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generation biodiesel. Ester content ranged from 81 to 93% wt depending on the raw material used for biodiesel synthesis.
Conclusions: A heterogeneous enzymatic biocatalyst, LOBE4, for effcient biodiesel production was successfully developed in a single step synthesis reaction using biosilicification technology. LOBE4 showed to be highly effcient in converting refined, non-edible and residual oils (with high water and free fatty acid contents) and ethanol into biodiesel. Thus, LOBE4 emerges as a promising tool to produce second-generation biofuels, with significant implications for establishing a circular economy and reducing the carbon footprint.

DE-3

Biochemical characterization of an immobilized pectinase in agar-alginate hydrogels for its potential use in winemaking

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Pectinases for the clarification of juices and wine is a traditional technology in winemaking process. Despite the excellent catalytic properties of pectinases, using them in free format presents some drawbacks such as poor stability under operating conditions and low efficiency of use due to recovery and reuse is not feasible. Therefore, immobilization of pectinase in a wide variety of carriers and methods is being considered interesting for clarification and depectinization, because of the increase in operational stability and the biocatalyst reuse. Enzyme entrapment within beads or other matrices from biodegradable polymers is one of the most convenient and effective techniques due to its biocompatibility, catalytic particles of regular size can be produced and low cost. The aim of the present study was to carry out a biochemical and kinetic characterization of an immobilized enological commercial pectinase in agar-alginate mixed biopolymers. In addition, technological effect of the biocatalyst as clarifying agent on Chardonnay grape musts was studied, and lyophilization was applied as conservation technique. Hydrogels (beads) were prepared by external gelation from aqueous solutions of sodium alginate and agar-agar (3%-5%) with pectinase (0.75% m/v), onto $CaCl_2$ solution (2.5% m/v) as crosslinking agent. Pectinolytic activity was assayed by measuring the amount of reducing sugars released from a pectin dispersion using 3,5-dinitrosalicylic acid (DNS) reagent. In previous works, beads were structurally characterized by Texture Profile Analysis (TPA), Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM), confirming the presence of the entrapped enzyme uniformly distributed throughout mixed biopolymer matrix. From the obtained results, it was be observed that immobilization procedure did not modify the optimal pH and temperature (pH=4.0 and 50 °C) for pectinase activity, comparing to free enzyme. Furthermore, immobilized pectinase showed good catalytic efficiency in conditions nearby to those of winemaking (pH of 3.6–4.0 and temperature of 20–30 °C), similar to free pectinase. Storage stability studies demonstrated that wet entrapped pectinase retained its initial enzymatic activity up to 4 weeks at 4 °C and maintained about 30% at 10 weeks, whereas that lyophilized hydrogels retained its original activity after 14 weeks of storage. Entrapped pectinase showed activity until at least six reaction cycles with 60% activity residual. Kinetic parameters, maximum reaction speed (v_{max}) and Michaelis-Menten constant (k_M) were 0.491 and 0.692 mmol/min and 3.77 and 3.69 mg/mL for both free and immobilized enzyme, respectively. The effect of entrapped pectinase on grape must clarification was tested on laboratory scale. The biocatalyst significantly decreased must turbidity to values of 68.5 NTU after 24 h at 20 ° C. Due to these good properties, the immobilized pectinase synthesized in this work could find applications in the grape must clarification. This biocatalyst could be easily recovered after clarification process, allowing its reuse and minimizing the production economic costs in wine industry.