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Enzyme as biocatalyst for synthesis of octyl ethanoate using acoustic cavitation: Optimization and kinetic study

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ABSTRACT

Octyl ethanoate, an aliphatic ester which is well-known for its use in different industries such as food, pharmaceutical, cosmetic etc. mainly as fixative, modifier and aromatic ester. This work focuses on the use of acoustic cavitation as ultrasound assisted lipase i.e. Novozym 435 catalyzed synthesis of Octyl ethanoate via transesterification of octanol and vinyl acetate in non-aqueous, solvent free reaction. Optimization of various parameters showed that a higher yield of 97.31% can be obtained at octanol to vinyl acetate ratio of 1:2 with 0.03% catalyst, temperature 40 °C and 300 rpm, with lower ultrasound power input of 50 W, at 25 kHz frequency and 50% duty cycle. Further, the time required for the maximum conversion is reduced to 20 min as compared to 90 min of conventional process. Likewise, the enzyme can be successfully reused seven times without loss of enzyme activity. Thus, ultrasound helps to enhance the enzyme catalyzed synthesis of flavors. Analysis of the initial rate and progress curve showed that reaction obeys ternary complex bi bi mechanism with inhibition by octanol.

1. Introduction

Enzymatic synthesis offers various advantages over chemical synthesis such as lower energy requirement, enhanced selectivity and ease of downstream processing with quality product (Kuma et al., 2004). Despite the fact aqueous enzymology is extensively used, non-aqueous enzymology has grown considerably in recent years for its successive application in pharmaceutical, food, agrochemical, drug, cosmetic industry, fine chemical, flavor and perfumery industry (Gutman et al., 1992). Chemical catalyzed reactions are less favored due to various problems such as poor reaction selectivity, formation of undesirable side products and requirement of harsh reaction conditions (Aragao et al., 2011). New immobilization techniques make it possible to use enzymes in industrial processes in a similar way to the use of classical catalysts for heterogeneous reactions. The heterogeneity of the reactions leads in mass transfer resistance this is because of the low diffusion rates of the reactant to the active sites of the enzymes. Hence, biochemical technique with using specific enzyme acquires consideration owing to strengthen environmental regulation clean and friendly approach of enzyme (Weitkamp et al., 2008) Lipases are the most widely investigated of all enzymes. Lipases are enzymes that catalyze the hydrolysis of oils and fats, under appropriate working conditions. It also present catalytic activities of esterification, transesterification, and alcoholysis reactions (Yadav et. al., 2009; Hasan et al., 2006; Rajendran et al., 2009). Novozym 435 is an immobilized lipase obtained from *Candida antarctica* which is used in various industrial applications mainly in formation of organic esters. Since it has stability at high temperature as well as over a wide pH range, easy handling and repeated use make immobilized lipases to be utilized in number of industrial reactions. Lipases are specific towards the ester bond (Yadav et al., 2008). Organic esters like Octyl ethanoate is major aliphatic flavoring ester present in melon, apricot as main flavoring component which serves as fixative and modifier. Traditionally, these esters are prepared by chemical synthesis and also by fermentation, which require number of purification steps and these are often expensive for commercial production (Kawamoto et al., 1987). Due to the overwhelming interest of market in natural products, biotechnology becomes an attractive way to produce flavors with natural aroma from natural precursors (Klibanov et al., 1986; Armstrong et al., 1989). Several studies on transesterification of primary and secondary alcohols by lipases have been issued. However, many papers have not actually explained with kinetics of the reaction (Alder et al., 1989; Brzozowski et al., 1991; Barnwell et., al, 2006; Martinelle et al., 1995; Goto et al., 1994). Application of vinyl or isopropenyl esters as the acylating agent for transesterification offers an effective solution to overcome equilibrium because the enol co-product is immediately transformed irreversibly into acetaldehyde or acetone (Segel et al., 1975; Berger et al., 1991). Thus, it was worthwhile to study kinetics and mechanism of the transesterification of octanol with vinyl acetate using lipase as

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biocatalyst. Even though, enzymes hold wide range of advantages they are not preferred by industries due to their slow rate of reaction and higher cost. So as to overcome these problems, various novel techniques such as microwave, super critical fluid were applied to increase rate of enzyme catalyzed reaction. The yields obtained with these techniques were comparably higher than conventional process but they also have short coming of sample subjection to higher temperature and formation of undesirable byproducts and frequent use of toxic organic solvent (Kulkarni and Rathod, 2015; Hiratake et al., 1988).

Ultrasound-assisted synthesis by using acoustic cavitation field is more recent approach to increase rate of enzyme catalyzed reaction which circumvent some drawbacks of conventional technique such as losses and degradation of volatile and thermo labile compounds, due to its working temperature most of time at or close to ambient temperature. Mechanism of sonication in chemical reaction in liquid solution offers specific activation which is centered on a physical phenomenon known as acoustic cavitation. Cavitation is a process in which mechanical activation directly damages the attractive forces of molecules in the liquid phase. Application of ultrasound consists of compression of the liquid which is followed by rarefaction (expansion), in which a sudden pressure drop creates small, oscillating bubbles of gaseous substances. These bubbles expand with each cycle of the applied ultrasonic energy until they reach an unstable size; they can then collide and/or violently collapse with generating large amount of energy. This energy helps in acceleration of reaction rates. Cavitation arises above a threshold level of sonication intensity that serves to generate voids by the shearing effect on fluid, and it suggests that a minimum intensity must be applied for any consequent effects to be observed (Peter et al., 1998). In many situations ultrasound-assisted synthesis is faster and more efficient than conventional process and provides high efficiencies, which does not require to be polar as is the case with microwave assisted synthesis. The main advantages of ultrasound-assisted synthesis versus other techniques such as Microwave assisted, Solid–liquid, Supercritical fluid are lower costs, due to the simplicity of the equipment required and the similar or better yields obtained most times (Wang et al., 1988).

