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LIPASE IMMOBILISED ONTO AMBERLITE XAD-7 FOR HYDROLYSIS OF OIL

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ABSTRACT

Lipase from *Candida rugosa* was immobilised onto Amberlite XAD-7 beads by physical adsorption. The hydrolytic activity of the immobilised lipase was monitored in the batch and continuous mode to assess for stability during repeated usage. Using olive oil (50% v/v in water) as the substrate, maximum hydrolysis can be obtained within 16 h at 28°C, using the batch mode. The immobilised enzyme can be repeatedly used for 20 cycles in a batch reactor system before activity dropped to less than 50% of initial activity. Using a packed bed system, the half-life was 80 cycles for the close system and 150 days for the open system.

INTRODUCTION

Lipase (glycerol ester hydrolase or triacylglycerol acylhydrolase, EC 3.1..1.3) catalyses random or regiospecific hydrolysis of triglycerides. Of greater interest is the reversible reaction in which glycerol ester and many other alcohol esters can be synthesised in organic media by this enzyme [1,2]. Although many enzymes have been utilised in large scale industrial processes, the use of lipases in this capacity is limited, due to its instability and the heterogeneous nature of its reactions. The technique of enzyme immobilisation may overcome the problem related to instability at high temperature and denaturation by organic solvents, in the industrial transformation of oleochemicals. Lipases from *Rhizopus arrhizus* has been immobilised onto diatomaceous earth[3] while the enzyme from *Thermomyces lanugonosus* has been immobilised to microporous membrane[4]. Kimura *et al.*[5] immobilised lipase within photocrosslinkable resin, as well as duolite, celite and controlled

pore glass. Huge-Jensen *et al.*[6] also used duolite resin to adsorb lipase. On the other hand, Pronk *et al.*[7] immobilised *Candida rugosa* lipase onto hydrophobic hollow fibres. Brady *et al.*[8] studied a series of support, with a hydrophobic nylon support (Accurel) showing high activity retention.

MATERIALS AND METHODS

Materials

Amberlite XAD-7 was obtained from Sigma Chemical Co. St. Louis, USA. All other reagents were of analytical grade. Lipase from *Candida rugosa* was also purchased from Sigma Chemical Co. and purified, as described below, before being immobilised.

Methods

Purification of lipase

Before immobilisation, commercial lipase was partially purified by 60% ammonium sulphate precipitation and the redissolved precipitate was fractionated through Sephadex G-200. A 15 fold purification (246 unit/mg) with a yield of 12% was obtained. It was homogeneous as judged by disk polyacrylamide-sodium dodecyl sulphate gel electrophoresis.

Immobilisation of lipase

One gram of Amberlite XAD-7 (air dried) was mixed with 2 ml of purified lipase (containing 4 mg protein and 984 unit of activity) and 8 ml water. The mixture was gently shaken for 30 min at room temperature. Immobilised lipase was washed with water and air-dried before use.

Substrate

For all studies, olive oil in water (1:1,v/v) was used.

Activity of enzyme

The activity of enzyme was measured by determining the free fatty acid liberated using olive oil as substrate[9]. Two point five ml substrate and 0.5 g immobilised enzyme was incubated at 28°C with shaking at 200 strokes per min with stroke diameter of 3.8 cm, for 30 min. The free fatty acids in the mixture were then estimated by titration with 0.05M NaOH to pH 10 using a Radiometer titration system (ABU 91 Autoburette/VIT 90 Titrator). One unit of lipase activity was defined as 1 μmole of fatty acid released per min.

Protein was determined by the method of Lowry *et al.*[10].

Progress of reaction

Fifty ml substrate (25 ml olive oil and 25 ml water) and 1 g immobilised lipase were used in a batch stirred reactor similar in configuration to that of Kimura *et al*[5]. The mixture was stirred to enable adequate mixing of water and oil phases as well as the immobilised enzyme beads. The experiment was carried out at 28° C. At 1 h interval, 1 ml sample was taken out and the free fatty acids released titrated.

