Contents lists available at ScienceDirect

Food and Bioproducts Processing

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

Sustainable production of the emulsifier methyl oleate by *Candida rugosa* lipase nanoconjugates

Nur Haziqah Che Marzuki[a], *Naji A. Mahat*[a], *Fahrul Huyop*[b], *Hassan Y. Aboul-Enein*[c], *Roswanira Abdul Wahab*[a,*]

[a] *Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*

[b] *Department of Biotechnology and Medical Engineering, Faculty of Biosciences and Medical Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia*

[c] *Pharmaceutical and Medicinal Chemistry Department, Pharmaceutical and Drug Industries Research Division, National Research Centre, Dokki, Giza 12622, Egypt*

ARTICLE INFO

Article history:
Received 22 April 2015
Received in revised form
15 August 2015
Accepted 18 August 2015
Available online 28 August 2015

Keywords:
Candida rugosa lipase
Nanoconjugates
Esterification
Immobilization
Multi-walled carbon nanotubes
Methyl oleate

ABSTRACT

Acid functionalization of multi-walled carbon nanotubes (F-MWCNTs) using a mixture of HNO_3 and H_2SO_4 (1:3, v:v) was used as support materials for the adsorption of *Candida rugosa* lipase (CRL) as nanoconjugates (CRL-MWCNTs) for producing methyl oleate. To evaluate the competency of the CRL-MWCNTs nanoconjugates, parameters viz. reaction time, surfactant as well as thermostability and reusability were investigated. The characterization of CRL-MWCNTs nanoconjugates using Fourier transform infrared spectroscopy, Field Scanning Electron Microscopy and Transmission Electron Microscopy revealed successful attachment of CRL onto the F-MWCNTs. Utilization of CRL-MWCNTs nanoconjugates resulted in a higher acid conversion in the synthesis of methyl oleate (79.85% at 11 h of reaction time) when compared with the free CRL i.e. an approximately 1.5-fold improvement over the free CRL. The highest percentage of esterification (83.62%) was observed following the use of non-ionic surfactant when compared with the anionic and cationic ones. The CRL-MWCNTs nanoconjugates could be used up to 5 cycles, retaining 50% of its residual activity. Since the preparation of the CRL-MWCNTs nanoconjugates was facile and cheap while producing reasonable yield, the CRL-MWCNTs nanoconjugates developed here were found as promising biocatalysts for the production of methyl oleate.

1. Introduction

Being one of the main components for manufacturing emulsifiers, detergents, intermediate stabilizers and wetting agents, esters such as methyl oleate are functionally important compounds in many industrial sectors (Long et al., 2013). However, the means of extracting such esters from plants and other natural sources are often too scarce or expensive for commercial use (Long et al., 2013). Pertinently, many disadvantages of utilizing the current chemical processes such as the use of corrosive acids and hazardous chemicals, requirement for high energy as well as counterproductive degradation of the produced ester under prolonged reaction time (Aranda et al., 2008) have been reported. Therefore, alternative methods that would overcome such disadvantages but at the same time enhance productivity need to be suggested. In this context, the enzymatic esterification using lipase for synthesizing methyl oleate may prove promising as an alternative route to the prevailing chemical production of esters (Pecnik and Knez, 1992; Mahmood et al., 2013). This is because enzymatic synthesis

* *Corresponding author*. Tel.: +60 75534148; fax: +60 75566162.
E-mail address: roswanira@kimia.fs.utm.my (R.A. Wahab) .

http://dx.doi.org/10.1016/j.fbp.2015.08.005

can be performed at ambient condition and the fact that improved production technologies and engineered enzymatic properties are two key elements desirable by industrial manufacturers (Iyer and Ananthanarayan, 2008).

Lipases (triacylglycerol ester hydrolysis EC 3.1.1.3) are one of the most adaptable classes of industrial enzymes with catalyzing capability in aqueous and organic solvents. Moreover, the enzymes can act on a broad spectrum of substrates, enantioselective as well as performing specific biotransformation (Treichel et al., 2010). Among others, *Candida rugosa* lipase (CRL) has been prevalently used in oil hydrolysis, transesterification, esterification and interesterification as well as catalyzing long chain of fatty acid esters (Abdul Rahman et al., 2012). However, the free form of CRL is (a) often unstable, (b) exhibiting low activity in organic solvents and (c) prone to deactivation when exposed to prolonged exposure of high temperature and extreme pH (Zhou et al., 2012). Therefore, immobilization of CRL onto multi-walled carbon nanotubes (MWCNTs) as supportive materials may prove useful for enhancing its activity and stability (Radzi et al., 2011).

