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Novel Aromatic Polymers for Immobilizing β-D-Glucosidase and Their Possible Application to Cellulolysis

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ABSTRACT

We have synthesized, by enzymic and chemical means, a variety of novel polyaromatic–enzyme complexes that are extremely stable and show promise in the conversion of cellulose to glucose. Thus we have prepared a number of homo- and heteropolymeric supports (involving L-tyrosine, pyrogallol, resorcinol, phloroglucinol, orcinol, catechol, protocatechuic acid, and various hydroxybenzoic acids) and discovered that, for example, a resorcinol-β-D-glucosidase copolymer has high stability combined with low K_m (10.5 m*M* vs commercial soluble β-D-glucosidase 9.3 m*M*) and high V_{max} values (104 μmol ρNP $mg^{-1}h^{-1}$ vs 85 μmol ρNP $mg^{-1}h^{-1}$). These properties are enhanced when the copolymer is complexed with bentonite clay. The kinetic constants of the resorcinol–β-D-glucosidase copolymer–bentonite complex were K_m = 9.6 m*M* and V_{max} = 73.5 μmol ρNP $mg^{-1}h^{-1}$. Stability has been assessed against proteolysis, organic solvents, elevated temperatures, storage, and incorporation into fresh soil.

A cellulase preparation from *Trichoderma viride* has also been copolymerized with a variety of phenolic macromolecules and displays varying degrees of stability and activity against carboxymethyl cellulose.

The resorcinol β-D-glucosidase–copolymer was immobilized on a PM10 ultrafiltration membrane (K_m = 16.8 m*M*; V_{max} = 42.4 μmol ρNP $mg^{-1}h^{-1}$) and showed enhanced thermostability, a broader pH range for maximal activity, and could be reused without loss of activity. An ultrafiltration cell, containing the membrane-immobilized resorcinol–β-D-glucosida se copolymer, can be operated as a continuous reactor with substrate flow rates from 0.1 to 0.7 mL min^{-1} without decrease in product formation.