Novelty of present study encompasses of first time introduction of solvent free condition for synthesis of octyl ethanoate. Solvent free condition leads to lowering down further downstream processing in purification process which directly reduces total cost of process. Additionally this study shows first time process intensification of synthesis of octyl ethanoate with the use of sonication particularly for synthesis of octyl ethanoate where sonication acts as pollution free and green technique. Thus, the objective of present study is to investigate effect of various reaction parameters such as substrate concentration, substrate mole ratio, enzyme loading, speed of agitation, temperature, ultrasonic frequency, power, effect of duty cycle on transesterification of octanol with vinyl acetate using immobilized Lipase (Novozym435) from *Candida antarctica*. The kinetic studies were also conducted with vinyl acetate as the acyl donor and *n*-octanol as the acyl acceptor.

2. Experimental section

2.1. Materials

Lipase B from *Candida antarctica* immobilized on a macro porous resin (Novozym 435) was acquired as a gift sample by Zytex, Mumbai. Octanol, vinyl acetate, *iso*-octane, *n*-butanol, oleic acid were purchased from S. D. Fine Chemicals Pvt. Ltd., Mumbai, India. *n*-decane were purchased from TCI Chemicals India Pvt. Ltd., Mumbai. Molecular sieves 5A° were purchased from Racro chemicals, Mumbai. All chemicals and enzymes were used without any further modification. *n*-decane was used as an internal standard in gas chromatography.

2.2. Instrumental setup

Instrumental set up of ultrasound assisted enzymatic transesterification reaction comprised of an agitated flat bottom glass reactor of 50 cm^3 capacity, furnished with four baffles and three bladed turbine impeller. The agitation was provided by means of electric motor with speed control system. The entire reactor assembly was immersed in an ultrasonic thermostatic water bath (Model No. 6-SL200H/DTC/DF – manufactured by Dakshin India Pvt. Ltd.) which was kept at preferred temperature with a precision of ± 1 °C. The reactor was set in the ultrasonic bath in such a fashion that there is a clearance of 2 cm between reactor bottom and ultrasound bath bottom (Kulkarni and Rathod, 2014). An archetypal reaction mixture entails of 1:2 mol of octanol and vinyl acetate, respectively. Aggregate reaction volume was maintained at 15 cm^3 without use of any solvent. The reaction mixture was agitated at 40 °C for 5 min at a speed of 150 rpm then, 0.1% (w/v) enzyme was added to initiate the reaction. Small amount of liquid samples without any catalyst particle were withdrawn at regular time intervals from reaction mixture. After completion of the reaction residual reaction mixture was filtered through Whatman filter paper and several times enzyme was washed with acetone. The washed enzyme was kept in a desiccator for 24 h and then further used for determination of enzyme activity. After complete transesterification, the reaction mixture consisted of product Octyl ethanoate, by product acetaldehyde and any traces of reactant material.

2.3. Enzyme activity

To analyze transesterification activity of an immobilized lipase 200 mg of vacuum dehydrated lipase material was added to conical flask enclosing a mixture of 0.32 mL oleic acid, 0.27 mL dry *n*-butanol in 3 mL dry isooctane and 0.05 mL distilled water. The flask was kept at a temperature of 30 °C with agitation speed of 250 rpm for 60 min The reaction was stopped with addition of 10 mL methanol and after addition of phenolphthalein indicator instantly, titrated against 0.05 M alcoholic NaOH (Tomke et al., 2015). One unit of enzyme activity is illustrated as 1 mol of oleic acid used up in reaction per min per mg lipase.

$$\text{Enzyme activity(Ea)} = \frac{V \times M \times 100}{E \times T}$$

where V = difference in volume in mL of NaOH between the blank and samples which is a measure of oleic acid consumed during reaction.

M = molarity of NaOH,

E = amount of enzyme employed in mg,

T = time of reaction in min.

2.4. Analytical method

2.4.1. Gas chromatography (GC)

Liquid samples collected during reaction were analyzed by GC (Chemito 8610) equipped with flame ionization detector using 4 m × 3.8 mm stainless steel column packed with 10% SE-30 stationary phase. The temperature program used was as follows: Initial temperature 60 °C for 0 min; 8 °C/min up to 100 ° C; 13 °C/min up to 190 °C; then steady temperature for 2 min. Nitrogen was used as carrier gas at a flow rate of 1 $cm^3\ min^{-1}$. The injector and detector temperatures were kept at 290 °C. *n*-decane was used as an internal standard which was added in reaction mixture.