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ORIGINAL PAPER

A. Krastanov

Continuous sucrose hydrolysis by yeast cells immobilized to wool

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Abstract A novel immobilized biocatalyst with invertase activity was prepared by adhesion of yeast cells to wool using glutaraldehyde. Yeast cells could be immobilized onto wool by treating either the yeast cells or wool or both with glutaraldehyde. Immobilized cells were not desorbed by washing with 1 M KCl or 0.1 M buffers, pH 3.5–7.5. The biocatalyst shows a maximum enzyme activity when immobilized at pH 4.2–4.6 and 7.5–8.0. The immobilized biocatalyst was tested in a tubular fixed-bed reactor to investigate its possible application for continuous full-scale sucrose hydrolysis. The influence of temperature, sugar concentration and flow rate on the productivity of the reactor and on the specific productivity of the biocatalyst was studied. The system demonstrates a very good productivity at a temperature of 70 °C and a sugar concentration of 2.0 M. The increase of the volume of the biocatalyst layer exponentially increases the productivity. The productivity of the immobilized biocatalyst decreases no more than 50% during 60 days of continuous work at 70 °C and 2.0 M sucrose, but during the first 30 days it remains constant. The cumulative biocatalyst productivity for 60 days was 4.8×10^3kg inverted sucrose/kg biocatalyst. The biocatalyst was proved to be fully capable of continuous sucrose hydrolysis in fixed-bed reactors.

Introduction

Immobilized cell systems have been applied for many biochemical processes and have been reviewed several times (Durand and Navarro 1978; Klein and Wagner 1978, 1983; Kolot 1981a, b; Fukui and Tanaka 1982; Vorlop and Klein 1983; Rosevear 1984; Phillips and Poon 1988; Klein and Ziehr 1990).

A. Krastanov
Department of Biotechnology, Higher Institute of Food Industry, 26 Maritza Blvd.,Plovdiv 4002, Bulgaria

In the production of high-quality inverted syrups, especially when produced from intermediate products of the sugar industry, enzymic hydrolysis is preferable, and attention has recently been directed almost entirely to the application of immobilized biocatalysts possessing invertase activity. In addition, enzymatic hydrolysis using invertase avoids the production of coloured by-products generated by processes involving acidic hydrolysis.

Although the immobilization of whole cells containing intracellular invertase offers eventual economic advantages compared to the immobilization of soluble invertase, the immobilization of whole cells has been more or less neglected. Cells with invertase activity have been immobilized successfully by inclusion in synthetic or natural hydrophilic gels: polyacrylamide (Ghosh and D'Souza 1989), polyhydroxyethylmethacrylate (Cantarella et al. 1992), alginate (Pira et al. 1991), gelatine (Parascandola et al. 1993), and calcium pectinate (Kurillova et al. 1992; Polakovic et al. 1993), without use of a carrier, by binding of cells with polyethyleneimine (Hasal et al. 1992a,b), as well as by adhesion and/or covalent linkage to the surface of insoluble carriers such as pumice (Cabral et al. 1984), tuff granules (Parascandola et al. 1987), glass (D'Souza et al. 1986), cotton fibres (D'Souza et al. 1988) and so on. Except for the aggregation of whole cells by polyethyleneimine (Hasal et al. 1992a, b), none of the methods mentioned here possesses the catalytic characteristics necessary for the industrial hydrolysis of sucrose: the ability to hydrolyse highly concentrated sucrose solutions (up to 70%), long-term operational stability of the enzyme activity at a temperature of 60–70 °C, long-term physicomechanical stability of the biocatalyst particles and minimum costs of the immobilization procedure.

This work presents a new method for the immobilization of yeast cells onto wool by adhesion using glutaraldehyde, and discusses the potential for its application to the industrial hydrolysis of highly concentrated sucrose solutions.

Materials and methods

Microorganism

For immobilization, cells of *Saccharomyces cerevisiae* with previously increased invertase activity in a medium containing 3% sucrose at 30 °C, for 8 h (Krastanov et al. 1995) were used. The dry-mass content of squeezed yeasts was 25% (w/w).

