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Immobilization of periodate oxidized invertase by adsorption on sepiolite

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Abstract: Periodate oxidized invertase was immobilized by adsorption on sepiolite. The obtained immobilized enzyme was more resistant to washing out by concentrated salt solution, and had an eight times higher half-life at 60 ºC than adsorbed native invertase. In packed bed reactor 50 % conversion of 500 g/dm^3 sucrose at 40 ºC and a flow rate of 1 bv/h was achieved. The specific productivity of the immobilized invertase was 0.187 kg/dm^3/h.

Keywords: invertase, periodate oxidation, sepiolite, immobilization, sucrose.

INTRODUCTION

Invert sugar from sucrose can be produced by acid hydrolysis or by using the enzyme invertase (E.C.3.2.1.26.). The enzymatic process has advantages over acid hydrolysis because neither color nor byproducts are obtained.

Inorganic materials have high storage, mechanical, thermal and microbial stability, and enable the possiblity of regeneration by pyrolysis which make them suitable for use as supports for enzyme immobilization. Invertase has previously been immobilized on inorganic supports by linking it covalently to modified silica gel[1] and activated clay[2] and by adsorption on tuff,[3] magnetite,[4] and sepiolite.[5]

Adsorption of enzymes on inorganic supports is a simple and cheap method of immobilization, but leakage of the enzyme from the support can occur. Covalent immobilization of enzymes on inorganic supports or crosslinking of the adsorbed enzyme by glutaraldehyde,[6] results in no leakage of the enzyme from the support, but the enzyme is partly inactivated, due to reaction of amino groups in the active site. Immobilization of invertase *via* its carbohydrate moiety by periodate oxidation does not affect the protein part of the enzyme molecule and hence there is no inactivation of the enzyme.[7–9]

A new procedure for the preparation of stabilized immobilized invertase by adsorption of previously periodate oxidized enzyme was developed. Further, the immobilized enzyme was characterized for use in the hydrolysis of concentrated sucrose solutions.

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EXPERIMENTAL

Sepiolite

Sepiolite was taken from several locations near Obrenovac (Serbia) and prepared by a previously described method.[5] Then the sepiolite was milled with a pestle and mortar. The milled sepiolite was rinsed with distilled water and the smaller particles were removed by fractional sedimentation. The particle diameters were between 0.1 mm and 0.5 mm.

Enzyme

Invertase was obtained from bakers yeast by purification using DEAE chromatography and gel filtration chromatography on Sephadex G-200.[9] The activity of the lyophilized enzyme was 250 IU/mg of solid.

Enzyme activity

The activity was determined in a 5 ml reactor at 1000 rpm in 50 mmol/dm^3 acetate buffer pH 4.7 in 100 g/dm^3 sucrose at 25 ºC. One unit of enzyme activity was the amount of enzyme which catalyzes the hydrolysis of 1 μmol of sucrose per minute under the test conditions. The reducing sugars formed were determined using dinitrosalicylic acid reagent.[10]

Periodate oxidation

Invertase was oxidized by incubating 2.0 mg/cm^3 of enzyme with 2 mmol/dm^3 sodium metaperiodate in acetate buffer pH 5.0 in the dark at 4 ºC for 6 h. The unreacted $NaIO_4$ was then removed with 10 mmol/dm^3 ethylene glycol for 30 min. The oxidized invertase was then dialyzed against 50 mmol/dm^3 acetate buffer pH 5.0 for 18 h.

Immobilization

0.1 g of sepiolite was incubated with 2 cm^3 invertase (5, 50 and 500 IU/cm^3) in 50 mM sodium acetate buffer pH 5.0 for two days at 4 ºC. Then the sepiolite was rinsed twice with 2 cm^3 0.1 M sodium acetate buffer pH 5.0 for 10 min.

Packed bed reactor

The experiments were carried out in a 10 cm^3 water-jacketed glass column (the diameter : height ratio was 1:3) at 40 ºC. The substrate solution was brought to 40 ºC before entering the column, and then pumped through the bed by means of a peristaltic pump. After steady state had been attained, the ratio of conversion was evaluated at the outlet of the column by determining the reducing sugars.

RESULTS AND DISCUSSION

The specific activity of immobilized enzyme increased with increasing amount of added enzyme, Fig. 1.

A specific activity of 130 IU/g for adsorbed native invertase and 180 IU/g for adsorbed periodate oxidized invertase was obtained when 10000 IU of enzyme was added per 1 g of sepiolite.

