Bioresource Technology 247 (2018) 568–575

Contents lists available at ScienceDirect

Bioresource Technology

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

# Conversion of levulinic acid into alkyl levulinates: Using lipase immobilized on meso-molding three-dimensional macroporous organosilica as catalyst

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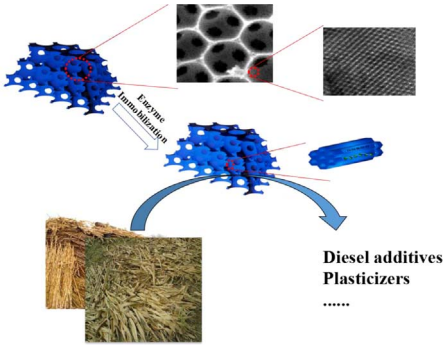
## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Keywords:*
Levulinic acid
Alkyl levulinates
Immobilized lipase
Meso-molding
Three-dimensional macroporous
Organosilica

## ABSTRACT

For conversion of biomass-derived levulinic acid into alkyl levulinates, a novel kind of lipase-based biocatalyst was prepared through immobilized lipase B from *C. antarctica* (CALB) on organosilica material with highly ordered 3D macroporous organosilica frameworks and a 2D hexagonal meso-structure (named 3DOM/m-OS) for the first time. The catalytic performance of the immobilized lipase (NER@3DOM/m-OS) was investigated. NER@3DOM/m-OS was used as biocatalyst to catalyze the esterification reaction between levulinic acid (LA) and *n*-butanol. Under optimized reaction conditions, 74.59% of ester yield was achieved after 12 h of reaction. NER@3DOM/m-OS was also used to production of other alkyl levulinates, the ester yields increased to 84.51% (octyl levulinate) and 91.14% (dodecyl levulinate), respectively. When NER@3DOM/m-OS was used repeatedly in batch reactions, the ester yields of *n*-butyl, octyl, and dodecyl levulinate could retain 46.18%, 82.33% and 81.25% after 9 reaction cycles, respectively, which was better than commercial lipase Novozym 435 under the same condition.

## 1. Introduction

Levulinic acid (LA) is a versatile renewable platform molecule considered to possess potential industrial applications due to its high chemical reactivity (Jeong et al., 2017). The levulinate esters obtained by esterification of LA with alcohols have various potential applications in diesel additives, plasticizers and flavoring industry (Chang et al., 2012; Jones et al., 2016; Nandiwale et al., 2013; Weingarten et al., 2012). Usually, the esterification reaction is catalyzed by an acid catalyst (such as sulphuric or polyphosphoric acid), but there are many

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http://dx.doi.org/10.1016/j.biortech.2017.08.134
Received 26 June 2017; Received in revised form 19 August 2017; Accepted 21 August 2017
Available online 10 September 2017
0960-8524/ 

disadvantages like highly acidic condition, high temperature, heavy down-streaming process, etc (Huang et al., 2016; Unlu et al., 2017). While enzymatic synthesis offers several benefits such as mild reaction conditions, low energy requirements, minimal waste disposal and no (less) by-product formation (Lee et al., 2010).

Lipases (triacylglycerol ester hydrolase, EC 3.1.1.3) are extensively used in catalyzing hydrolysis, alcoholysis, esterification, and transesterification reactions due to their excellent chemo-, regio-, and stereoselective properties (Foukis et al., 2017; Singh and Mukhopadhyay, 2012). However, its industrial application is often hampered by low stability, poor reusability and high cost (Jiang et al., 2014a; Jiang et al., 2014b). In order to overcome these drawbacks, facilitate product purification, and realize the repeated or continuous use of lipases, native lipases are usually immobilized on solid supports (Bencze et al., 2016; Tielmann et al., 2014). Among the solid supports reported to date, mesoporous silica materials appear to be the ideal candidates for enzyme immobilization due to their controllable morphologies, mesostructures, porosities, and ease of functionalization (Gao et al., 2013). Hundreds of reports regarding the immobilization of lipases on different mesoporous silica materials and their related applications have been published within the last two decades (Ali et al., 2017; Popat et al., 2011; Sun et al., 2014; Zheng et al., 2017). While mesoporous silica materials are extensively investigated, only a few literatures have been published about physically or covalently immobilized lipases onto three-dimensionally ordered macroporous (3DOM) silica materials (Jiang et al., 2014a; Jiang et al., 2014b). Owing to the interconnected pores, enhanced pore volume and limited tortuosity, 3DOM silica materials are recognized as a promising platform for enzyme immobilization (Gornowich and Blanchard, 2012; Jiang et al., 2015). In our previous report, we have prepared lipase CLEAs in the macropores of 3DOM silica. Whether in aqueous or organic phases, the lipase CLEAs in 3DOM silica exhibited excellent thermal and mechanical stability (Jiang et al., 2014a; Jiang et al., 2014b). However, the lower specific surface area of 3DOM silica than that of the mesoporous silica may limit the broader applications. Thus, we prepared meso-molding 3DOM silica materials (3DOM/m-S) for lipase immobilization (Jiang et al., 2016). Compared to lipase immobilized on 3DOM silica, the thermal and long-term storage stabilities of lipase immobilized on 3DOM/m-S in organic solvent were improved. This can be attributed to the confined space of the mesopores on the 3DOM/m-S supports that may provide the stability necessary for lipase to retain its activity and selectivity in organic solvents (Jiang et al., 2016). Considering these clear advantages and the growing interest in 3DOM/m-S, immobilization of lipase on meso-molding 3DOM supports deserves further exploration.

