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Evaluation of the activity of β -glucosidase immobilized on polydimethyl siloxane (PDMS) microfluidic flow injection analyzer with embedded optical fibers

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Abstract

β -glucosidase from almonds was immobilized on a polydimethylsiloxane (PDMS) microdevice by covalent chain using 3-aminopropyltriethoxysilane and glutaraldehyde. Enzymatic activity was evaluated using p-nitrophenyl- β -D-glucopyranoside dissolved in a 0.01 M pH 5.0 phosphate solution at 45 °C measuring the reaction product (p-nitrophenol) at 410 nm. The microdevice consisted of two parts: the one part where the enzymatic reaction was carried out and a second part where pH was adjusted at 10, with NaOH. The reaction product was measured at the microchip exit using two optical fibers which were aligned facing each other with a gap of 7 mm, between both tips using guides located perpendicular to the flow outlet. A water bath was used to carry out the enzymatic reaction on the microdevice at 45 °C. The enzymatic surface of the PDMS microdevice was 1.15 cm² and the immobilized β -glucosidase amount on the microdevice was of 1.17 μ g/cm². The calculated kinetics parameters were: K_m 2.5 mM; V_{max} 2.2 mM/min; K_{cat} 908.3/min and K_{cat}/K_m 363.3/mM min. The immobilized enzyme is very stable decreasing only 5 % the first 15 days; on the 30th day, the activity was 69 %, regarding the initial activity.

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