Invert sugar formation with *Saccharomyces cerevisiae* cells encapsulated in magnetically responsive alginate microparticles

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Invert sugar (an equimolar mixture of glucose and fructose prepared by sucrose hydrolysis) is a very important food component. We have prepared magnetically responsive alginate microbeads containing entrapped *Saccharomyces cerevisiae* cells and magnetite microparticles which can be easily separated in an appropriate magnetic separator. The microbeads (typical diameter between 50 and 100 µm) were prepared using the water-in-oil emulsification process. The prepared microbeads containing yeast cells with invertase activity enabled efficient sucrose conversion. The biocatalyst was quite stable; the same catalytic activity was observed after one month storage at 4 °C and the microbeads could be used at least six times.

**1. Introduction**

Sucrose, which is a disaccharide, can be broken down into a 1:1 mixture of glucose and fructose, known as invert sugar [3]. It is a very important food component because it is sweeter and more soluble than granular sucrose is. Invert sugar is used mainly by food manufacturers to retard the crystallization of sugar in high-viscosity solutions [1]. It is used in the production of non-crystallizing creams, jams, artificial honey, and in confectionery industry and to a lesser extent in the industrial production of liquid sugar [2,3].

Industrial production of invert sugar is usually based on the acid or enzymatic hydrolysis of sucrose. Acid hydrolysis is based on the application of appropriate strong mineral or weak organic acids [4]. The disadvantage of acid hydrolysis is the possible presence of impurities in the product introduced by uncontrollable parameters during conversion. On the other hand this conversion can also be achieved by enzymatic action of invertase on sucrose with a conversion efficiency of almost 100% without the inherent disadvantages of acid hydrolysis.

Invertase or β-D-fructofuranosidase (E.C. 3.2.1.26) has a long-established use for both bulk conversion of sucrose and *in situ* hydrolysis of sucrose in confectionery. The enzyme is usually obtained from baker’s yeasts (*Saccharomyces cerevisiae*). The isolated enzyme can be used for sucrose hydrolysis both in the free and immobilized form [2,5]; however, the isolation process increases the cost of purified enzymes.

In order to lower the production costs the whole *S. cerevisiae* cells can be used as a biocatalyst. Immobilized cells are usually used; in most cases readily available biopolymers such as alginate [6] or gelatine [6,7] are utilized as matrix for cells entrapment, while modified jute fabric was used for yeast cells adsorption [8].

The biocatalyst particle size can significantly influence the yeast cells catalytic activity. The diameter of many biocatalysts usually ranges between several hundreds micrometers and a few millimeters [9], which enables simple separation of the biocatalyst from the reaction mixture. However, immobilization of yeast cells into smaller particles can lead to the increase of the conversion rate. In order to simplify the manipulation with small biocatalyst particles, magnetically responsive cells-containing beads are of special interest, because they also enable work in difficult-to-handle samples, such as suspensions [10,11]. Magnetic properties of the beads are usually caused by the presence of magnetic iron oxide nano- and microparticles.

In this paper we describe the preparation of magnetically responsive alginate microbeads containing *S. cerevisiae* cells and their application for the conversion of sucrose into the mixture of glucose and fructose.

**2. Materials and methods**

**2.1. Materials and equipment**

Sodium alginate was from BDH Laboratory Supplies, United Kingdom. Magnetite (iron (II, III) oxide, declared particle size <5 µm) was from Aldrich, USA. Sucrose, calcium chloride and
sodium chloride were obtained from Lachema, Czech Republic. Sunflower oil and baker’s yeast were purchased in a local market. Compressed yeast cells (S. cerevisiae) were suspended in 0.15 M NaCl and the homogenous suspension was centrifuged at 2500 rpm for 5 min; after pouring off the supernatant the remaining pellet was used as a starting material (“wet yeast cells”). Flat magnetic separator was from Qiagen, USA. Kit for the detection of glucose (Glucose GOD 250, Lachema, Czech Republic) was based on conversion of glucose to gluconate and hydrogen peroxide using glucose oxidase; in the presence of peroxidase, hydrogen peroxide reacted with 4-aminophenpyrine and 3-methylphenol to form a colored product, which was measured at 498 nm.