Materials

Chemically untreated carded wool was used for the immobilization of yeast cells. The wool was washed in water and air-dried. The glutaraldehyde used for immobilization was of technical grade (25% solution in water, Fluka, Switzerland). Other chemicals were of reagent grade and were purchased from local sources.

Immobilization

Yeast cells (500 mg wet weight) were washed in saline and then twice in distilled water. Washed yeast cells were suspended uniformly in 20 ml distilled water. Dry wool (500 mg) was soaked in the yeast cell suspension with gentle shaking on a water bath shaker for 30 min at a temperature of 35 °C. After a washing in distilled water, the biocatalyst was air-dried at room temperature.

Immobilization of glutaraldehyde-treated yeast cells on wool

To 18 ml yeast suspension, obtained as described above, 2 ml 25% glutaraldehyde solution was added. The sample was continuously gently shaken for 110 min at room temperature. After washing, centrifugation and resuspension of yeast cells, the immobilization was performed on 500 mg wool as described above.

Immobilization of yeast cells on glutaraldehyde-treated wool

Distilled water (18 ml) was added to 500 mg wool together with 2 ml 25% glutaraldehyde solution. The treatment was continued for 100 min at room temperature with shaking. The carrier was intensively washed in water and air-dried. The yeast cells were immobilized on glutaraldehyde-treated wool with gentle shaking on a water bath shaker for 30 min at a temperature of 35 °C (as described above).

Immobilization of glutaraldehyde-treated yeast cells on glutaraldehyde-treated wool

Glutaraldehyde-treated cells and glutaraldehyde-treated wool were obtained as described above. Dry glutaraldehyde-treated wool (500 mg) was soaked in a glutaraldehyde-treated yeast cells suspension with gentle shaking on a water bath shaker for 30 min at a temperature of 35 °C. After a wash in distilled water, the biocatalyst was air-dried at room temperature.

Reactor

For the continuous hydrolysis of sucrose, a tubular reactor was used with a fixed layer of biocatalyst (220 mm high, 12 mm diameter). The reactor was equipped with a jacket connected to a water-bath circulator for the temperature control. The reactor was tightly filled with wool (1200 mg), washed and soaked in water at pH 4.7. The carrier was fixed between two adjustable sieves. The suspension from glutaraldehyde-treated yeast cells (50 mg/ml) with increased invertase activity was forced through the reactor at a rate of 2.0 ml min^{-1} for 150 min. Every 30 min the flow direction of the yeast suspension was changed from up to down and vice versa. After the end of the procedure, the biocatalyst was washed in water until no yeast cells could be detected in the washing water.

Continuous hydrolysis of sucrose

The direction of the substrate flow was from the bottom to the top of the reactor, and the solution was pumped through by a peristaltic pump. The efficiency of the hydrolytic process was evaluated by the productivity of the reactor, which can be defined as:

$$P(\mu\text{mol min}^{-1}) = \frac{[\text{Glc}]Q}{180}$$

where Q is the flow rate (ml min^{-1}).

The productivity shows the quantity of sucrose hydrolysed or glucose liberated in the reactor in unit time (1 min).

Analytical methods

The efficiency of yeast cell binding was expressed as a function of the invertase activity. The invertase activity of immobilized cells was assayed by incubation of 100 ml preparation in 10 ml sucrose solution (0.03 mol l^{-1} in 0.05 mol l^{-1} acetate buffer, pH 4.6) for 10 min at 30 °C with gentle shaking. The free glucose was estimated using the glucose oxidase method (Dahlgvist 1961).

Results

Immobilization of cells on wool

Four approaches for immobilizing yeast cells on wool were used. The efficiency of immobilization by the various methods, expressed as a function of invertase activity, is shown in Fig. 1.

