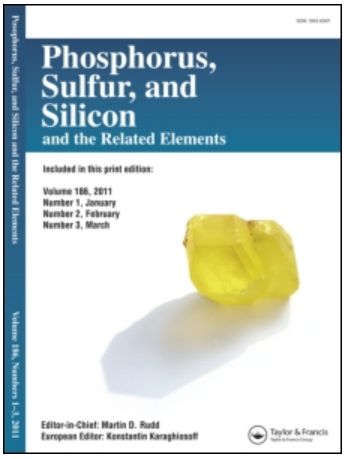

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Enzymatic Synthesis of Phosphoric Monoesters with Alkaline Phosphatase in Reverse Hydrolysis Conditions

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ENZYMATIC SYNTHESIS OF PHOSPHORIC MONOESTERS WITH ALKALINE PHOSPHATASE IN REVERSE HYDROLYSIS CONDITIONS

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Abstract

Title compounds were synthesized on a preparative scale using alkaline phosphatase, orthophosphoric monoester phosphohydrolase E.C. 3.1.3.1, in reverse hydrolysis conditions. Optimization for one of the 25 phosphoryl acceptors investigated (glycerol) shows that up to 55% synthesis yield can be obtained using a large excess of substrate, conditions in which the enzymatic activity remains high. From the results obtained with different phosphoryl group donors, phosphate, pyrophosphate and polyphosphates and with enzymes of different sources, it comes up that the best results are obtained with pyrophosphate and with the weakly purified calf intestine alkaline phosphatase. The extent of enzymatic hydrolysis of the donor can be reduced owing to the existence of two different pH optima for the two reactions, phosphorylation and hydrolysis. The synthesis can be also performed using inert co-solvents which allow to reduce the amount of acceptor used, as long as Zn^{++} is added to the reaction medium. The results are discussed in terms of the catalytic mechanism of alkaline phosphatase.

The application was performed using immobilized enzyme on cellulose. The kinetic parameters of the fixed enzyme was determined and compared to the free one. The inhibition by substrates was shown to be drastically lowered and the fixed enzyme was tested in a recycled reactor which gave improvement in yield of glycerol phosphate.