To determine leakage of the enzyme from the support 0.1 g of immobilized enzyme was rinsed with 10 cm^3 1 M NaCl in 50 mM sodium acetate buffer pH 5.0 for 4 days at 4 ºC, Table I.

After rinsing with 1 M NaCl, only 14.2 % of the initial activity of adsorbed native invertase was retained, while adsorbed periodate oxidized invertase retained 91.6 % of its initial activity. This could be explained by the formation of oligomers of enzyme molecules of the periodate oxidized invertase on the surface of the sepiolite. This is possible be-

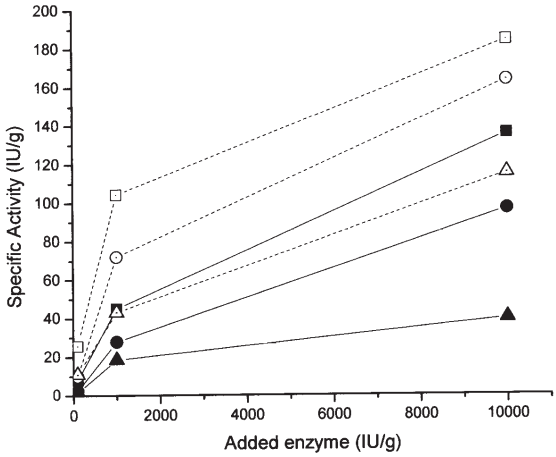

Fig. 1. Dependence of the specific activity of immobilized native (—) and periodate oxidized (···) invertase on the amount of added enzyme for three different charges of sepiolite 1 (□), 2 (○), 3 (Δ).

cause periodate oxidized invertase has aldehyde and amino groups in the same eznyme molecule so that they could react and form oligomers.[11] Such cross-linked enzyme molecules on the surface of a support are difficult to wash out because of the chelate effect, which has been reported in the literature for invertase adsorbed on alumina and further cross-linked with glutaraldehyde.[6]

TABLE I. Retention of the activity of immobilized enzyme after rinsing with 1 M NaCl at pH 5.0 and 4 ºC for 4 days

Enzyme	Relative activity before rinsing/%	Relative activity after rinsing/%
Native invertase	100	14.2
Periodate oxidized invertase	100	91.6

Such conclusions are further confirmed by the thermostability of the immobilized enzyme, Fig. 2.

The half life at 60 ºC for the adsorbed periodate oxidized enzyme was 8 times higher than that for native adsorbed invertase (16.6 min compared to 2.0 min). This further con-

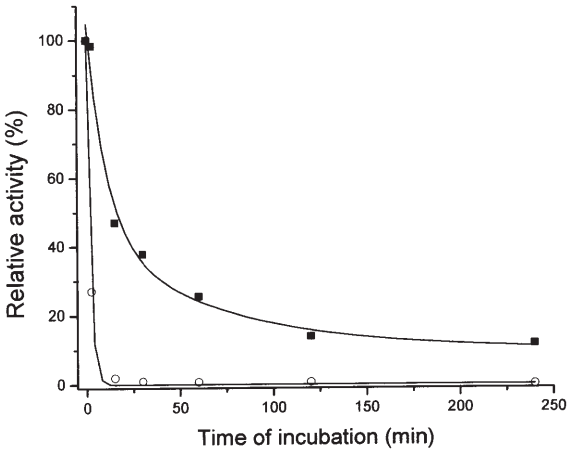

Fig. 2. Dependence of the relative activity of immobilized invertase on time of incubation at 60 ºC in 50 mM sodium acetate buffer pH 4.7. Adsorbed periodate oxidized invertase (■) and adsorbed native invertase (○).

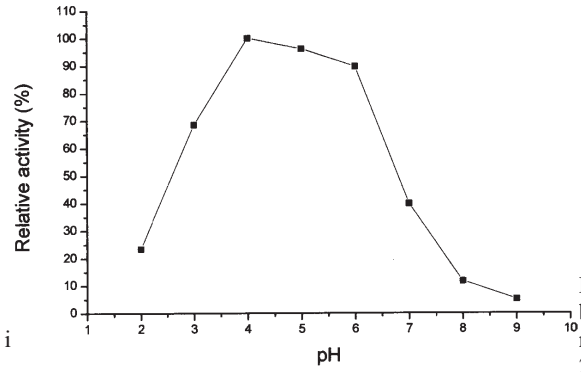

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Fig. 3. Dependence of the activity of immobilized periodate oxidized invertase on pH, n 50 mM sodium citrate phosphate buffer at 25 ºC.

