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ORIGINAL ARTICLE

Esterification for butyl butyrate formation using *Candida cylindracea* lipase produced from palm oil mill effluent supplemented medium

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Abstract The ability of *Candida cylindracea* lipase produced using palm oil mill effluent (POME) as a basal medium to catalyze the esterification reaction for butyl butyrate formation was investigated. Butyric acid and *n*-butanol were used as substrates at different molar ratios. Different conversion yields were observed according to the affinity of the produced lipase toward the substrates. The *n*-butanol to butyric acid molar ratio of 8 and lipase concentration of 75 U/mg gave the highest butyl butyrate formation of 63.33% based on the statistical optimization using face centered central composite design (FCCCD) after 12 h reaction. The esterification potential of the POME based lipase when compared with the commercial lipase from the same strain using the optimum levels was found to show a similar pattern. It can be concluded therefore that the produced lipase possesses appropriate characteristics to be used as a biocatalyst in the esterification reactions for butyl butyrate formation.

1. Introduction

Lipase (EC 3.1.1.3) is an important industrial enzyme originally characterized by the ability to hydrolyze triglycerides at the oil–water interface, this enzyme also catalyzes a number of useful reactions, such as esterification, transesterification, acidolysis, alcoholysis, aminolysis and resolution of racemic mixtures (Gandhi, 1997; Reetz, 2002; Villeneuve et al., 2000). Microbial lipases are endowed with interesting characteristics such as action under mild conditions, stability in organic

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solvents as well as high substrate specificity (Snellman et al., 2002).

Lipases from different sources show different substrate specificities, however for commercial utilization of a specific microbial lipase, it is important to achieve high yield with higher activity and good stability in various solvent systems. Thus, good stability of the lipase helps in its exploitation in various fields of application, and also aids in extending its shelf half-life and its use cycles (Shu et al., 2010). Based on this, lipase catalyzed reactions do not require homogeneous lipase preparations; instead a certain degree of purity enables efficient and successful usage (Saxena et al., 2003).

Lipase-catalyzed reactions (e.g. esterification) have several benefits over the chemically catalyzed reactions. The most notable one is the transformation at moderate temperature and pressure under neutral pH conditions with no unwanted side reactions (Rajendran et al., 2009). Also, using lipases to carry out the esterification reaction lessens the need for several post-reaction separation processes, which can contribute in reducing the overall operating costs (Yahya et al., 1998). Lipases have been efficiently utilized for direct esterification and transesterification reactions in organic solvents to produce esters of glycerol (Chang et al., 1999), aliphatic alcohols (Abbas and Comeau, 2003), terpenic alcohols (Yadav and Lathi, 2004) such as amyl isobutyrate (Bezbradica et al., 2006), ethyl valerate (Salina et al., 2011), ethyl esters (Sun et al., 2009), glyceride of oleic acid (Kaushik et al., 2010), among others. Lipase from *Candida cylindracea* has been found to be among the most versatile biocatalysts owing to its high activity both in hydrolysis and synthesis (Linko and Wu, 1996; Wong et al., 2000). Thus, several studies proved that the enzyme based technology especially for the production of esters can be used to replace chemical processes both theoretically and practically (Babu and Divakar, 2001; Deng et al., 2003).

Esters of short chain fatty acids and alcohols such as butyl butyrate are important components of natural aromas that have been extensively used as flavor components in the food, beverage and pharmaceutical industries (Santos and de Castro, 2006). Based on these demands and benefits, this study explores the esterification reaction for butyl butyrate formation using *C. cylindracea* lipase that was produced using an inexpensive medium containing palm oil mill effluent. This can justify the development of this medium for the production of lipase if the produced enzyme has characteristics suitable for various applications, since most lipase catalyzed synthesis of different flavor components are performed by commercially available lipase preparations. Thus, attempt was made to optimize the *n*-butanol to butyric acid molar ratio and lipase concentration using face centered central composite design (FCCCD) in order to obtain the highest ester yield.

2. Materials and methods

All reagents used in this study were purchased from Sigma–Aldrich (USA) and were of analytical grade. Palm oil mill effluent (POME) was collected from West Oil Mill of Sime Darby Plantation Sdn. Bhd., Carey Island, Malaysia in clean containers and immediately brought to the laboratory and stored at 4 °C. *C. cylindracea* ATCC 14830 was obtained from American Type culture collection. The strain was grown on potato dextrose agar plates at 28 °C for 4 days and subcultured every 2 weeks. It was then maintained and preserved at 4 °C.

2.1. Production of lipase in palm oil mill effluent (POME) based medium

The fermentation medium used in this study was based on our previous study (Salihu et al., 2011a), containing POME sample of 1.0% (w/v) total suspended solids (TSS) supplemented with 0.2% (v/v) olive oil, 0.65% (v/v) Tween-80 and 0.45% (w/v) peptone, adjusting the initial pH to 6.0. The fermentation was carried out in 2-L Biostat (Sartorius BBI Systems) bioreactor filled with 1-L of POME based medium and sterilized in situ at 121 °C and 15 psi for 15 min. The bioreactor was inoculated with 2.2% (v/v) of actively growing cells of *C. cylindracea* (10^8 cells/ml) from 48 h-Erlenmeyer flask cultures. Standard operating conditions (temperature, agitation and aeration) were set based on the developed process optimization by full factorial design using an incubation period of 36 h (Salihu et al., 2011b).

2.2. Determination of lipase activity and protein concentration

Lipase activity was carried out according to the method reported by Gopinath et al. (2005). The cell free extract (culture supernatant) was used as a source of crude extracellular lipase and assayed quantitatively using *p*-nitrophenyl palmitate (*p*NPP) as the substrate. One lipase unit (U) was defined as the amount of enzyme that liberated 1 μmol *p*-nitrophenol per milliliter per minute under the standard assay conditions. Extracellular protein was determined using the Bradford method (1976). Bovine serum albumin (BSA) standard curve was used in estimating the protein concentration.

2.3. Lipase-catalyzed butyl butyrate formation

Reaction mixture was prepared according to Kiran et al. (2000), which consists of butyric acid (160 mM) and *n*-butanol (320 mM) in *n*-heptane and incubated at 35 °C with a specific lipase concentration for definite periods of incubation. The mixture was stirred in an orbital shaker at 150 rpm. This was then followed by addition of methanol (2 ml) as a quenching agent. Decrease in butyric acid content was measured by titrating the reaction mixture against 20 mM NaOH using phenolphthalein indicator. The progress of esterification was monitored by determining the residual acid content and the yield was calculated as described by Deng et al. (2003) using the equation:

$$Y = \frac{M\alpha - M}{M\alpha - M\beta} \times 100 \quad (1)$$

where Y is the yield of butyl butyrate, Mα and M are amounts of NaOH consumed by titration of the mixture at the beginning (0 h) and the end of the reaction respectively, and Mβ is the amount of NaOH consumed by titration of the mixture without butyric acid. The conversion percentage obtained by gas chromatography (based on direct product formation) and titrimetric analyses (estimation based on residual acid content) were found to be in good agreement.