It was established that the cells were adsorbed well on the wool even without use of binding agents. The glutaraldehyde proved to be very convenient for immobilization of yeast cells on wool. Best results were obtained when cells previously treated with glutaraldehyde were immobilized on untreated wool. These results correspond to those of D'Souza and Kamath (1988) who immobilized cells on cotton using polyethyleneimine. The invertase activity of yeast cells was found to be affected

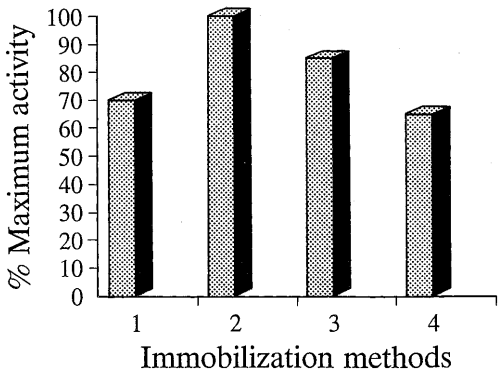

Fig. 1 Immobilization of yeast cells to wool. *1* Control wool + control cells. *2* Control wool + glutaraldehyde-treated cells. *3* Glutaraldehyde-treated wool + control cells. *4* Glutaraldehyde-treated wool + glutaraldehyde-treated cells

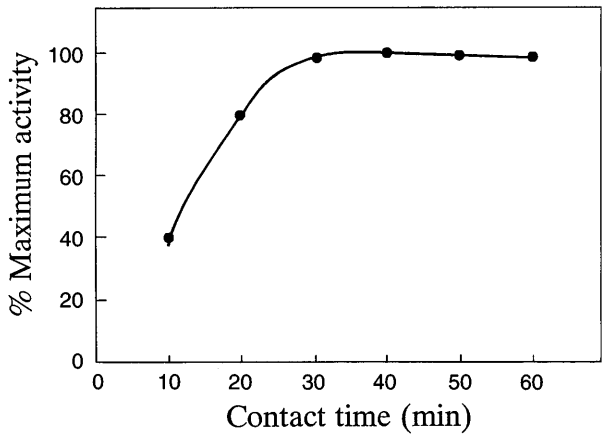

Fig. 2 Effect of contact time of yeast cells with wool

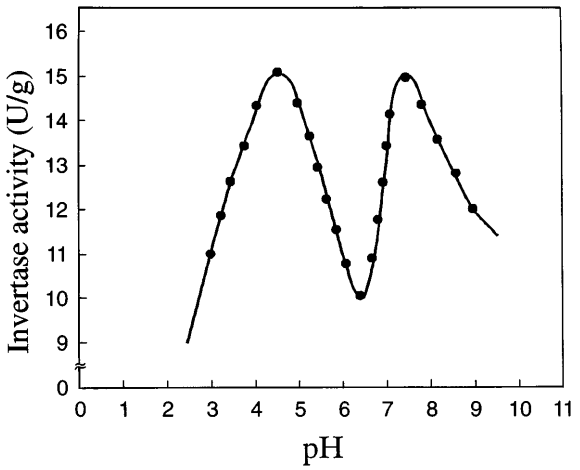

Fig. 4 Optimization of pH for immobilization of yeast cells to wool

by glutaraldehyde. Washing the cells bound to wool with 1 M KCl solution or with 0.1 M buffer, pH 3.5–7.5, does not result in cell desorption. The immobilized cells were also treated with 50% ethyleneglycol solution (v/v). An approximately 10% desorption of cells was found only when untreated cells were adsorbed on wool treated with glutaraldehyde.

The effect of the time of contact between glutaraldehyde-treated cells and wool is shown in Fig. 2. It was found that 30 min contact was sufficient to achieve a dynamic balance between adsorbing and desorbing cells and the optimal binding. However a significant adhesion was observed in the first 10 min of the process.

The effect of cell concentration on the immobilization is shown in Fig. 3. The adsorption of the yeast cells depends on a cell concentration up to 25 mg/ml, and above this it remains constant. Higher binding at low cell concentration may be achieved with an increase of the time of contact.

The adsorption and the desorption of the cells on the surface of the carrier depend on the basic state of the latter. The dynamic balance normally observed between the adsorbed and the free cells depends considerably on pH and the ionic strength of the solution (Bar et al. 1986). In order to study this dependence, a binding of the cells with the carrier in a medium with different pH values was performed. The effect of pH of the medium on the cell binding (invertase activity) during the immobilization is shown in Fig. 4. It is characteristic that here two peaks are found. Preparations immobilized at pH 4.2–4.6 and pH 7.5–8.0 show the highest activity. This phenomenon could be explained by the different kinds of functional groups that were activated at these pH values and which, in both cases, participate in the formation of ionic bonds between the cells and carrier.

