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Optimization and characterization of lipase catalysed synthesis of xylose caproate ester in organic solvents

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ABSTRACT

The lipase catalysed synthesis of xylose caproate ester was performed by condensation of xylose, an aldopentose and caproic acid in organic solvents. A dual-solvent system containing DMSO and acetone (1:10 v/v) was used to determine the optimal conditions for the reaction. Different reaction parameters (solvent system, reaction time, substrate molar ratio and the amount of enzyme loaded) were studied. The highest conversion rate (64%) was obtained within 24 h with the optimal conditions of 16% (w/v) Novozym 435 and a molar ratio of xylose to caproic acid of 1:4.

1. Introduction

Xylose is an aldopentose that is obtained from the hydrolysis of hemicellulose [1]. Hemicellulose and cellulose are major components of biomass [1]. Thus, they are abundantly available as cheap and renewable feedstock. In recent years, sugar esters have gained much interest [2–6] especially with regards to their use as surfactants with wide range of hydrophilic-lipophilic balance (HLB) values. Sugar esters are non-ionic surfactants [7] and are non-irritant, non-toxic [6,8], biodegradable [9], economical and are tasteless in nature [10]. The properties of sugar esters have enabled their widespread use in many household products such as detergents, oral care products, food, cosmetics and also in pharmaceutical industries [2]. Some sugar esters have been reported to possess biological properties and have found to be used as antimicrobial, antitumor [11], antibacterial [9], miticidal [9], and insecticidal agents [6,8]. Previous reports have described the synthesis of sugar esters *via* both chemical and enzymatic methods. Esterification of sugar and fatty acid using conventional methods often shows poor regio-selectivity [2,12] and hence the development of enzymatic synthesis was proven to be beneficial with regards to regioselectivity and specificity [13]. Furthermore, enzymatic synthesis requires mild conditions and allows for easy separation of the products. Lipase has also been reported as a good biocatalysts in synthesis reactions especially in organic solvents and non-aqueous solvents [2]. For these reasons, considerable work has been dedicated to synthesis new sugar esters via enzymatic methods using lipase as the biocatalyst in the reaction system. To date, there have been no reports on the synthesis of the xylose caproate ester using the enzymatic method. Hence, with this objective in mind, we report herein the synthesis of xylose caproate and the optimized reaction conditions for maximum conversions.

All reactions were carried out using Novozym 435 (immobilized lipase B from *Candida antarctica*). As previously reported, Novozym 435 has great capability and stability towards the esterification reaction at the same time, allows high percentage conversions of esters [14]

2. Materials and methods

2.1. Materials

Commercial lipase, Novozym 435 (immobilized lipase B from *Candida antarctica*) was obtained from Novo Nordisk A/S (Denmark). Commercial xylose was purchased from Acros Organics (USA) and caproic acid was purchased from Fluka (Germany). Solvents (acetone, DMSO, hexane, *tert*-butanol, ethyl acetate) were purchased from SYSTERM (Malaysia), Fluka (Germany) and Merck (Germany). Molecular sieve 3 Å was purchased from Acros Organics (USA). Filter paper was purchased from MACHEREY-NAGEL (Germany) and TLC sheets were purchased from Merck (Germany).

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All chemicals were commercially available and of analytical grade unless specified.

2.2. Lipase catalysed esterification of xylose

In a glass vial, xylose (0.15 g, 1 mmol) was dissolved in DMSO (200 μL) at 60 °C for 30 min. Caproic acid (500 μL, 4 mmol) and acetone (2 mL) were added and stirred. Novozyme 435 (16%, w/v) and 3 Å molecular sieve (0.03 g) was then added into the mixture and stirred at a fixed rotational speed (300 rpm) and temperature (60 °C). The volume of the reaction mixture was about 2.5 mL in total and the glass vial was shaken in an Eppendorf Thermomixer. After 24 h, the mixture was transferred into a 15 mL Eppendorf tube and centrifuged for 15 min at 4000 rpm. The clear supernatant layer was transferred into a clean conical flask and filtered using filter paper.

2.3. Analytical method

A 10 μL aliquot of the mixture was diluted with acetone before being applied onto a TLC sheet (Kieselgel-60, Merck, Germany). The plate was developed using ethyl acetate: hexane (1:1). Subsequently, the plate was dipped into potassium permanganate solution (1.5 g of $KMnO_4$, 10 g K_2CO_3, and 1.25 mL 10% NaOH in 200 mL water) and heated at 60 °C for 5 min. Brown spots indicated the presence of caproic acid, xylose and possible esters. A spot at $R_f = 0.02$ indicated the presence of xylose, a spot at $R_f = 0.80$ indicated the presence of caproic acid, and spots at $R_f = 0.24$, 0.40 indicated the presence of esters.

