WAX ESTER SYNTHESIS CATALYZED BY A LIPASE IONICALLY ADSORBED ON FUNCTIONALIZED CHITOSAN HYDROGEL

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Wax esters are important organic compounds composed of long-chain alcohols and carboxylic acids widely used in cosmetic, pharmaceutical and lubricant industries. They can be extracted from animal and plant materials. However, natural wax esters are rare and very expensive for commercial exploitation (Lima et al., 2018). Synthetic esters have been considered as being promising substitutes to the natural wax esters. The production of synthetic wax esters has been performed by chemical or enzymatic routes (Okura et al., 2020). The production of organic compounds catalyzed by immobilized lipases is very attractive because catalyze reactions under mild and environment friendly conditions (Lima et al., 2018; Okura et al., 2020). In this study, a non-commercial ion-exchange prepared via sequential activation of chitosan, a byproduct from seafood industry waste, with glutaraldehyde and functionalization with glycine (Gly-GA-Chit) was used to immobilize lipase from *Thermomyces lanuginosus* (TLL). This immobilized derivative was used as biocatalyst in wax ester synthesis. This is the first study dealing with the application of immobilized lipase on chitosan-based support to catalyze wax ester synthesis.

Gly-GA-Chit was prepared according methodology described by Okura et al. (2020). The adsorption of was performed by mixing 1 g of support with 19 mL of TLL solution (5 mM buffer sodium acetate pH 4.0) using an initial protein loading of 65 mg/g of support. Maximum immobilized protein concentration at equilibrium was of around 54 mg/g. Esterification reactions were performed in screw-capped glass bottles (100 mL) incubated in an orbital shaker at 50 °C and 240 rpm by 70 min containing 6 mL of reaction mixture (0.5 M of each reactant – alcohol and palmitic acid in iso-octane medium) and immobilized TLL at 10% m/v of reaction mixture. Samples (100 µL) of the reaction mixture were periodically withdraw and titrated with 30 mM NaOH solution using phenolphthalein as the indicator to determine conversion percentage (Lima et al., 2020). Reusability tests were also performed after nine successive batches of 90 min each under such experimental conditions.

The effect of acyl acceptor on the ester synthesis was firstly evaluated. Maximum palmitic acid conversion of around 80.3 ± 0.6% was obtained by using isoamyl alcohol, followed by hexanol (74.7 ± 1.3%), decanol (73.2 ± 2.3%), and 2-ethyl-hexanol (70.3 ± 3.2%). Thus, subsequent tests were performed in order to determine the reaction time required to attain maximum conversion percentage. As it can be observed in Fig. 1A, maximum conversion of 85% after 90 min of reaction was obtained. Initial reaction rate (determined from the slope of the palmitic acid consumption *versus* reaction time – Fig. 1A inset) was around of 8.3 mM/min of reaction that corresponds to 2.13 g/L.min.

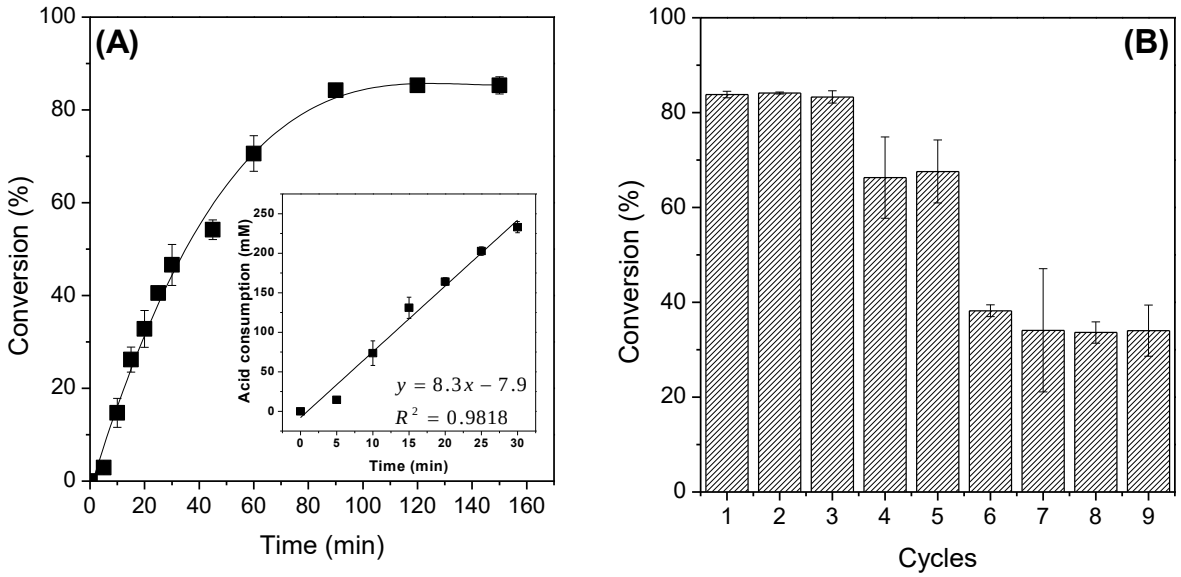

Figure 1 – Effect of reaction time on the isoamyl palmitate synthesis – Inset is the determination of initial reaction rate (A), and reusability tests of ester synthesis after successive batches (B).

Reusability tests (nine cycles of 90 min each) were performed under fixed experimental conditions above described. According to Fig. 1B, the prepared biocatalyst retained all of its original activity after three successive cycles. After (4^{th} and 5^{th} cycles), ester synthesis decreased to 65%, followed by a sharp decrease of its activity (ester conversion of ≈35%) which was kept at this level until 9^{th} cycle. This indicates possible distortion of the 3D structure of TLL or desorption of enzyme molecules from the support microenvironment due to accumulation of water molecules generated during successive reactions.

KEY-WORDS: Lipase immobilization; Ion-exchange support; Chitosan; Wax ester.

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