

Sucrase in immobilized cells of *Cucumis sativus* L.

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ABSTRACT: Cell suspension cultures of *Cucumis sativus* L. – cucumber were permeabilized by Tween, hexadecyltrimethylammonium bromide, hexadecylpyridiniumbromide ethanol and/or immobilized by glutaraldehyde. The highest invertase activity was at pH 4.4 and temperature 53°C. The hydrolysis of the substrate was linear for 5 h reaching 60% conversion. The cells displayed high sucrase activity and convenient physico-mechanical properties.

Keywords: glutaraldehyde; sucrase; cell permeabilization; cell immobilization; *Cucumis sativus* L.

Plant cells were first immobilized by BRODELIUS et al. (1979). Immobilization techniques have a great impact on technology nowadays. In the last decades, several methods for fixation of biocatalysts have been developed. Enzymes, living or nonliving microorganisms, animal and plant cells, as well as combined systems have been bound within/or to carrier materials (HULST, TRAMPER 1989; FÖRSTER 1994; STANO et al. 1998). Immobilization of cells or enzymes represents an effective way of obtaining highly efficient enzyme catalysts important for biotransformation processes (KLIBANOV 1983). Many matrices from synthetic polymers or biological materials have been used for the immobilization of cells. The most widely used technique is the application of gel particles of agar, agarose, kappa-carrageenan, collagen, chitosan, polyacrylamide, polyurethane or cellulose (HULST, TRAMPER 1989; TAMPION, TAMPION 1987). The spontaneous adhesion or covalent binding of cells to the surfaces of insoluble carriers were also examined (PARASCANDOLA et al. 1987; ROGALSKI, LOBARZEWSKI 1995). Recently, the use of polyvinyl-alcohol and glutaraldehyde (WU, WISECARVER 1992; HASAL et al. 1992), or Tween and glutaraldehyde (STANO et al. 1998) for cell immobilization has been investigated.

Sucrase as invertase (β -D-fructofuranosidase EC 3.2.1.26) is called catalyses the hydrolysis of sucrose to glucose and fructose. The studied enzyme is also used in processes leading to mixtures of glucose and fructose (invert sugars) enabling the successive production of fructose-containing preparations (MANSFELD et al. 1992; SCHLEE, KLEBER 1991; PŠENÁK et al. 1981).

The development of new techniques of immobilization of biocatalysts is tightly connected with the progress of biotechnological processes. Because of the known fact that the cell wall slows down the transport of many com-

pounds from and into the cell we were interested in exploring possibilities of the cell wall permeabilization. We assume that immobilized cells of plant origin could play a similar role in biotechnological processes as representatives of various microorganisms.

In this paper we focused our attention on the study of the effect of permeabilization on enzymic hydrolysis of sucrose using immobilized cells of *Cucumis sativus* L. cv. Natali.

MATERIALS AND METHODS

Tissue cultures

Long-term callus cultures were derived from seedlings of *Cucumis sativus* L. cv. Natali and continuously subcultured every 2–3 weeks on MURASHIGE-SKOOG (1962) medium as was described by ANDRIAMANTY et al. (2000) and NEUBERT et al. (1999).

Cell permeabilization

Cell suspension was filtered through a nylon cloth and 10 g fresh mass of cells was suspended in 50 ml of 0.15 mol/l NaCl with: 5% Tween 20, (SIGMA) 5% Tween 80, (SIGMA) 30% ethanol, 50% ethanol, 0.1% hexadecyltrimethylammonium bromide (Fluka), 0.1% hexadecylpyridinium chloride (Fluka). Permeabilization proceeded for 3 h under moderate stirring at a laboratory temperature. The cells were filtered off and washed with 2,500 ml of distilled water and 1,500 ml of 0.15 mol/l NaCl solution.

Cell immobilization

The permeabilized cells were immediately suspended in 30 ml of 0.15 mol/l NaCl and immobilized using glutaraldehyde by crosslinking as described by PÓOR et al.

(1998). The immobilized cells were washed with 3,000 ml of 0.15 mol/l NaCl solution and separated by filtration.

Determination of fresh and dry mass

Fresh and dry masses of cells in living suspension culture and in immobilized cells were determined gravimetrically. For determination of dry mass, samples were dried to constant mass at 105°C.

Influence of temperature

The effect of temperature on enzymatic activity was tested in the range from 20°C to 100°C, as described in Enzyme assay (see below).

Effect of some sugars on enzyme activity

The effect of glucose, fructose, cellobiose, galactose and gluconolactone on the activity of invertase in suspension cell culture and in immobilized cells was tested in 1, 5, 10 and 20 mol/l concentrations, resp.

pH optimum

The effect of pH on enzymatic activity was tested in the range from pH 4.2 to 5.2, using 0.2 mol/l Mc Ilvaine buffer.

Glucose utilization

The immobilized cells and cell suspensions were exposed to the initial glucose concentration of 200 mg/1,000 ml in culture media (MURASHIGE, SKOOG 1962) without the presence of sucrose. The concentration of glucose was determined by the method of TRINDER (1969).

Enzyme assay

The enzyme activity was determined by a modified method of TRINDER (1969) using sucrose as the substrate. The reaction mixture contained 0.1 g of wet cells, 0.4 mol/l sucrose in 2 ml of 0.2 mol/l Mc Ilvaine buffer pH 4.4. The mixture was incubated at 30°C for 1 h. The control contained temperature (100°C) inactivated cells. The cells were separated from the reaction mixture, dried and enzyme activity was calculated per

1 g of dry mass. The enzyme activity was expressed in katals. Protein contents were determined by the method of BRADFORD (1976) using bovine serum albumin as the standard protein.

Cell viability

The cell viability was determined by the method of DIXON (1991) with 2,3,5-triphenyltetrazoliumchloride (TTC), fluoresceindiacetate and with an oxygen electrode.

RESULTS AND DISCUSSION

The immobilization of isolated enzymes and permeabilization followed by immobilization are techniques widely used in technologies (BRODELIUS et al. 1979).

After the immobilization of the cells using glutaraldehyde gentle plasmolysis occurs as well as aggregation of the cells. The viability of the immobilized cells was determined by the respiratory activity measured with an oxygen electrode, and by vital staining (using 2,3,5-triphenyltetrazoliumchloride and fluoresceindiacetate). It was observed that cells immobilized by glutaraldehyde are not viable. Glucose was also utilized only by cell suspension, but not by immobilized cells (Fig. 1).

The permeabilization of the studied cells by Tween 80, Tween 20, ethanol, hexadecylpyridinium chloride and hexadecyltrimethylammonium bromide, resp., led to a decrease in protein content while the enzyme activity showed a moderate increase with the exception of samples in which ethanol was used, and the specific activity increased in all tested samples. By glutaraldehyde crosslinking a noticeable decrease in the enzyme activity was found (Table 1). The immobilized cells, like viable cells, had a pH optimum of invertase at 4.4 and a minor peak of activity also appeared at pH 5.3 (Fig. 2). By cell wall permeabilization of yeasts a very significant increase in the phenylalanine ammonialyase (PAL) activity was observed (SRINIVASAN-NAGAJYOTHI et al. 1994).

By cell wall permeabilization of the suspension culture no noticeable increase in invertase nor in the α - and