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Process Biochemistry 40 (2005) 1525–1530

PROCESS BIOCHEMISTRY

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Continuous production of monoacylglycerols by glycerolysis of palm olein with immobilized lipase

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Received 12 April 2003; accepted 12 December 2003

Abstract

Nine commercial lipases from *Pseudomonas* sp. (lipase PS), *Pseudomonas fluorescens* (lipase AK), *Candida rugosa* (lipase AY), *Rhizopus delemar* (lipase D), *Mucor javanicus* (lipase M), *Rhizopus oryzae* (lipase F), *C. rugosa* (lipase OF) *Alcaligenes* sp. (lipase PL) and *Chromobacterium viscosum* (lipase LP) were screened for production of monoacylglycerols (MAG). Lipase PS was the most suitable enzyme for glycerolysis of palm olein with glycerol. This lipase had hydrolytic activity 10.42 U/mg and provided a high yield of MAG with 28.05% at 45 °C. Celite, silica gel, $CaCO_3$, Accurel EP100 and activated charcoal were used as supports to immobilize lipase PS. Accurel EP100 (<200 μm) was the best support. The optimum conditions for immobilization included the enzyme concentration of 50 U/ml and immobilization temperature at 30 °C for 30 min. When 5.0 ml enzyme solution was mixed with 0.5 g support the immobilized activity was 0.23 U/mg support and immobilized yield was 45.38%. The immobilized lipase PS (IM-PS) on Accurel had optimal activity at 45–65 °C and more than 90.0% of the activity remained after incubated at 45 °C for 24 h. In batch production 20.74% MAG was obtained at 45 °C for 24 h. The continuous production of MAG was performed with IM-PS (350 U) in the packed-bed reactor (PBR) (0.68 cm ID, 25 cm long) and a continuous stirred-tank reactor (CSTR) (4.5 cm ID, 6.0 cm height) for 96 h at 45 °C. When the flow rate of the substrate mixture was 0.02 ml/min the average yields of MAG were 14.01 and 14.34% in PBR and CSTR, respectively.

Keywords: Glycerolysis; Immobilized lipase; Monoacylglycerols; *Pseudomonas* sp.; Lipase

1. Introduction

Monoacylglycerols (MAG) are the most widely used emulsifiers in food, pharmaceutical, and cosmetic industries [1]. In the pharmaceutical industry, MAG are used as binders in tablets and as emollients for transdermal, slow-release drugs [2]. In the food industry, MAG are the most common food emulsifiers for bakery products, magarines, dairy products, confectionary and sauces, etc. [2]. In the cosmetic industry, they are used as texturizing agents and for improving the consistency of creams and lotions [3]. Monopentadecanoylglycerol is used as a hair care additive [4]. In addition, owing to their excellent lubricant and plasticizing properties, monoglycerides are used in textile processing, production of plastics and formulation of oil for different types of machinery [5].

Currently, MAG are manufactured on an industrial scale by continuous chemical glycerolysis of fats and oils at high temperature (220–250 °C) employing inorganic alkaline catalysts under a nitrogen gas atmosphere [6]. The products produced by this strategy have several drawbacks [4]. A molar excess of glycerol is used and because the reaction temperature is greater than 220 °C, dark-colored by-products with an undesirable flavor are formed. Moreover, the yield of MAG is rather low (30–40%) [7]. Molecular distillation is necessary because MAG need to be highly pure in the food industry, since they have better emulsifying properties than a mixture of different acylglycerols [4]. Recently, many approaches have been investigated in the enzymic synthesis of MAG [4]. These are selective hydrolysis using 1,3-regiospecific lipases [8], esterification of fatty acids or transesterification of fatty esters with glycerol [9], and the glycerolysis of fats or oils [10].

At present, the main disadvantage of the use of lipase in industrial applications is the cost of the enzyme. To over-

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doi:10.1016/j.procbio.2003.12.002

come this problem, the lipase is employed in immobilized form because it would allow the reutilization of the enzyme. By immobilizing the enzyme, it is possible to operate enzymic processes continuously. Enzyme immobilization has been accomplished by chemical and physical attachment to solid surfaces [11]. Many supports, such as calcium carbonate ($CaCO_3$) [12], Celite [13], ion exchange resin [3] and Accurel [14] have been used for immobilized lipase. Several approaches for the synthesis of MAG by immobilized lipase have been reported [15–17]. In particularly, glycerolysis of palm oil with glycerol by immobilized lipase has been used for MAG production [7,18,19]. However, the reactions were performed in a batch stirred-tank reactor (BSTR) using a solid phase system. Thus, continuous production was impossible.