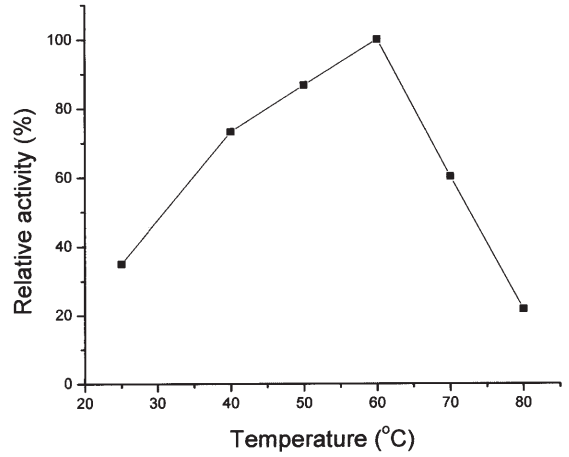

Fig. 4. Dependence of the activity of immobilized periodate oxidized invertase on temperature in 50 mM sodium acetate buffer pH 4.7 and 100 g/dm^3 sucrose.

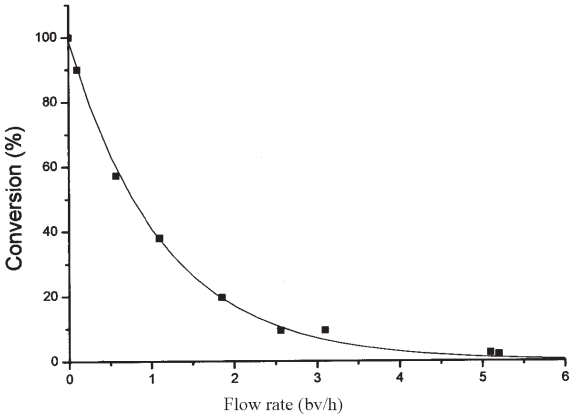

Fig. 5. Dependence of the degree of sucrose conversion on flow rate for 2 cm^3 of immobilized periodate oxidized invertase in a thermostated packed bed reactor in 50 mM sodium acetate buffer pH 4.7 and 500 g/dm^3 of sucrose at 40 ºC.

firmed that periodate oxidized invertase was stabilized by cross-linking on the surface of the sepiolite.

The immobilized periodate oxidized invertase had a pH optimum between 4 and 6, Fig. 3.

The temperature optimum of immobilized invertase was 60 ºC, Fig. 4.

In a thermostated packed bed reactor at 40 ºC and 500 g/dm^3 of sucrose in 50 mM sodium acetate buffer pH 4.7, 90 % conversion of sucrose was obtained at a flow rate of 0.1 bv/h (bed volume per hour), and 50 % at a flow rate of 1 bv/h, Fig. 5.

The specific productivity of immobilized invertase for 90 % degree of conversion was 0.045 kg hydrolyzed sucrose per 1 dm^3 of immobilized invertase per 1 hour. For a degree of conversion of 50 %, the specific productivity was 0.188 kg/dm^3/h. The productivity was two times higher than previously reported in the literature for adsorbed native invertase on sepiolite.[5]

CONCLUSIONS

A new procedure for immobilizing invertase on sepiolite by oxidizing it with periodate before adsorption has been developed. The obtained immobilized invertase was more tightly bound to the sepiolite, and more stable against temperature than adsorbed native invertase. The specific productivity of the immobilized invertase was two times higher than that previously reported in the literature.

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ИЗВОД

ИМОБИЛИЗАЦИЈА ПЕРЈОДАТНО ОКСИДОВАНЕ ИНВЕРТАЗЕ АДСОРПЦИЈОМ НА СЕПИОЛИТУ

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Перјодатно оксидована инвертаза је имобилизована адсорпцијом на сепиолиту. Добијени имобилизовани ензим је био резистентнији на испирање концентрованим растворима соли и имао је осам пута већи полуживот на 60 ºC од адсорбоване нативне инвертазе. У проточном цевастом реактору добили смо 50 % конверзију раствора сахарозе концентрације 500 g/dm^3 на 40 ºC и при протоку од 1 bv/h. Специфична продуктивност имобилизоване инвертазе је била 0,187 kg/dm^3/h.

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