Journal of Environmental Chemical Engineering 4 (2016) 1797–1809

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece

Comparative study on hydrolysis of oils by lipase immobilized biocatalytic PS membranes using biphasic enzyme membrane reactor

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ARTICLE INFO

Article history:
Received 21 December 2015
Received in revised form 4 February 2016
Accepted 5 March 2016
Available online 7 March 2016

Keywords:
Lipase
Immobilization
Green chemistry
Bioreactor

ABSTRACT

In the present study the hydrolysis of olive, palm and castor oil had been carried out to yield valuable free fatty acids by using immobilized *Candida rugosa* lipase. This study was designed to achieve high yield of lipase immobilization by investigating different factors (applied pressure, time, and lipase concentration) for ultrafiltration process of immobilization. A biphasic enzyme membrane bioreactor was fabricated having aqueous-organic two-phase system with lipase immobilized polysulfone. Effect of operating variables on the performance of this biphasic EMR was investigated with the hydrolysis of olive oil, palm oil and castor oil.

It was found that the optimal conditions for highest degree of hydrolysis (%) were temperature 37 °C, pH 8.0, at concentration of 0.05 M in iso-octane and initial reaction time was 24 h. In optimal conditions, degree of hydrolysis for olive oil, palm oil and castor oil was 38.5%, 35.4% and 21.2% respectively. Reusability feature was also studied and only ~20% decrease in activity was observed after five cycles.

1. Introduction

Lipase (triacylglycerol ester hydrolase, EC 3.1.1.3) has many industrial applications, in particular, hydrolysis of vegetable oil is gaining interest in the oleochemical industry as it offers advantages over conventional chemical reactions [1–4].

Glycerol and fatty acids are widely used as raw materials in food, cosmetics and pharmaceutical industries [5]. Castor oil is a unique vegetable oil as it contains high amounts (90%) of a hydroxy monounsaturated fatty acid named ricinoleic acid. This industrially important acid can be obtained by hydrolysis of castor oil [6]. Existing methods for production of fatty acid are based on chemical and physical methods, they hydrolyze oils at temperature and pressure of 250 °C and 50 bar, respectively. It results into extremely dark fatty acids and discolored aqueous glycerol solution due to polymerization of fat and byproducts formation. Both the hydrolysis and the subsequent distillation of fatty acids to produce pure products are energy intensive process [7,8].

These different disadvantages of conventional hydrolysis processes can be avoided by lipase-catalyzed process. Lipase catalyzed hydrolysis is an energy saving method that only requires a mild temperature, simple operational procedure and low cost and minimize thermal degradation of the products. The natural fatty acids produced from the natural enzymatic techniques are more preferable, especially in food, cosmetics and pharmaceutical industries. The use of highly active lipase from *Candida rugosa* has been widely studied for the purpose of fat and oil hydrolysis due to its high activity, both in hydrolysis as well as synthesis [9,10]. The use of immobilized lipase offers many advantages such as enzyme reusability, high thermal and operational stability of enzyme through a range of pH values and ionic concentrations, less downstream process and predictable production yield. They have higher temperature optima compared to their native counterparts as immobilization provides a more rigid external backbone for lipase molecule [11–14].

Membrane technology has developed during the last two decades and its applications have expanded in different industrial sectors [7,15] (viz. chemical, petrochemical, food, pharmaceuticals, electronics, biotechnological etc.). Membrane separation processes are considered most suitable and economical amongst all. Similarly, membrane bioreactors are considered most suitable for carrying out enzyme catalyzed reactions as it offers synergistic effect of enzyme technology i.e. mild reaction conditions, high catalytic activity and inherent substrate selectivity as well as membrane separation. Polysulfone membrane has excellent resistance to inorganic acids and bases and can be used for food, water and medical applications. It is hydrophobic in nature and it was experimentally proven that adsorption of lipase is higher on hydrophobic membranes than hydrophilic ones [16].

As lipase substrates (oils and fats) have limited solubility in water, the Enzyme Membrane Reactors (EMRs) are biphasic

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http://dx.doi.org/10.1016/j.jece.2016.03.007
2213-3437/

reactors and lipase is known to act on oil–water interface. The EMRs are ideal for such situation as the immobilized membrane forms the boundary between the two phases.

In the present study, lipase was immobilized by the ultrafiltration process and most lipase molecules were trapped in the pores and surface of PS membranes. It was followed by post treatment with glutaraldehyde crosslinking agent. As lipase immobilized by physical adsorption can easily leached out from support, resulting in enzyme loss and poor operational so, crosslinking with glutaraldehyde give stability to immobilized lipase and minimize leaching. The lipase loading was up to 2.05 mg/cm^2 in this work. The most suitable PS (Polysulfone) asymmetric membranes (laboratory made) are used as the support for lipase immobilization. An enzymatic biphasic membrane reactor using PS membrane was introduced for hydrolysis of olive oil, palm oil and castor oil. The reactor offered high specific surface area, simultaneous reaction, reuse of the enzyme and continuous operation of the process.

