

P002 Immobilisation and diphenolase activity of polyphenoloxidase coupled on Eupergit-CM.

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Polyphenoloxidase has been immobilised on Eupergit-CM. The immobilisation variables temperature, type and concentration of buffer, mass of support, volume and concentration of enzyme, and time of coupling have been assayed. The storage stability at different temperatures, and the operational stability with several uses on different samples of the same substrate, have also been studied. The diphenolase activities of the free and immobilised enzymes have been compared towards anionic, cationic, zwitterionic and non ionic o-diphenolic substrates of polyphenoloxidases. L-Dopa had the lowest Km, whereas 4-tButhylcatechol showed the highest Vmax. The assays have been carried out on an immobilised enzyme recycling reactor. Assay methods used the detection of 4-tButhyl-oBenzoquinone or the corresponding MBTH-oQuinone adducts. The results obtained will be useful for further biocatalytic applications of immobilised polyphenoloxidase on diphenols analysis, degradation and enzymatic synthesis of phenolic derivatives.

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