Screening covalent immobilization of a recombinant cutinase for Esterification and Hydrolysis reactions in solvent-free reaction media

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The objectives of this work are the screening of the supports used for covalent immobilization of a recombinant cutinase and to evaluate the respective catalytic ability in solvent free reaction media, as well as to evaluate the operational stability of the enzyme.

Many studies have indicated that an enzymatic catalysis can be reached in the presence of organic solvents.[1] However, due to of the toxicity and flammability of organic solvents, enzymatic catalysis in a solvent-free reaction medium is important in industrial applications. Several studies have reported that immobilized enzymes can effectively catalyse the hydrolysis and synthesis reactions in these conditions. [2]

A new genetically modified *Escherichia coli* strain produced and secreted the cutinase to the production medium and the culture conditions and composition were studied and optimized. The influence of yeast extract, tryptone, the enzyme transcription inductor (IPTG), MgSO4, and ampicillin concentrations and the volume of the headspace given for aeration were also studied. [3] This recombinant cutinase was immobilized covalently on commercial supports. The amount of enzyme and the time of contact were adjusted to increase the amount of immobilized enzyme. The supernatant enzyme (enzyme that has not been immobilized on supports) was characterized by stereolytic activity (p-NPB)[4] and total protein (Bradford method)[5]. The esterification ability of immobilized cutinase on epoxy/butyl methacrylate, epoxy methacrylate and amino methacrylate supports in the synthesis of propyl laureate from Lauric acid and n-propanol (at 50ºC and 200 rpm) and hexyl caproate from caproic acid and n-hexanol (at 30ºC and 200 rpm) were studied. The reaction conversion was determined by assay of acid not consumed through titration method based on phenolphtalein indicator. The tributyrin hydrolysis was catalysed by cutinase covalently immobilized on supports: silica Grace, silica Kieselgel, vinyl, silica, Grace amino, epoxy silica Grace and epoxy/butyl methacrylate at 30 ºC under soft magnetic stirring at constant pH using 702 SM Titrino. The hydrolysis reaction extension was quantified by titration of butyric acid generated.

Stability of cutinase immobilized on the epoxy/butyl methacrylate support showed a constant degree of conversion (around 90%) after 29 reuses, which demonstrates a high operational stability in the synthesis of propyl laureate. Studies of re-utilization of cutinase preparations in hydrolysis reactions are being developed. We can conclude that the esterification and hydrolysis reactions catalysed by cutinase in solvent-free systems are efficient and promoted with stable cutinase covalently immobilized on some supports.

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