THE USE OF GRAFT COPOLYMERS AS ENZYME SUPPORTS FOR APPLICATIONS IN BIOREACTORS: IMMOBILIZATION OF INVERTASE ON PP-G-AA

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Introduction

Current methods for immobilizing enzymes for use in bioreactors include adsorption or covalent attachment to a support, microencapsulation and entrapment within a membrane or gel. The ideal immobilization method should employ mild chemical conditions, allow for large quantities of enzyme to be immobilized, provide a large surface area for enzyme-substrate contact within a small total volume, minimize barriers to mass transport of substrate and product, and provide a chemically and mechanically robust system. The graft copolymers prepared by ionizing radiation satisfies all of these criteria. The radiation-induced graft polymerization techniques seems to be most convenient process to introduce carriers with active sites on polymeric matrix. The advantages of the method are that almost every polymer may be used as support and that a wide variety of monomers can be grafted onto the polymeric surface. The industrial applications of graft copolymers, both potential and actual, have expanded to include a wide variety of industries from wastewater treatments to biotechnology processes. In countries where the main sources of sugar are beet or cane, inverted sugar syrup, which can be obtained by acid or enzymic hydrolysis, is a valuable commercial product for the food industry (due to its low crystallization rate and high sweetening power), apart from its use as raw material for the production of glucose and frutose. In this work, we wish to report the results obtained when invertase was immobilized onto polypropylene (PP) spheres grafted with acrylic acid (AA) by simultaneous irradiation method.

Experimental

The graft copolymer poly(propylene-graft-acrylic acid) (PP-G-AA) was obtained by the irradiation of a glass ampoule containing the AA in MeOH (35% v/v) and PP spheres (ϕ = 3 mm) with γ-rays from ^{60}Co source at a dose rate of irradiation of 0.31 $kGy.h^{-1}$ and a total dose of 12 kGy. The enzyme invertase (Fluka, 100 $U.mg^{-1}$) was covalently immobilized on the PP-G-AA surfaces after activation of the carboxyl groups of the obtained graft copolymer according the azide method. The sucrose hydrolysis solution (50 $g.L^{-1}$ in 0.025 $mol.dm^{-3}$ sodium acetate buffer, pH 5.0) was carried out for 3-6 h at 25 °C under constant stirring. For monitoring the hydrolysis, 0.5 mL samples were taken every 1h and transferred to a polarimeter (Jasco model D 370) for the measurements of frutose contents.

Results and Discussion

The invertase immobilization process described in this work gave a coupling yield of 82% when the grafting yield of 70% was attainned. When immobilized invertase was used on enzymic hydrolysis, the convertion to frutose syrup attained 51% after reactional time of 6 h. In the case of soluble free invertase, the isomerization reached 58%. Thus, it's may be concluded that the invertase retained 88% of the original activity after the immobilization process. This may be due to interactions between the active site of the enzyme with the functional groups present on the polymeric support[1]. Thus, the synthesized PP-G-AA spheres appears to be a promising invertase carrier for application in biorreactors.

References

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