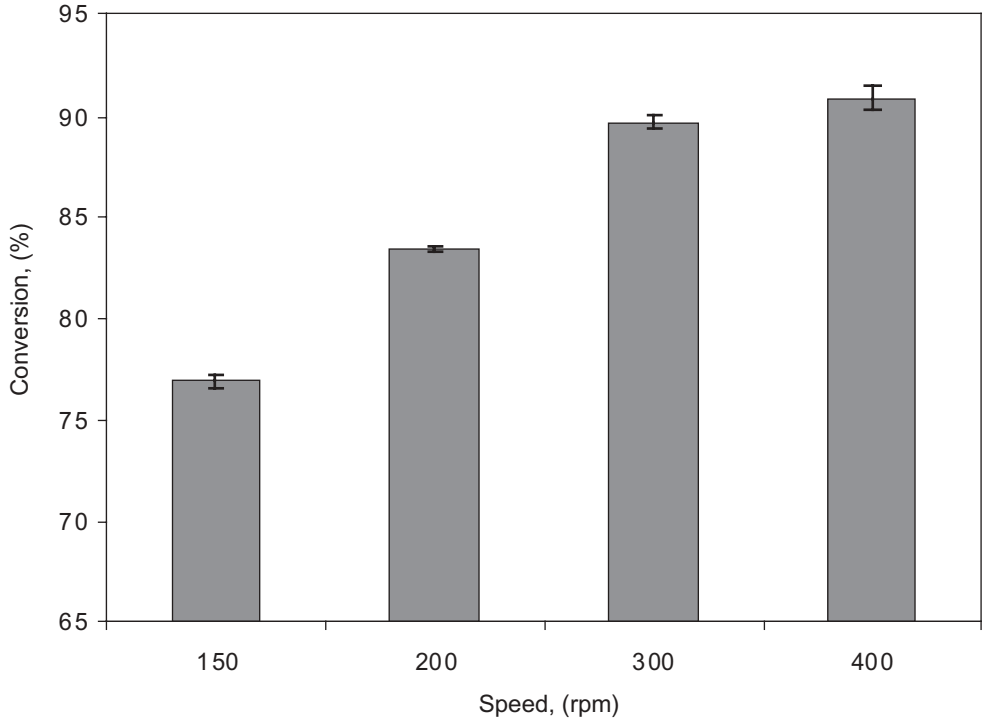

Figure 3. Effect of agitation speed on the esterification of DHSA and 1-octanol. Reaction conditions: reaction time, 3 hr; temperature, 50°C, enzyme concentration, 15% (w/w).

Effect of Number of Propeller Blades

Another purpose of the study was to find the most appropriate number of propeller blades for a simple 5-litre stirred tank reactor. The highest conversion to esters was achieved with a three-bladed propeller (94.1%) used as mixing device than with a two-bladed (92.9%) or four-bladed (91.7%) propeller. The three-bladed propeller provided good mixing with no stagnant areas in between the blades, but with four blades the plates were too close together and acted as a single wide blade, and flow to the middle was restricted, power input reduced and the tank became inadequately agitated (Akkara *et al*., 1999).

Operational Stability of Immobilized Enzyme in the Reaction System

In this work, the residual immobilized enzyme activity after repeated use was assessed in terms of conversion for each cycle. The enzyme still retained over 70% of its original activity even after five cycles (*Figure 4*). However, it then decreased more steeply to 50% after a further cycle, possibly due to contamination by traces of the substrates or products remaining in the matrix. This finding is in agreement with Liu and Shaw (1995) on the synthesis of propylene glycol monoesters.

CONCLUSION

This work shows that octyl-9,10-dihydroxystearate can be produced in large scale by Lipozyme IM-catalyzed reaction with 90% conversion. The optimal reaction conditions were a temperature 50°C, reaction time of 3 hr, catalyst concentration of about 10% (w/w) and agitation speed of 300 rpm.

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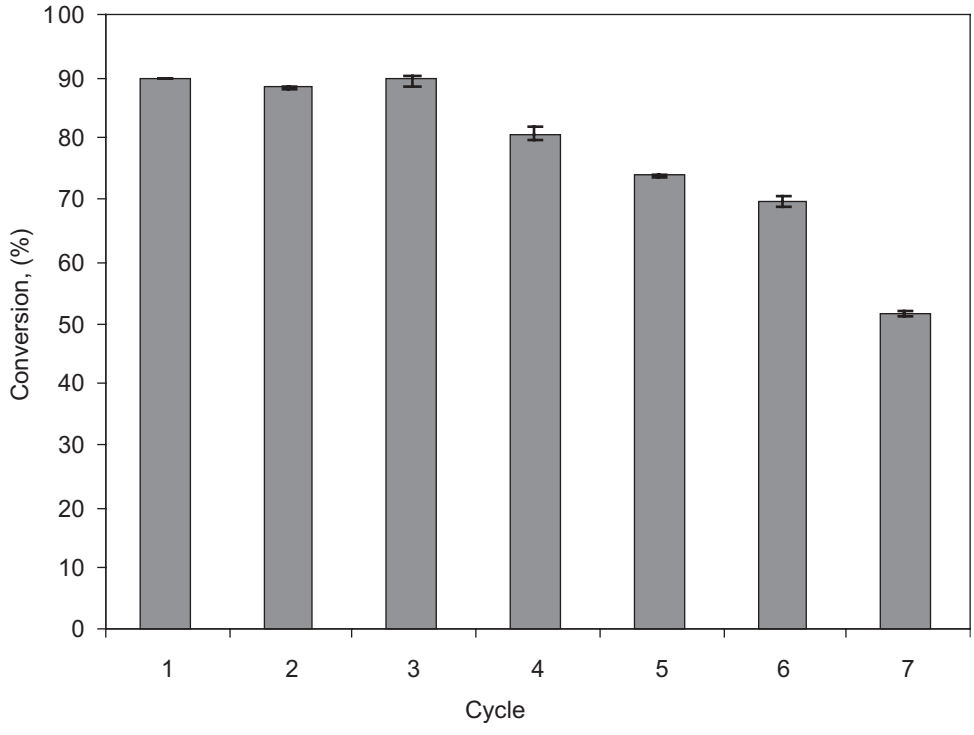

Figure 4. Reusability test for the immobilized enzyme in the esterification reaction system.

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