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WAX ESTER SYNTHESIS CATALYZED BY A HOMEMADE HETEROGENEOUS BIOCATALYST AND ITS PHYSICOCHEMICAL CHARACTERIZATION

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ABSTRACT – *Commercial lipase from Thermomyces lanuginosus (TLL) has been immobilized on glutaraldehyde-activated rice husk particles via covalent attachment. The immobilized biocatalyst was used to synthesize cetyl oleate (wax ester) via direct esterification of oleic acid and cetyl alcohol. The influence of relevant factors on ester synthesis, such as presence or lack of hydrophobic organic solvents and reaction time has been evaluated. Under optimal experimental conditions, it was observed maximum ester conversion of 90.2 ± 0.6% in 9 h of reaction time in hexane medium by using 1 M of each reactant (cetyl alcohol and oleic acid). Similar conversion (91.5 ± 0.8%) in a solvent-free system was also obtained within 24 h of reaction. The biocatalyst retained 85% of its initial activity after twelve cycles within 9 h of reaction in hexane medium. The physicochemical properties of purified ester have been determined in accordance with ASTM standards. The results indicate that the prepared biocatalyst has great potential for wax ester synthesis due to its satisfactory catalytic activity and operational stability.*

1. INTRODUCTION

Wax esters are important organic compounds widely used in cosmetic, pharmaceutical and lubricant industries. They can be extracted from animal and plant materials (Rani *et al.*, 2015; Lima *et al.*, 2018). However, natural wax esters are rare and very expensive for commercial exploitation. In this sense, synthetic esters from inexpensive renewable resources as vegetable oils, animal fats and their derivatives (free fatty acids) have been considered as promising substitutes to natural wax esters (Alves *et al.*, 2016; Lima *et al.*, 2018).

The production of synthetic wax esters has been performed by chemical or enzymatic routes (Lima *et al.*, 2018). The production of valuable compounds by enzymatic route is very attractive because it involves reactions under mild and environment friendly conditions. The enzymatic production of wax esters has been preferentially performed by using immobilized lipases on solid supports due to facilitating their reuse and purification of products (Rani et al., 2015; Alves *et al.*, 2016; Lima *et al.*, 2018). In the present study, commercial TLL was immobilized on glutaraldehyde-activated rice husk particles via covalent attachment. The homemade heterogeneous biocatalyst was used in cetyl oleate (wax ester) synthesis by direct esterification of oleic acid and cetyl alcohol.

2. MATERIALS AND METHODS

2.1. Materials

TLL, cetyl alcohol and oleic acid were acquired from Sigma-Aldrich (St. Louis, MO, USA). Rice husk was acquired from Arroz Rei Ouro Ltda. (Itajubá, MG, Brazil). Glutaraldehyde solution at 25% v/v was purchased from Vetec Química (São Paulo, SP, Brazil). All other chemical reagents were of analytical grade from Vetec Química.

2.2. Support activation and biocatalyst preparation via covalent attachment

10 g of rice husk were ground in a knife-mill for 10 min and sieved to obtain particles size of 75–90 µm. Subsequently, the support was suspended in 28 mL of a solution composed of 16.8 mL of glutaraldehyde solution (25% v/v) and 11.2 mL of 200 mM buffer sodium phosphate pH 7.0 and kept under continuous stirring in an orbital shaker (200 rpm) within 24 h at room temperature (Mendes *et al.*, 2011). The activated support was then incubated in 90 mL of TLL solution previously prepared by mixing 22.2 mL of commercial TLL solution (protein concentration of 18 mg/mL) with 67.8 mL of 100 mM buffer sodium phosphate pH 7.0 (initial protein loading of 40 mg/g of support). The suspension was kept under mechanical stirring (200 rpm) within 48 h of incubation at room temperature. Maximum immobilized protein concentration of 27.5 ± 1.8 mg/g of support was obtained.

2.3. Esterification reaction

Esterification reactions were performed in screw-capped glass bottles with capacity of 100 mL containing 6 mL of reaction mixture and biocatalyst. The suspensions were incubated in a temperature-controlled orbital shaker under fixed stirring speed (240 rpm), biocatalyst concentration (15% m/v), reaction temperature (50 °C) and acid:alcohol equimolar ratio (1:1) (Lima *et al.*, 2018). Samples (100 µL) of the reaction mixture were periodically withdraw, dissolved in 10 mL of a solution acetone:ethanol (volume ratio 1:1) and titrated with 30 mM NaOH solution using phenolphthalein as the indicator to determine the unreacted oleic acid concentration and, thus, ester conversion percentage (Alves *et al.*, 2016). The influence of relevant factors on the ester synthesis as presence or not of organic solvent (hexane) and reaction time was evaluated. Under optimal conditions, ester synthesis in a solvent-free system was also performed. The operational stability of the heterogeneous biocatalyst was evaluated after twelve successive cycles of reaction in hexane medium.