Operational stability

Batch system : The reaction system and composition were similar to those used in the progress of reaction experiment above. At the end of each cycle (24 h for convenience) the substrate was completely drained from the reactor, and replaced with an equivolume of fresh substrate. Two ml sample was assayed for free fatty acid released at the end of each cycle.

Packed bed reactor

Closed system : The system is similar to the batch system except that the substrate was pumped through a column (1.5 x 17 cm) packed with immobilised enzyme beads (5 g). One hundred ml substrate was continuously recycled through the column at 0.12 ml/min. At the end of 24 h, the substrate was replaced with an equivolume fresh substrate. Two ml sample was taken for assay at the end of each cycle.

Open system : The open system is similar to the closed system except that the substrate was not recycled (single pass technique). At specific time interval, 2 ml eluant was collected and assayed for free fatty acid liberated. Flow rate was 0.5 ml/min.

RESULTS AND DISCUSSION

Amberlite XAD-7 is one of the commercially available absorbents and classified as a polymer with an intermediate polarity. It is a porous polymer with a high surface area and has a good physical durability suitable for batch or column operations. It has been used for immobilisation of lipases[11] though immobilisation was based on adsorption of protein from buffered solution. As such, the activity of immobilised lipase obtained by adsorption in buffered and non-buffered lipase solutions was tested. The activity of immobilised lipase is 10% less (when the adsorption was carried out in acetate, pH 5), and 20% less (when the adsorption was carried out in tris-HCl, pH 7.0), than the activity

of the derivatised enzyme obtained from adsorption of protein in water. Ionic strength of the protein solution obviously played an important role in the adsorption process but the authors did not investigate further. The hydrophobicity or hydrophilicity of the matrix surface has a critical function in the adsorption capacity and both characteristics have been considered as being advantages for lipase immobilisation. The high lipase activity on this polymer suggests that it has suitable surface properties that enable the water-oil emulsion to approach the enzyme on the polymer. The more hydrophobic surface needed for lipolytic reaction has been suggested by Kimura *et al.*[5] and our experiments showed that the activity of the enzyme was low on the less polar polymer (Amberlite XAD-2 and XAD-4) than Amberlite XAD-7 although considerable amount of the protein enzyme has been immobilised. It is felt that there is a certain point where both characteristics meet, to achieve maximum adsorption as well as to attain optimum activity. Under the condition of immobilisation 100% protein was adsorbed. However, the specific activity obtained was about 200 unit/g resin. This is about 20% of the input activity. It was also found that if crude enzyme were used without further purification, the activity of the immobilised enzyme prepared would be very low. The physical adsorption technique often causes little or no conformational changes on the enzymes, or seldom destroys the active centre of the enzymes. The technique is usually simple and effective. In several cases, the activity of immobilised enzyme is higher than free enzyme[12,13]. It has also been reported that enzyme immobilised by this technique is stable during the storage[8]. However, the main disadvantage is that adsorbed enzyme may leak from matrix during operation since the binding force between enzyme and carrier is fairly weak. To test the stability of this immobilised enzyme three techniques were used, i.e. batch, close and open systems and the results are shown in Figures 2, 3 and 4. Studies on incubation period showed that maximum conversion can be achieved within 16 h (Figure 1) under the conditions studied. Repeated use of the immobilised lipase showed a steady but gradual loss in activity possibly due to leaching. Figure 2 shows that nearly 20 cycles could be achieved before the activity dropped to less than 50% of initial activity.