Under normal pH conditions most of the cells have a net negative charge. This property has been used to adsorb cells on particulate, synthetic (Yoshioka and Shimamura 1986) or modified cellulose ion exchangers (Bar et al. 1986).

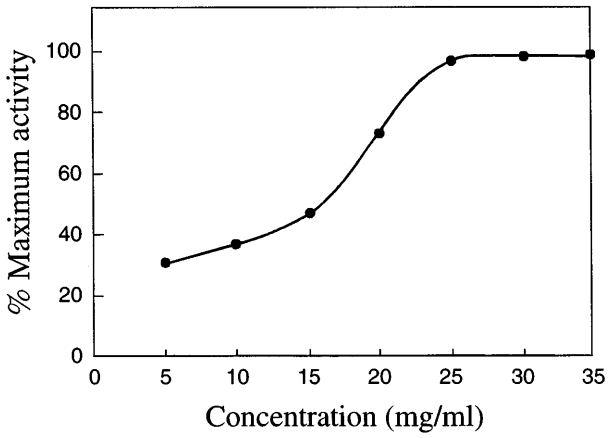

Fig. 3 Optimization of yeast cell concentration for immobilization

Continuous hydrolysis of sucrose by cells immobilized on wool in a bioreactor

The most important operational parameters from the point of view of full-scale continuous sucrose hydrolysis in a fixed-bed reactor are the productivities of the reactor and the long-term stability of the enzyme activity.

The aim of this experimental study was to demonstrate continuous sucrose hydrolysis by means of a biocatalyst in a tubular fixed-bed reactor. The following process parameters were explored: an inlet sucrose concentration of 0.5–2.0 M, a reaction temperature of 40–70 °C, a sucrose solution pH of 4.6, and conversion higher than 0.5.

The effect of the temperature on the productivity of the biocatalyst is shown in Fig. 5. A maximal productivity was found at 70 °C. Increasing the substrate flow rate above 6 ml min^{-1} increases the productivity but is of no interest because the conversion drops below 0.5.

The effect of inlet sucrose concentration on the productivity is shown in Fig. 6. At a flow rate of 6 ml min^{-1},

constant biocatalytic productivity was achieved at a sucrose concentration of 0.5–1.5 M, while 2 M sucrose solution hydrolysed at this flow rate also increased the productivity. But a flow rate for which the rate of conversion is under 0.5 is of no practical interest. At low sucrose concentration the biocatalyst fabricated in the bioreactor in this study demonstrated a high productivity at a flow rate of 3–4 ml min^{-1}. In these cases the productivity tends to decrease with increase of flow rate.

The productivity of a reactor for the enzymic hydrolysis of sucrose depends directly on its construction and on the quantity and the volume of the biocatalyst respectively (the volume of the wool). The effect of the layer height of the biocatalyst on the productivity was studied in a tubular reactor with continuous action, a constant diameter (12 mm) and a variable height. The results are shown in Fig. 7. Increasing the height (or the volume of the wool) of the biocatalyst layer increases the productivity of the bioreactor exponentially. The productivity increases 14 times when the height of the wool layer increases 6 times.

The stability of the invertase activity of the biocatalyst was examined in a continuous fixed-bed reactor at

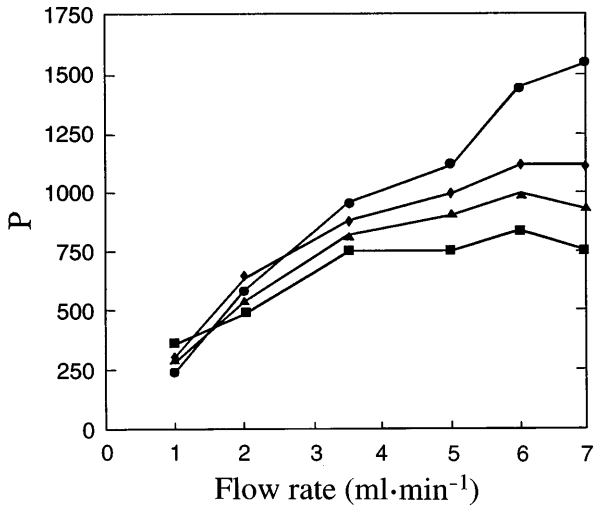

Fig. 5 Effect of substrate flow rate on productivity (*P*) in a fixed-bed reactor. Inlet sucrose concentration, 2.0 M; pH 4.6; biocatalyst concentration, 0.135 g/ml; temperature, (◆) 40 °C, (■) 50 °C, (▲) 60 °C, (●) 70 °C