2.4. Physicochemical characterization of cetyl oleate

Physicochemical properties of purified cetyl oleate as kinematic viscosity at 40 and 100 °C (ASTM D445), viscosity index (ASTM D2270), acid value (ASTM D664), pour point (ASTM D97), flash point (ASTM D93), and specific gravity (ASTM D891) were determined following ASTM standard methods (ASTM, 2005).

3. RESULTS AND DISCUSSION

According to Fig. 1, maximum conversion percentage of 91.5 ± 0.8% during 24 h of reaction in a solvent-free system was observed, while similar conversion percentage was

obtained within 9 h of reaction performed in hexane medium. These results could be attributed to low accessibility of reactant molecules to the biocatalyst microenvironment due to their high viscosity in a solvent-free system. However, the use of hexane as a hydrophobic solvent has reduced the reaction mixture viscosity, thus improving the mass transfer to the enzyme active sites. Thus, operational stability tests were performed in hexane medium.

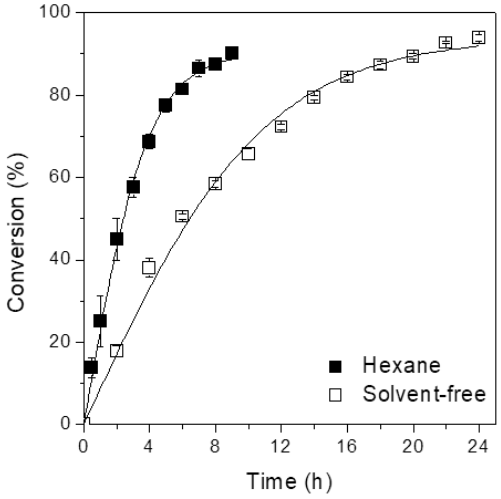

Figure 1 – Comparative ester synthesis performed in hexane and solvent-free systems.

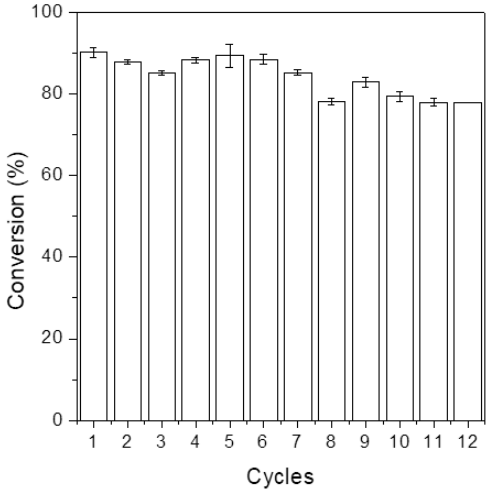

Figure 2 – Operational stability tests after successive cycles of reaction in hexane medium under optimal conditions.

Table 1 – Characterization of the physicochemical properties of purified cetyl oleate and jojoba oil (natural wax).

Properties	Units	Cetyl oleate[a]	Jojoba oil[b]
Kinematic viscosity (40 °C)	mm^2/s	18.13	17.1
Kinematic viscosity (100 °C)		4.74	–
Viscosity index	Dimensionless	198.7	210
Acid value	mg KOH/g	3.2	1.0–2
Specific gravity at 20 °C	Dimensionless	0.85	0.86
Pour point	°C	25	9–23
Flash point	°C	283	293– 295

a – This study

b – Rani et al. (2015)

The most important advantage of applying immobilized lipases on an industrial scale is its use in subsequent cycles. Operational stability tests of the immobilized biocatalyst were

performed under optimal experimental conditions, as aforementioned. According to Fig. 2, immobilized TLL fully retained its initial activity up to the 8th cycle of reaction. Afterwards, a slight decrease of around 15% in its activity was observed, which has been kept up to the 12th cycle. This high operational stability could be due to stabilization of the three-dimensional structure of TLL immobilization on glutaraldehyde-activated rice husk particles and a proper elimination of residual compounds (unreacted reactants and products) in the biocatalyst microenvironment during the washing steps (Alves *et al.*, 2016).

Physicochemical properties of synthesized ester have been determined herein, as shown in Table 1. These results were compared with those of jojoba oil, a natural wax commonly used in cosmetic formulations (Rani *et al.*, 2015). By making a comparison with jojoba oil, some characteristics are also similar, such as kinematic viscosity at 40 °C and pour and flash points, thus suggesting that the synthesized cetyl oleate can be used as wax ester.

In this study, it has been proposed a process to synthesize cetyl oleate by esterification reaction in a solvent system. Immobilized TLL via covalent attachment on glutaraldehyde-activated rice husk particles was successfully used as biocatalyst. Physicochemical properties of purified cetyl oleate showed its potential application as a wax ester. The prepared biocatalyst was highly stable after twelve cycles of reaction. These results show that immobilized TLL on a low-cost and renewable support can be a promising biocatalyst to be used in wax ester synthesis.

4. ACKNOWLEDGEMENTS

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