Similar trend can be seen with the packed bed reactor. In the case of the close system, nearly 80 cycles can be achieved before 50% loss in activity (Figure 3) whereas the half-life for the open system is about 150 days (Figure 4). It is interesting to note that the maximum conversion achieved in the batch and close system was about 65% of the maximum theoretical value, whereas in the open (single pass technique), the conversion was around 90% of theoretical value. It seems to indicate product inhibition effect. In the experiment by Kimura *et al.*[5] the process was carried out for 10 cycles but for a longer reaction time (48 h). We obtained 90% hydrolysis, a result comparable with that of Kimura *et al.*[5] Pronk *et al.*,[7] using lipase immobilised

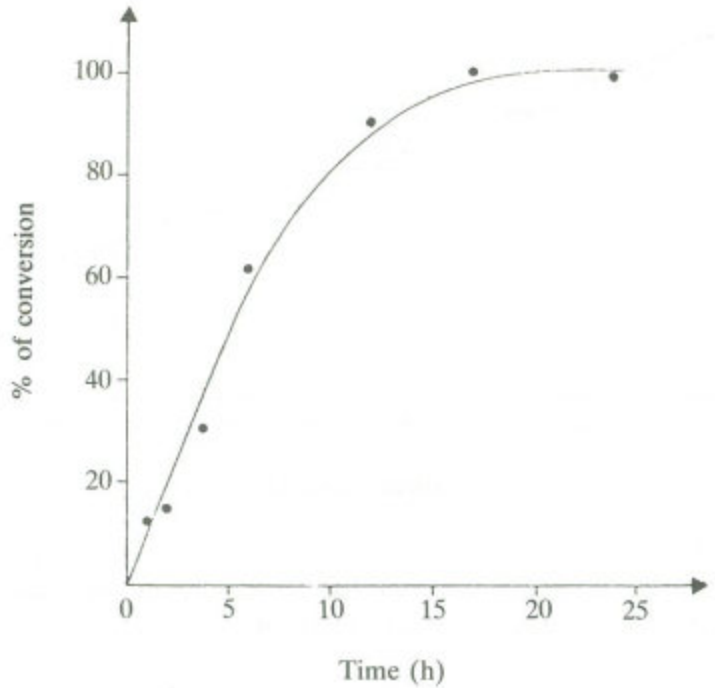

Fig. 1 Hydrolysis of triglycerides by immobilised lipase. The maximum conversion achieved was around 65% of the maximum theoretical value.

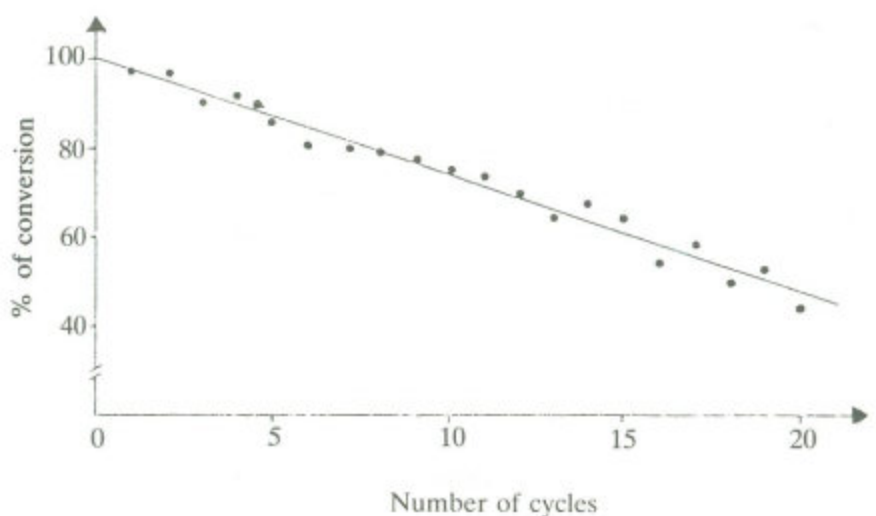

Fig. 2 Repeated use of immobilised lipase by batch wise assay. The maximum conversion achieved was about 65% of the maximum theoretical value. Each cycle was for 24 h duration.