The aims of this research were to select lipase for glycerolysis and optimize immobilization as well as investigate continuous glycerolysis of palm olein for MAG production by immobilized lipase in packed-bed reactor (PBR) and stirred-tank reactor (CSTR).

2. Materials and methods

2.1. Materials

Lipase PS (*Pseudomonas* sp.), lipase AK (*Pseudomonas fluorescens*), lipase AY (*Candida rugosa*), lipase D (*Rhizopus delemar*), lipase M (*Mucor javanicus*), lipase F (*Rhizopus oryzae*) were gifts from Amano Pharmaceutical Co. Ltd., Nagoya, Japan. Lipase OF (*C. rugosa*) and lipase PL (*Alcaligenes* sp.) were gifts from Meito Sangyo Co. Ltd., Japan. Lipase LP (*Chromobacterium viscosum*) was gift from Asahi Chemical Industry Co. Ltd., Japan. The supports were Celite 545 (200 μm) from Wako Pure Chemical Industries, Ltd., Silica gel 60 (200 μm) from Merck Co. Ltd., and $CaCO_3$ (Softon 3200) from Shiraishi Calcium Co. Ltd. Polypropylene powder EP100 (Accurel) was a gift from Akzo Nobel (Obernburg, Germany). Activated charcoal was purchased from Fluka Chemical Co. Ltd. Palm olein was purchased from Morakot Industry Co. Ltd., Thailand. All other chemicals were also obtained from commercial sources.

2.2. Immobilization

The support in powdered form (0.5 g) was added to 5.0 ml lipase solution containing approximately 100 U/ml enzyme and stirred with a magnetic bar at 100 rpm for 1 h. Afterwards, 5.0 ml of 0.1 M phosphate buffer pH 7.0 was added and the suspension was filtered through a Buchner funnel. The immobilized enzyme was washed on the filter paper with another 5.0 ml of 0.1 M phosphate buffer pH 7.0 and dried in a vacuum desiccator for 8 h. For this immobilization study, the immobilized yield was calculated using the following formula:

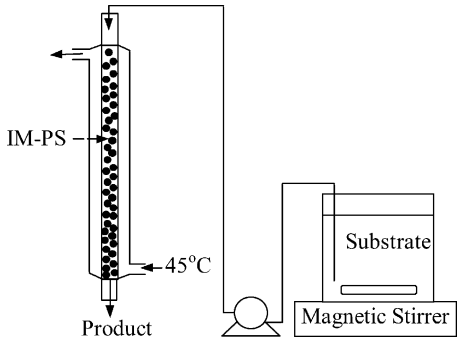

Fig. 1. Schematic diagram for continuous glycerolysis of palm oil by IM-PS in PBR at 45 °C.

$$\text{immobilized yield} = \frac{\text{total immobilized activity (U)}}{\text{total initial soluble enzyme activity (U)}} \times 100$$

2.3. Glycerolysis

Glycerolysis experiments were carried out in batch and continuous systems. The substrate mixture consisted of palm olein and glycerol containing 4.0% (w/w) water. The glycerol to palm olein molar ratio was 2.7 [13]. In the batch system, the substrate mixture was mixed with soluble or immobilized lipase with a magnetic stirrer at 300 rpm. In the continuous system, the substrate mixture was stirred at 300 rpm by magnetic stirrer and introduced into the PBR or CSTR reactor with a peristaltic pump. The reaction was maintained at 45 °C by water circulation. For the PBR, the immobilized lipase (1500 mg) was packed in a jacketed column (0.68 cm ID, 25.0 cm long). The substrate mixture was mixed well and introduced to the top of the column at a flow rate of 0.02 ml/min and the product was removed at the bottom of the column (Fig. 1). For the CSTR, the immobilized lipase (1500 mg) was placed in a jacketed cylindrical vessel (4.5 cm ID, 6.0 cm height) and agitated at 300 rpm. The substrate mixture was introduced to the top of the vessel with 0.02 ml/min of the flow rate and the product was removed at the bottom of the vessel (Fig. 2).

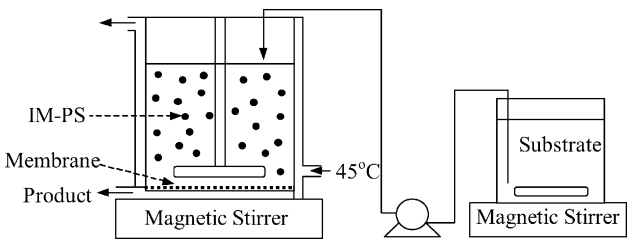

Fig. 2. Schematic diagram for continuous glycerolysis of palm oil by IM-PS in CSTR at 45 °C.