The surface properties of the chosen support also play a key role for the stabilization and the performance of the immobilized lipase (Hartmann and Kostrov, 2013). As is well known, lipases have a hydrophobic domain in the form of a lid, which is responsible for their interfacial activation (Rehm et al., 2010). Thus, supports with hydrophobic surfaces could improve lipase immobilization and enhance the activity and stability. For example, periodic mesoporous organosilicas (PMOs) are demonstrated to be excellent supports for lipase immobilization. Compared to lipase immobilized on pure silica supports, PMOs showed promising adsorption capacities and the immobilized lipase exhibited improved activity and stability (Zhou et al., 2012, 2011). In this regard, we believe that the designed meso-molding 3DOM organosilica which combined the advantages of meso-molding 3DOM supports with PMOs can provide a very promising platform for lipase immobilization. However, to the best of our knowledge, no literature concerning enzymes immobilized on meso-molding 3DOM organosilica has been published until now.

Thus, for the first time, the meso-molding 3DOM organosilica (3DOM/m-OS) materials were prepared by using polystyrene (PS) colloidal crystals as the hard template and amphiphilic triblock copolymers (P123) as the soft template. The obtained 3DOM/m-OS materials that combine the advantages of both macropores and mesopores may provide potential alternative to conventional 3DOM materials. Moreover, the framework of organosilica could improve the catalytic performance of lipase. Note that lipase B from *C. antarctica* (CALB) lacks hydrophobic lid (Trodler and Pleiss, 2008), but it still has a hydrophobic domain. Hydrophobic materials could improve activity of CALB by driving immobilization and substrate accessibility (Shah et al., 2015). Thus, CALB was chosen as model enzyme and immobilized onto 3DOM/m-OS by the adsorption approach (CALB@3DOM/m-OS) and the 'nanoscale enzyme reactor' approach (NER@3DOM/m-OS), respectively (Kim et al., 2008). The catalytic performances (activity, thermal stability, and long-term stability) of the two immobilized lipases were investigated in detail. Subsequently, using NER@3DOM/m-OS as biocatalyst, the esterification between LA and *n*-butanol was studied by optimizing temperature, LA/*n*-butanol molar ratio and enzyme amount. Furthermore, the esterification and reusability between LA and alcohols with different chain length were also explored extensively.

## 2. Materials and methods

### 2.1. Materials

Lipase B from *C. antarctica* in liquid form and Novozym 435 (N435) were purchased from Novozymes. Triblock copolymer $PEO_{20}PPO_{70}PEO_{20}$ (P123) was purchased from Sigma-Aldrich. 4-nitrophenyl palmitate (p-NPP) was purchased from Alfa Aesar Chemical Co., Ltd. (Tianjin, China). Levulinic acid was purchased from Tokyo Chemical Industry Co., Ltd. (Shanghai, China). Bis(triethoxysilyl)ethane (BTEE) was purchased from Xiya Chemical Reagent Co., Ltd. (Shandong, China). Glutaraldehyde (GA), tetraethyl orthosilicate (TEOS), HCl (37%), lauric acid, isooctane, ethanol, and acetone were purchased from Fengchuan Chemical Reagent Co., Ltd. (Tianjin, China). *N*-butanol, octanol and dodecanol were purchased from Damao Chemical Reagent Co., Ltd. (Tianjin, China). All the chemicals and reagents were analytical grade.

### 2.2. Preparations of 3DOM/m-OS

PS colloidal crystals with diameter of 500 nm were prepared as previously reported (Holland et al., 1998). Subsequently, the 3DOM/m-OS was synthesized by dual templating method, where P123 and close-packed ordered PS colloidal crystals were as the mesostructure-directing agent and the hard template for a 3DOM structure, respectively. Firstly, P123 of 1 g was added into BTEE of 2 mL under magnetic stirring at 50 °C. Then, 0.05 M HCl of 200 μL and distilled water of 1 mL were added into the above mixture with vigorous stirring for several minutes to obtain precursor solution. After that, an amount of PS colloidal crystals were half-immersed into the above precursor solution. Subsequently, the composites were kept in vacuum overnight at room temperature. In order to remove templates by solvent extraction, the composites were redispersed in ethyl acetate and anhydrous ethanol, and refluxed in a Soxhlet apparatus for 3 days.

### 2.3. Determination of enzyme activity

The hydrolytic activities of the lipase samples were measured by hydrolysis of p-NPP. The reaction was measured spectrophotometrically at 410 nm. One unit (U) of hydrolytic activity was equivalent to the amount of enzyme which catalyzed the production of p-nitrophenol (p-NP) of 1 nmol from p-NPP per minute under standard assay conditions.

Esterification activity was determined by esterification of lauric acid (100 mM) and octanol (250 mM) in isooctane (5 mL) at 40 °C for 30 min. One unit (U) of esterification activity was defined as the amount of enzyme that consumed lauric acid of 1 μmol per hour under assay conditions.