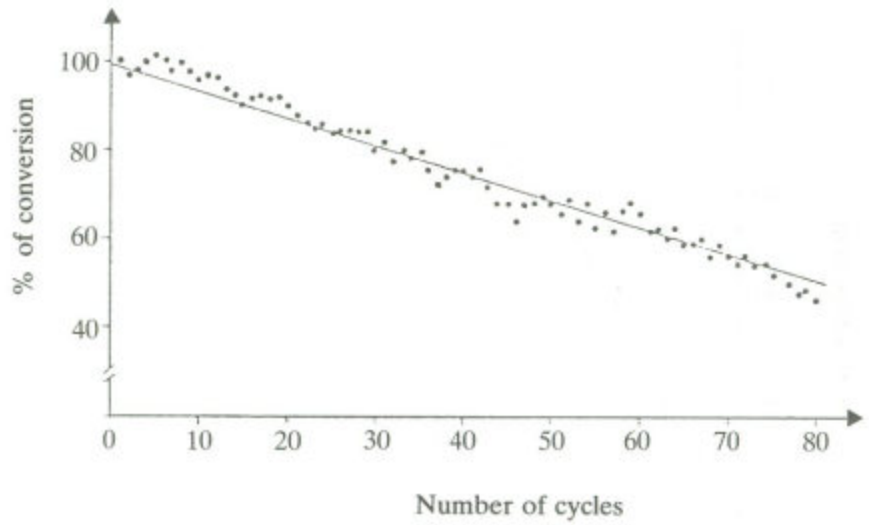

Fig. 3 Continuous hydrolysis of triglycerides in a packed bed system (closed). The maximum conversion achieved was about 65% of the maximum theoretical value. Each cycle was for 24 h duration.

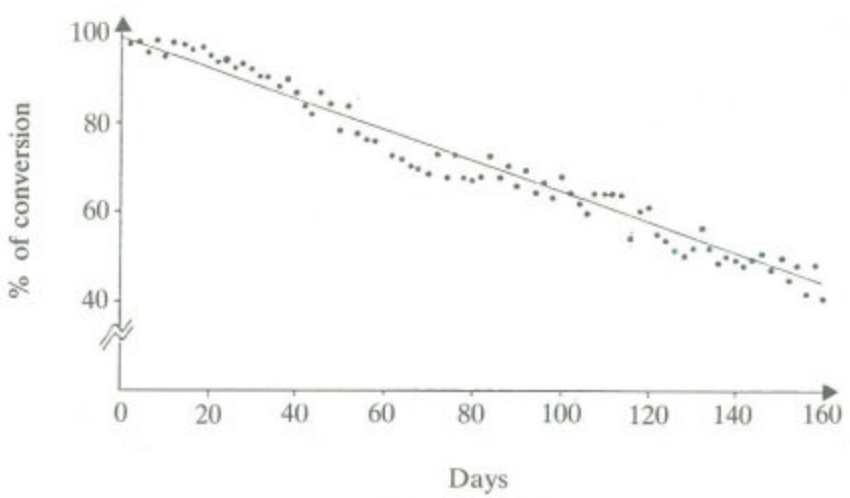

Fig. 4 Continuous hydrolysis of triglycerides in a packed bed system (open). The maximum conversion achieved was about 90% of the maximum theoretical value. Sampling was done on daily basis.

onto membrane, obtained a half-life of 43 days. Its operational temperature was 30°C. Brady *et al.*[8] showed that lipase immobilised onto Accurel fibers has a half-life of 200 h. Ibrahim *et al.*,[11] who used XAD-7 to immobilise lipase, showed that the adsorbed enzyme can be used for 2 runs with 100% activity, but the half-life was extended to 7 runs if the adsorbed enzyme was derivatised with glutaraldehyde. Hoq *et al.*,[14] also using hydrophobic membrane reactor, obtained a half-life of only 2 days at the operational temperature of 40°C.

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