2. Experimental

2.1. Materials

Polysulfone polymer (Udel P-3500, Solvay Advanced Polymer, India), non-woven Polyester fabric (Filtration Sciences Corporation, USA) and di-methyl formamide (DMF) (Qualigen, India) were used for membrane fabrication. *C. rugosa* lipase (EC 3.1.1.3) (Sigma-Aldrich, USA), and glutaraldehyde (SD fine Chem., India) were used for immobilization. Folin reagent (SD fine Chem., India), di-sodium tartarate (SD fine Chem., India), BSA fraction V (Sigma-Aldrich, USA) were used for protein estimation. Acacia powder (SD fine Chem, India), refined olive oil (SRL, India), Palm oil, castor oil, Iso-octane (SD fine Chem, India), hexane, and heptane were procured.

In all the experiments reverse osmosis treated water is used.

3. Methods

3.1. Preparation of polysulfone membrane

Asymmetric ultrafiltration membranes were prepared from polysulfone polymer by phase inversion technique. Homogeneous solution of 15% w/w PS in solvent dimethyl formamide (DMF) was prepared by continuous stirring. The solution was cast with the help of a proto-type casting machine in uniform thickness on a support such as non-woven polyester fabric and immediately coagulated in the gelation solution (water mixed with sodium lauryl sulfate). The membranes were kept in the gelation bath for at least 3 h to complete the phase inversion process. Then membranes were washed and dried. The process parameters viz. composition of polymer solution, types of polymer solvent, thickness of polymer film, composition of gelation solution etc. are required to be controlled precisely to get membrane of required porosity and pore structure.

3.2. Immobilization and estimation of amount of lipase on membranes

Membranes of different polymer concentration (13, 15 or 18% w/w) were used for the immobilization of lipase by ultrafiltration process. The set up was presented in our earlier report [17]. Again lipase loading was optimized using different concentration of lipase i.e. 2, 2.5 and 3 mg/ml. Membrane area 9.1 cm^2 were taken for ultrafiltration using lipase solution prepared in 0.1 M of phosphate buffer of pH 7.0 for 12 h at 0.1 MPa pressure. Membranes were then removed from ultrafiltration kit and washed with water. The membranes were then immersed in aqueous solution of glutaraldehyde (2.5%) for 4 h to stabilize the lipase immobilized on it. We had optimized the crosslinker's concentration for lipase immobilization in our earlier reports [18]. Then the samples were removed from glutaraldehyde solution and washed with reverse osmosis treated water to remove loosely adhered lipase from the membrane surface. We quantified the correct immobilization by estimating protein in washings also. The process was repeated and checked for protein content till no protein was found in decant.

The lipase immobilized biocatalytic membranes were stored in buffer solution.

The amount of lipase immobilized on the membrane was calculated from the difference in concentration of lipase in the initial and decanted solutions. The concentration of lipase in solutions was determined in accordance to Lowry method of protein estimation using Bovine Serum Albumin as standard protein [19].

3.3. Biphasic enzyme membrane reactor

The membrane reactor was H-shaped. The two arms were filled with organic and aqueous phases. It was made up of glass and consisted of two identical flat channels with arrangement for holding membrane (membrane area: 9.1 cm^2, membrane shape: circular disc) between two compartments. The lipase immobilized membranes were fixed at the junction of the two arms. As the two arms were filled with two phases it is called biphasic one. The two phases were separated by a biocatalytic membrane on which lipase was immobilized. It was a combination of a bioreactor and membrane separation step. Membrane bioreactor offers in-situ separation capability that is lacking in other types of reactors. Fig. 1

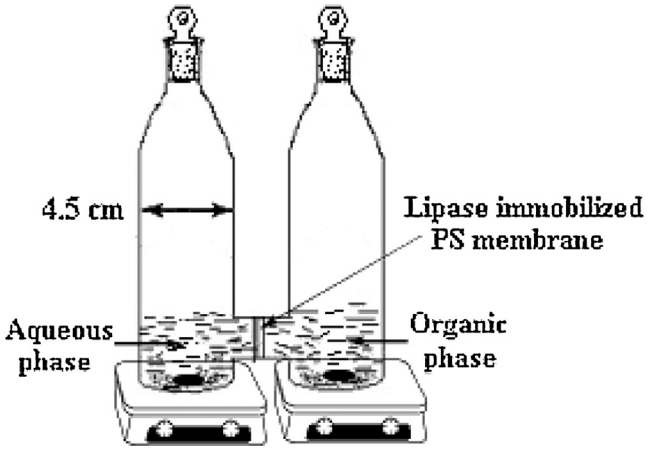

Fig. 1. Experimental set-up of biphasic membrane reactor.