2.2. Preparation of magnetically responsive beads with encapsulated yeast cells

The millimeter-sized alginate beads were prepared as follows. Sodium alginate solution (2%; 2 ml) was thoroughly mixed with magnetite (20 mg) and then 1 g of “wet yeast cells” was added. The thoroughly mixed suspension was dropped through a pipette tip in 5% CaCl$_2$ solution. After 10 min the beads were magnetically separated and transferred to 1% CaCl$_2$ for 30 min. The magnetic calcium alginate beads were stored in 0.15 M NaCl containing 0.05 M CaCl$_2$ at 4°C. Approximately 74 pieces of magnetic alginate beads were obtained.

To prepare magnetic alginate microbeads, 2 ml of 2% sodium alginate, 20 mg of magnetite and 1 g of “wet yeast cells” were mixed in a test tube. After addition of 8 ml of sunflower oil, the suspension was thoroughly vortex mixed for 5 min to create magnetic alginate microbeads. Then 10 ml of 5% CaCl$_2$ was added and mixing continued for another 2 min. The sunflower oil was removed by several washings with 1% CaCl$_2$ using flat magnetic separator. The prepared magnetic calcium alginate microbeads were stored in 0.15 M NaCl with 0.05 M CaCl$_2$ at 4°C. Approximately 2.6 ml of sedimentsed microbeads was prepared.

Invertase-free magnetic microbeads were prepared in order to test non-specific sucrose conversion. Invertase-free yeast cells were prepared by steam boiling of the cells suspension (in 0.15 M NaCl) for 15 min. After centrifugation at 2500 rpm for 5 min the pellet was used for magnetic alginate particles preparation.

2.3. Experimental setup

All experiments were performed with magnetic alginate microbeads; in selected experiments also millimeter-sized alginate beads were used. Before experiments magnetic microbeads were transferred into reaction mixture (0.1 M acetate buffer pH 5 with 0.05% CaCl$_2$) for 20 min. Different concentrations of sucrose (range 5–50%) and amounts of magnetic beads were used. The total reaction volume was 15 ml. The reactions were performed at room temperature under shaking at 125 rpm using Rotamax 120 (Heidolph, Germany). After reaction magnetic alginate microbeads were separated from reaction medium using flat magnetic separator. The concentration of formed glucose was assayed by a commercial kit. Each experiment was repeated four times and the results were statistically evaluated; each point in the graphs represents an arithmetic mean±SD. The following parameters were tested:

(a) Time dependence of sucrose conversion. This experiment was performed using both magnetic alginate microbeads and millimeter-sized alginate beads. Constant amounts of magnetic beads (corresponding to 200 mg “wet yeast cells”) were incubated in reaction medium containing 20% sucrose for different periods of time (from 5 min to 2 h).

(b) The amount of yeast cells. Different amounts of microbeads (corresponding to 86–688 mg “wet yeast cells”) were placed in reaction mixtures containing 20% sucrose and incubated for 30 min.

(c) Initial concentration of sucrose. Constant amounts of magnetic microbeads (corresponding to 172 mg “wet yeast cells”) were incubated in reaction mixtures containing sucrose (concentration range 5–50%) for 30 and 90 min.

(d) Repeated application of magnetic microbeads. Constant amounts of magnetic microbeads (corresponding to 172 mg “wet yeast cells”) were used six times for the conversion of sucrose at different concentrations (5–20%) for 30 min.

(e) Biocatalyst stability. The magnetic microbeads were stored in 0.15 M NaCl containing 0.05 M CaCl$_2$ at 4°C and their ability to sucrose conversion was monitored during one month. The reaction conditions were similar as in (d), the initial sucrose concentration was 20%.