Currently, the focus in enzyme immobilization technology is shifting toward the use of nanosized materials as supports due to their high specific surface areas (Radzi et al., 2011). An enzyme immobilization process involves attachment of free or soluble enzymes onto different types of supports (Khan and Alzohairy, 2010; Mohamad et al., 2015a) to enhance structural stability, activity, specificity and selectivity (Cesar et al., 2007). Immobilization of CRL would extend reaction life and lead to the excellent binding capacity, favorable physicochemical properties as well as biological compatibility of MWCNTs (Zhang and Henthorn, 2010). The role of the multi-walled carbon nanotubes (MWCNTs) as support materials in enhancing activity and stability of enzymes has been clearly indicated in literature (Tavares et al., 2015). The improved stability of immobilized enzymes is due to the formation of stable multipoint interactions between lipase molecules and support materials (Metin, 2013). Such process may result in such enzymes becoming too stable or even loss of activity (Metin, 2013). In this context, a relatively simple cost-effective physical adsorption resulting from weaker unspecific forces is preferred for facilitating easy separation of the biocatalyst from the reaction mixture, without compromising its productivity (Mohamad et al., 2015a). Due to its outstanding mechanical, electrical and thermal properties as well as biocompatibility to CRL (Tan et al., 2012), the surface of acid functionalized MWCNTs (F-MWCNTs) was used for immobilizing the free CRL. Since other studies reported about the use of chemically mediated processes for enzyme immobilization that may be harmful to human and associated with high production costs, while the CRL-MWCNTs evaluated here remained cost-effective and environmentally friendly; its application deserves consideration. Considering a variety of experimental conditions for optimizing the production of methyl oleate, productivity and stability of the physically adsorbed CRL (CRL-MWCNTs) with that of free CRL alone were duly examined.

2. Experimental

2.1. Materials

MWCNTs were prepared using a chemical vapor deposition method. Lipase from *C. rugosa* lipase Type VII (1140 units/mg), sodium hydroxide, sulphuric acid (99%), nitric acid (99%), potassium phosphate buffer pH 7.0 and phenolphthalein were purchased from Sigma–Aldrich (St. Louis, USA). Surfactants, Triton X-100, Tween-80, hexadecyltrimethylammonium bromide (HTAB), cetyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS) and dioctylsulfosuccinate sodium salt (AOT) were also acquired from Sigma–Aldrich (St. Louis, USA). Analytical grade methanol, oleic acid and *iso*-octane were procured from QReC Chemicals (New Zealand). Distilled and deionized water was produced in our lab and used without further purification.

2.2. Methods

2.2.1. Purification and functionalization of MWCNTs

For purifying the MWCNTs, the as-synthesized MWCNTs (1 g) were transferred into a 100 mL round bottom flask containing 4.0 M HCl (60 mL) and refluxed with stirring for 24 h at 100 °C. Upon cooling to room temperature, the liquid was decanted and the MWCNTs were washed repeatedly with distilled water. The suspension of MWCNTs was pelleted down by centrifuging (6000 rpm) for 5 min and the liquid was decanted. The process was repeated until no residual acid was detected after which the purified MWCNTs were dried overnight in an oven at 60 °C (Yudianti et al., 2011).

For functionalizing the MWCNTs, the purified MWCNTs were refluxed in a mixture of concentrated H_2SO_4:HNO_3 (3:1, v/v) for 6 h at 100 °C. The mixture was left overnight to ensure deposition of the F-MWCNTs. Then, the suspension was diluted and rinsed with distilled water until no residual acid was detected and subsequently dried at 80 °C (Yudianti et al., 2011).

2.2.2. Immobilization of CRL onto F-MWCNTs by physical adsorption

The produced F-MWCNTs (100 mg) were suspended in a 50 mL flask of potassium phosphate buffer pH 7.0 (20 mL) that contained CRL (3 mg/mL) and incubated at 4 °C for 3 h with constant stirring at 150 rpm. The suspension was centrifuged for 10 min at 6000 rpm, the liquid decanted and the unbound proteins were removed by repeated washing with potassium phosphate buffer pH 7.0 until no evidence of hydrolytic activity was detected in the washings. The CRL-MWCNTs nanoconjugates were lyophilized overnight and stored at 4 °C until further use (Peng et al., 2013).

2.2.3. Characterization of CRL-MWCNTs

The CRL-MWCNTs were characterized using Fourier-transform infrared (FT-IR) and Field Emission Scanning Electron Microscope (FESEM). For infrared analysis, a mass of sample was ground thoroughly with potassium bromide (1:100 ratios) and the resulting powder was pressed onto a transparent pellet using a hydraulic press. The FT-IR spectra were obtained using a spectrophotometer (Perkin Elmer) in transmission mode between 400 and 4000 cm^{-1} with a resolution of 4 cm^{-1}. Morphology of the synthesized MWCNTs, F-MWCNTs and CRL-MWCNTs were examined using FESEM (JEOL JEM-6700F), which operated at an accelerating voltage of 5 kV and electric current of 10 μA. Prior to examination, a sample was mounted on the surface of a silicon wafer and sputter-coated with a thin film of gold to avoid charging under the electron beam.