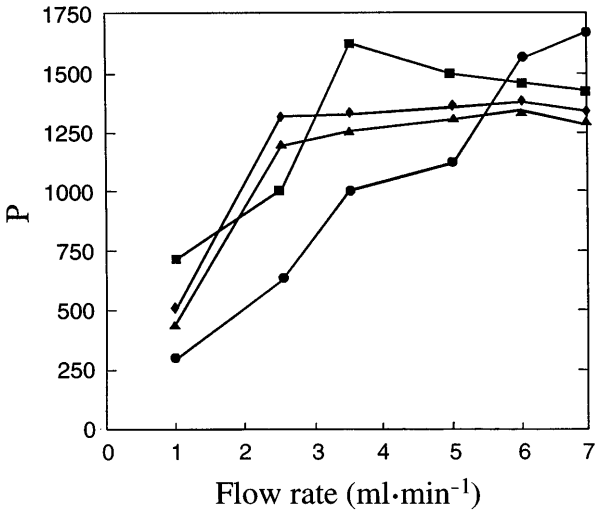

Fig. 6 Effect of substrate flow rate on productivity (*P*) in a fixed-bed reactor. Temperature, 70 °C; pH 4.6; biocatalyst concentration, 0.135 g/ml; inlet sucrose concentration, (◆) 0.5 M, (■) 1.0 M, (▲) 1.5 M, (●) 2.0 M

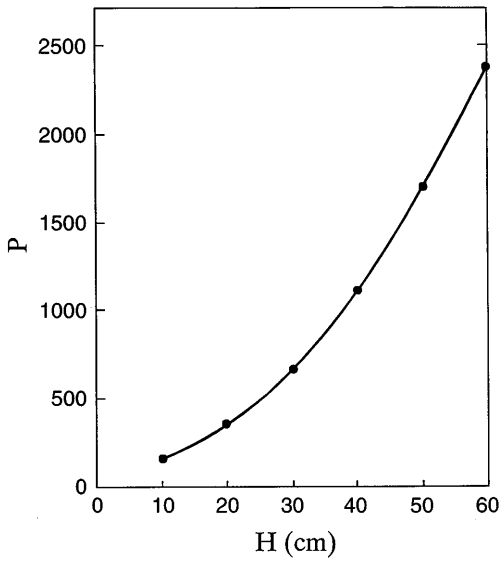

Fig. 7 Effect of biocatalyst layer height (*H*) on productivity in a fixed-bed reactor. Substrate flow, 2.5 ml min^{-1}; inlet sucrose concentration, 2.0 M; temperature, 70 °C; pH 4.6; biocatalyst concentration, 0.135 g/ml

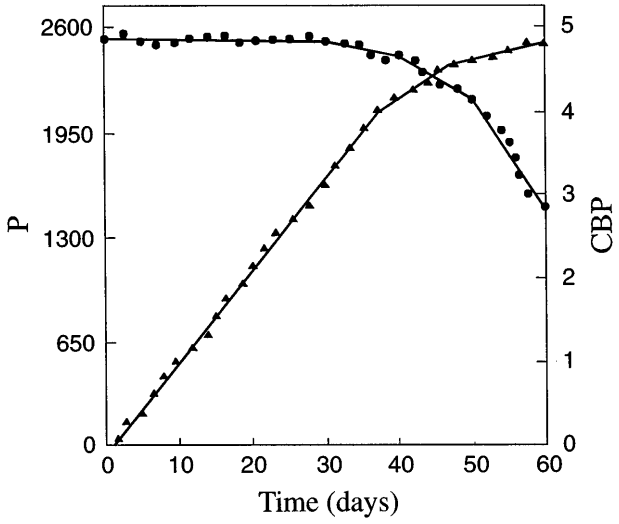

Fig. 8 Long-term continuous sucrose hydrolysis. Inlet sucrose concentration, 2.0 M; temperature, 70 °C; pH 4.6; biocatalyst layer height 660 mm; sucrose solution flow rate, 2.5 ml min^{-1}; biocatalyst concentration, 0.135 g/ml; ● productivity; ▲ 10^3 × cumulative biocatalyst productivity (*CBP*)

