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EXPERIMENTAL ARTICLES

Some Properties of the Xylose/Glucose Isomerase of Immobilized *Arthrobacter* sp. Cells

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Abstract—The study of the xylose/glucose isomerase–containing *Arthrobacter* sp. B-5 cells immobilized in cobalt hydroxide gel showed that immobilization increases the substrate affinity of the enzyme, its thermo- and pH-optima of action and stability, and makes the addition of stabilizing cobalt ions to the isomerization medium unnecessary.

Key words: bacteria, *Arthrobacter*, xylose/glucose isomerase, immobilization, properties.

Xylose isomerase (D-xylose ketol-isomerase, EC 5.3.1.5), also known as glucose isomerase, catalyzes the isomerization of D-xylose into D-xylulose in vivo and, with lower efficiency, of D-glucose into D-fructose in vitro. The enzyme is employed in the industrial production of glucose–fructose syrup [1], which is used as a natural sweetener in nutriceutics and special diets.

The industrial production of glucose–fructose syrup is based on the use of immobilized crude xylose/glucose isomerase or cells containing this enzyme. Clinton Corn Processing company (United States) was the first to implement the isomerization of glucose into fructose on an industrial scale (in 1974). The production of glucose–fructose syrup is presently the most large-scale biotechnology based on immobilized biocatalysts.

Great research efforts are now being made to improve the available biotechnologies of glucose–fructose syrup production [1, 2]. In particular, new methods for enzyme immobilization are being developed, since immobilization considerably affects the physicochemical properties of xylose/glucose isomerase [3–7]. Appropriate immobilization media may decrease the optimum pH of xylose/glucose isomerase and enhance its thermostability. It is these properties of xylose/glucose isomerase that are considered to be basic in devising new efficient biotechnologies for the production of glucose–fructose syrup [8].

Earlier, we selected the *Arthrobacter* sp. strain B-5 that efficiently produced xylose/glucose isomerase, studied the major physiological and biochemical properties of this strain, determined the optimal conditions of glucose isomerization into fructose by intact cells, and immobilized them in cobalt hydroxide gel [9–12].

The aim of this work was to study the main physicochemical properties of the xylose/glucose isomerase–containing *Arthrobacter* sp. B-5 cells immobilized in cobalt hydroxide gel.

MATERIALS AND METHODS

The xylose/glucose isomerase–producing strain *Arthrobacter* sp. B-5 was obtained from the Collection of Microorganisms at the Institute of Microbiology, National Academy of Sciences of Belarus.

The strain was grown in a liquid nutrient medium containing (%) glucose, 1.0; peptone, 1.0; yeast extract, 0.7; K_2HPO_4, 0.3; and $MgSO_4 \cdot 7H_2O$, 0.1. The initial pH of the medium was 6.8. The medium was inoculated with aqueous suspensions of bacteria grown on a peptone–yeast extract agar at 28–30°C for 3 days. The initial culture density was (0.5–1) × 10^7 cells/ml. After 2 days of cultivation at 28–30°C, cells were harvested by centrifugation at 6000 *g* for 15 min and washed with distilled water. The bacterial biomass (2.5 g) with an 80–90% moisture content was suspended in a solution containing cobalt acetate in a relative amount of 50% of the dry weight of the biomass. The pH of the suspension was adjusted to 9.5 by adding the necessary amount of 10% ammonia solution. This procedure brought about the formation of cobalt hydroxide gel, in which *Arthrobacter* sp. B-5 cells were immobilized. The gel was washed with distilled water and then with 0.2 M K,Na-phosphate buffer, dehydrated at 28–30°C, and crushed. The preparation thus obtained was stored at room temperature.

The effect of temperature, pH, and the concentration of Co^{2+} and Mg^{2+} ions on the xylose/glucose isomerase activity of the immobilized cells was studied within the temperature range 40–90°C, pH 5.0–11.0, and the ion concentration range 0–50 mM.

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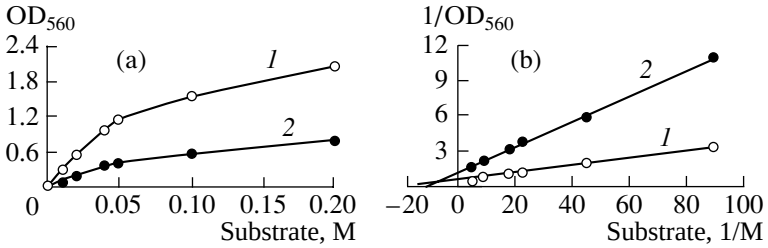

Fig. 1. (a) The Michaelis–Menten and (b) Lineweaver–Burk double reciprocal plots of the isomerization rate of (*1*) D-xylose and (*2*) D-glucose by the xylose/glucose isomerase of immobilized *Arthrobacter* sp. B-5 cells.