2.4. Isolation and purification of the xylose ester

The molecular sieve and lipase were first separated from the reaction mixture and the remaining solvent was evaporated off by a vacuum pump at a fixed temperature (50 °C). The components left were subsequently loaded into the silica gel column. Eluted solvent consists of hexane and ethyl acetate (7:3 v/v) was added onto the column chromatography and the solvent was collected every 8 mL prior analysed by thin layer chromatography containing hexane: ethyl acetate (1:1 v/v). The fraction containing spots at $R_f = 0.24$ is collected. The solvent was evaporated off thereafter, and the residue was collected and undergo re-purification step on Silica Gel 60 eluted by a mixture of hexane and ethyl acetate (7:3 v/v).

2.5. Analysis and characterization

Percentage of conversion was measured by titration with 0.1 M NaOH (aq) solution in the presence of phenolphthalein as an indicator. The colourless solution turned pink as the end point was obtained. The ester produced was expressed as equivalent to the acid conversion [15–17]. The amount of acid reacted was calculated from the data obtained for the control samples (without enzyme) and test samples (with enzyme) [16]. The percentage of conversion of each sample was obtained in triplicate.

Percentage conversion [16,17]:

$$\frac{(Vc - Vs)}{Vc} \times 100 \quad (1)$$

Where,

Vs is the volume of NaOH (with enzyme) in mL and Vc is volume of NaOH used (without enzyme) in mL

Eq. (1): Calculation for the percentage of conversion of the sugar ester.

2.5.1. FTIR, GCFID, GCMS and NMR

IR spectra of the reaction mixture were recorded on a Nicolet Magna Infra-Red Spectrophotometer (Perkin Elmer, Waltham, MA). The reaction mixture was prepared by using universal attenuated-total reflectance (UA-TR). The reaction synthesis was scaled up before undergoing further purification and characterization. The purified sample was further characterized by Gas chromatography flame ionization detector (GC-FID). The analysis was carried out by a semi polar column BP-10 (0.33 mm × 50 mm × 0.25 μm) with nitrogen as the carrier gas. The injector and detector temperature was set at 280 °C and 310 °C respectively. The initial oven temperature was set up at 100 °C. Then, the temperature was increased at 8 °C/min and kept at 240 °C for 3 min, after which, the rate was increased to 10 °C/min and kept at 300 °C for 30 min. Gas chromatography/mass spectroscopy (GC–MS) experiments were carried out on a Shimadzu (model GC 17A; model MS QP5050A, Tokyo, Japan) instrument equipped with a non-polar column (fused silica capillary column SGE BPXS, 30 m × 0.25 mm ID × 0.25 μm thickness). ^1H and ^{13}C NMR spectra were recorded on a BRUKER Ascend 700 spectrometer where 100 mg of the sample was diluted with DMSO-d_6 for analysis.

3. Results and discussion

3.1. Influence of variable in sugar synthesis

Our previous study [4] shows that the rate of conversion was influenced by the shaken speed. Normally, stirring rate can increase the mass transfer rate of the substrate to the active site before the product subsequently release from an active site of enzyme. However, vigorous shaking at high speed can damage the surface of immobilized enzyme. The damaged of the surface cause the released of free enzyme to the reaction media. Moreover, high speed is unsuitable in industrial application and large scale studies. Therefore, the lowest shaken speed was chosen as suitable rotational speed according to our previous researched.

Temperature also has significant effects in reaction synthesis. Previous report shows the reaction profile temperature range between 40 and 60 °C. Higher temperature has improved the substrate solubility thus promote the higher conversion rate [4,17]. The effective collision between reactant and enzyme at temperature 60 °C happened when more energy was applied. Another previous report shows that further increased the temperature leads to thermal deactivation of the enzyme [8]. Instead of thermal deactivation, the solvent system (acetone) has a low boiling point (56 °C). Higher temperature (more than 60 °C) allowed the evaporation of the solvents and making it unsuitable to be applied to the system. Therefore, fixed rotational speed (300 rpm) and temperature (60 °C) was applied since our previous study has shown strong evidence that both variables gives significant effect at low speed and high temperature in the reaction synthesis.

3.2. Influence of solubility on esterification

Carbohydrates, including sugars, are poorly soluble in almost all solvents except wate [18]. It has been reported that high polarity solvents such as DMSO are able to dissolve sugars [4]. In order to understand the sugar solubility and its importance towards the reaction synthesis, various types of solvents were screened. Table 1 shows the solvents used for dissolving xylose in order to react with caproic acid.

Table 1 shows that the lower the log P, the higher the polarity of the solvent, hence only DMSO and water was able to fully dissolve the sugar. Meanwhile, as the log P values increase, the solubility rate decreases correspondingly. Previous published reports have stated