3. Results and discussion

The magnetically responsive yeast cells-containing alginate microbeads had spherical shape, with diameters ranging between 20 and 150 μm; however, the diameters of majority of microbeads were 50–100 μm (Fig. 1). It was shown that the vortex speed and agitation time had significant effect on the size of alginate microbeads; more intensive agitation led to the formation of smaller microparticles (data not shown). The biocatalyst microparticles could be easily separated using appropriate magnetic separators. The prepared beads were stable during one month storage at 4°C. For comparison, larger yeast cells-containing alginate beads (diameter 2–3 mm) were prepared using the standard procedure.

The type (size) of the particles influenced dramatically the velocity of sucrose conversion and the formation of invert sugar (Fig. 2). The same amount of yeast cells (200 mg “wet yeast cells”) encapsulated in alginate microbeads formed significantly higher amounts of invert sugar (measured as the formation of glucose) in comparison with millimeter beads for the same time period. Using cells-containing microbeads, all sucrose present in the reaction mixture was converted into invert sugar approximately after 1 h. In comparison, the same amount of cells entrapped in millimeter-sized beads converted only 12.3% of sucrose in 60 min and 39.4% in 90 min. Based on these results the next experiments were performed only with microbeads and reaction time was chosen to be 30 min.

Fig. 1. Magnetically responsive alginate microbeads containing entrapped Saccharomyces cerevisiae cells and magnetite microparticles. The scale bar corresponds to 50 μm.
The velocity of sucrose conversion was not only dependent on the type of particles but also on amount of yeast cells in the reaction mixture (Fig. 3). The total sucrose conversion (starting concentration: 20%) was achieved in the presence of 688 mg yeast cells in the reaction mixture during 30 min. Approximately 50% sucrose conversion was obtained by using 172 mg of yeast cells.

The initial concentration of sucrose influenced considerably the ability of yeast cells entrapped in microbeads to form invert sugar (Fig. 4). Complete sucrose conversions were achieved in 5% and 10% sucrose solutions during 30 min incubation, using 172 mg "wet yeast cells", while 20% sucrose solution was converted during 90 min incubation. In the case of 30% sucrose the conversion was 36.7% and 82.2% after 30 and 90 min, respectively. Increased sucrose concentrations (40% and 50%) caused its lower conversion to glucose and fructose (55.9% and 31.4%) by the prepared biocatalyst during 90 min incubation; similar behavior (the substrate inhibition kinetics) was observed for both free and immobilized invertase [2].

The reuse of immobilized biocatalysts is one of the necessary assumptions for their potential applications in food and biotechnology industries. The efficiency of invert sugar formation was tested during six cycles using three different initial sucrose concentrations (5%, 10% and 20%) (Fig. 5). The microbeads exhibited the same invert sugar formation efficiency in all six cycles in the whole range of tested sucrose concentrations.

The prepared magnetically responsive yeast cells-containing alginate microbeads were stable during one month storage at 4 °C in the presence of calcium ions; there was very low change of invert sugar formation during this period.

To check the possible non-enzymatic sucrose conversion by the prepared biocatalyst, invertase-free alginate beads were prepared. It was shown that dead cells entrapped in alginate microbeads had no effect on sucrose conversion, and the presence of glucose was not detected.

4. Conclusions

From this study it is evident that one of the cheapest and most safe biological materials, *Saccharomyces cerevisiae* cells, together with magnetite microparticles entrapped into alginate microbeads can easily convert sucrose into glucose and fructose. The prepared magnetically responsive biocatalyst in the form of microparticles exhibited high activity (substantially higher in comparison with yeast cells entrapped in alginate millimeter-sized particles prepared in the standard way) and high stability, and can be used repeatedly. The magnetic properties of the prepared material enable simple separation of this microparticulate biocatalyst using appropriate magnetic separators. The sucrose hydrolysis by intracellular invertase exhibited substrate inhibition kinetics, similarly as described for free and immobilized invertase. Such a biocatalyst could be efficiently used for the formation of invert sugar in appropriate food technology processes.

Due to the presence of variety of enzymes in the entrapped yeast cells, this inexpensive biocatalyst can also be used for other biotechnology processes.

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