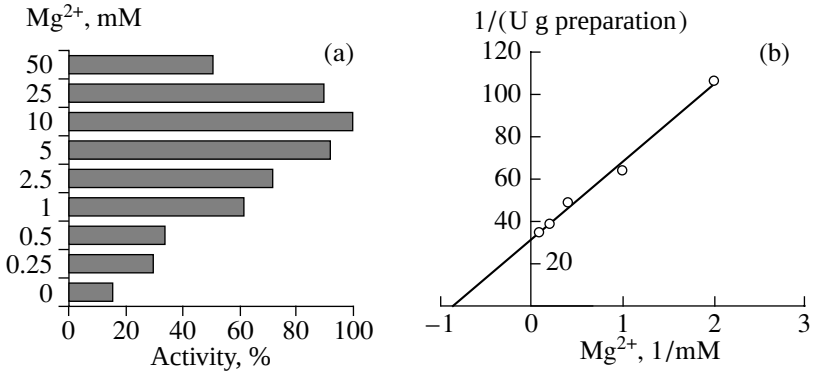

Fig. 2. (a) The Michaelis–Menten and (b) Lineweaver–Burk double reciprocal plots of the xylose/glucose isomerase activity of immobilized *Arthrobacter* sp. B-5 cells as a function of the concentration of Mg^{2+} ions.

The thermostability of xylose/glucose isomerase in the immobilized cells was determined from the residual activity of the enzyme assayed after heating the preparation in 0.2 M K,Na-phosphate buffer (pH 7.8) at 70 and 80°C for 15 to 300 min.

To determine the pH stability of xylose/glucose isomerase in the immobilized preparation, it was incubated at pH 5.0 to 11.0 in 0.2 M citrate–phosphate, 0.2 M K,Na-phosphate, or universal buffer system (depending on pH) at 70 and 80°C for 1 h; washed with distilled water and then with 0.2 M K,Na-phosphate buffer (pH 7.8); and assayed for the residual activity of the enzyme.

The Michaelis constant (k_m) of xylose/glucose isomerase in the immobilized cells was determined from the Lineweaver–Burk double reciprocal plot.

The stability of xylose/glucose isomerase in the immobilized cells during storage was determined by measuring the enzymatic activity of the immobilized cells stored at room temperature (≤25°C) over a period of 2 years. The activity was measured first at one-month and then at three-month intervals.

Isomerase activity was measured in a reaction mixture containing 0.2 ml of 1 M substrate solution, 0.5 ml of 0.2 M K,Na-phosphate buffer (pH 7.8), 0.1 ml of 0.1 M $MgSO_4 \cdot 7H_2O$, 0.2 ml of immobilized preparation, and distilled water to a total volume of 2 ml. The mixture was incubated at 70°C for 1 h. One unit of enzymatic activity (U) was defined as the amount of enzyme that isomerizes 1 μmol of D-glucose into D-fructose in one minute. Isomerase activity was expressed either in U/mg preparation or in OD_{560}. The relative activity was expressed as a percent of the control.

Fructose was analyzed by the Dische and Borenfreund method [13]. Protein was quantified by the Bradford method [14]. The dry matter content was determined refractometrically.

The data presented in this paper are the means of two–three independent experiments performed in triplicate.

RESULTS AND DISCUSSION

There is controversy in the literature as to the effect of immobilization on the kinetic parameters of xylose/glucose isomerase. For instance, researchers reported that immobilization augmented the Michaelis constant of the xylose/glucose isomerase of *Bacillus coagulans* and *Streptomyces thermonitrificans* [6, 15], decreased that of the xylose/glucose isomerase of *S. flavogriseus* [16], and did not affect the Michaelis constant of the enzyme from *S. phaeochromogenes* [17] and *Actinomyces olivocinereus* [3].

Our measurements showed that the Michaelis constants of the immobilized *Arthrobacter* sp. xylose/glucose isomerase for D-glucose and D-xylose are equal to

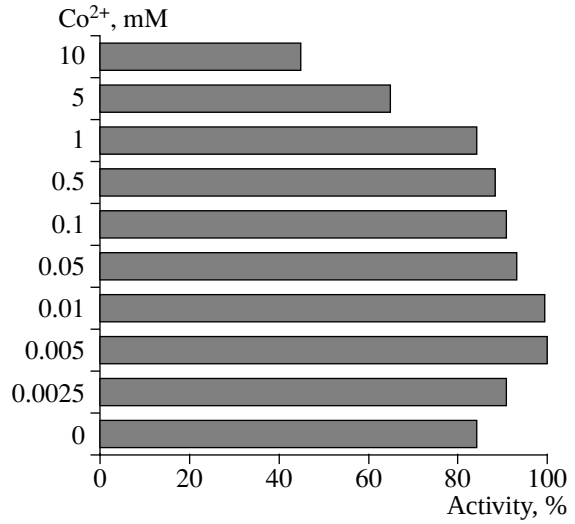

Fig. 3. The effect of Co^{2+} ions on the xylose/glucose isomerase activity of immobilized *Arthrobacter* sp. B-5 cells.