constant substrate flow rate, constant reaction temperature, and constant inlet sucrose concentration. The total duration of the test was 60 days (Fig. 8). The initial value of the productivity was 2500 mol min^{-1}, and it remained almost unchanged for 25–30 days, after which a sharp decrease started. The final value of the cumulative productivity was 4.8×10^3 kg inverted sucrose/kg biocatalyst. This amount is 2.5 times higher than that reported by Hasal (Hasal et al. 1992a, b) while using half as much biocatalyst.

Discussion

Microbial cells have been immobilized by adsorption using different techniques (Mattiason 1983). Unlike most of the studies reported earlier, the glutaraldehyde-treated yeast cells immobilized on wool were found to adhere strongly to the wool surface. The precise mechanism of immobilization in this system is not clear. It is possible, however, that in addition to the ionic or hydrophobic interactions, some other factors may be involved. Giving the protein structure of the carrier, we suggest that glutaraldehyde acts as a binding link between NH_2 groups of the cell surface as well as between NH_2 groups of the cell surface and the carrier. However, it is recognized that the balance of short-distance interactions like hydrogen bonds, dipole interactions and Van der Waals' interactions at the particle–liquid–particle interfaces play an important role in stabilizing the immobilized cells. This technique of adsorbing yeast cells thus offers an easy method for immobilizing cells by adsorption on various supports with free NH_2 groups.

Unlike the materials for immobilizing yeast cells described in previous publications, the wool not only has a more compact structure and is easily manipulated, but is also more reliable in the fabrication of various reactor geometries for continuous use. A high flow rate is possible even when viscous solutions are used. This is a great advantage of the system presented, compared to cells immobilized on cotton cloth (D'Souza and Kamath 1988), when highly concentrated sucrose solutions are hydrolysed.

The data presented above prove that the flow rate, the concentration of sucrose and the temperature both limit and determine the productivity of the biocatalyst in a frame fixed-bed tubular reactor with yeast cells immobilized on wool. An increase in temperature from 40 °C to 70 °C results in an important increase in productivity. These results show the importance of temperature for enzymatic sucrose hydrolysis.

An extremely interesting result found here is the exponential increase of the productivity when the height of the biocatalyst layer increases. This fact changes our ideas about a universal criterion for evaluating reactors for continuous sucrose hydrolysis with immobilized cells. Each reactor must be evaluated separately depending on its construction.

The operational stability of the biocatalyst investigated in this work is better than in the immobilized cell systems of Horbach et al. (1989) and Hasal et al. (1992. a, b). It may be concluded that the stability of biocatalyst activity reported here is quite satisfactory for continuous sucrose hydrolysis at reaction temperatures of 60 °C–70 °C. Furthermore, the risk of microbial contamination of the substrate solution or of the biocatalyst itself is eliminated at this temperature and this substrate concentration, and no side-reactions resulting from the metabolism of glucose by the yeast can occur. The resistance of the biocatalyst particles is excellent. The viscous sucrose solution does not affect the fixed wool.

The experiments summarized in this work show that the biocatalyst with invertase activity, obtained by immobilization of whole yeast cells on wool, is completely satisfactory for the continuous hydrolysis of a highly concentrated sucrose solution in reactors with a fixed layer on an industrial scale. The productivity value, the operational stability of invertase activity, and the mechanical stability of the biocatalyst are feasible for the successful full-scale operation of the biocatalyst. The low cost of the immobilization procedure makes the possibility of producing, inverted syrups by means of the biocatalyst economically very attractive.

The inverted syrups produced by sucrose hydrolysis, using the biocatalyst described, do not contain undesirable reaction by-products, inorganic salts, etc., so they can be used in the food industry. Moreover, not only pure sucrose solutions but also non-refined sugar, e.g. clairce, can be inverted without difficulties.

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