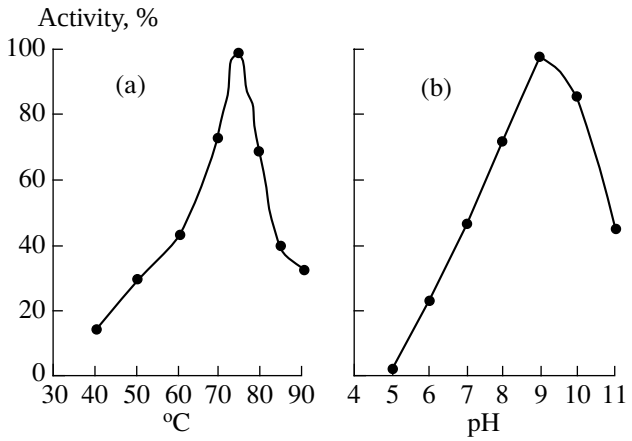

Fig. 4. The effect of (a) temperature and (b) pH on the xylose/glucose isomerase activity of immobilized *Arthrobacter* sp. B-5 cells.

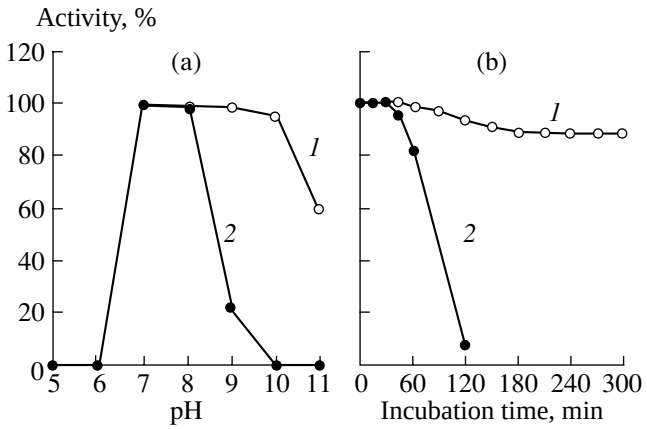

Fig. 5. (a) The pH and (b) the thermal stability of the xylose/glucose isomerase of immobilized *Arthrobacter* sp. B-5 cells exposed to (*1*) 70 and (*2*) 80°C.

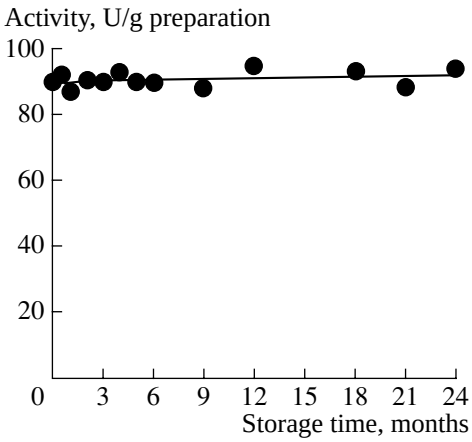

Fig. 6. The dynamics of the xylose/glucose isomerase activity of immobilized *Arthrobacter* sp. B-5 cells during storage at room temperature.

0.100 and 0.073 M, respectively (Fig. 1). These k_m values considerably differ from those determined for the xylose/glucose isomerase of intact cells (0.58 and 0.23 M, respectively) [10].

According to earlier observations [10], Mg^{2+} ions activate the xylose/glucose isomerase of *Arthrobacter* sp., while Co^{2+} ions stabilize it. The optimal concentration of Mg^{2+} ions for immobilized xylose/glucose isomerase turned out to be 10 mM (Fig. 2a). The apparent k_m of the immobilized enzyme for Mg^{2+} was found to be 1.14 mM (Fig. 2b).

Co^{2+} ions virtually did not affect the activity of immobilized xylose/glucose isomerase within the range 0.0025–0.1 mM and slightly inhibited it at higher concentrations (Fig. 3).

The maximum activity of xylose/glucose isomerase in intact *Arthrobacter* sp. cells was observed at 70°C and pH 8.0 [10], whereas the immobilized enzyme exhibited maximum activity at 75°C and pH 9.0 (Fig. 4). Reportedly, immobilization increased the temperature optimum of the *Lactobacillus brevis* 74 xylose/glucose isomerase [4], diminished it in the case of the enzyme of *S. flavogriseus* [16], and did not influence the temperature optimum of the enzyme from *Actinoplanes missouriensis* [18]. Similarly, immobilization exerted diverse effects on the optimum pH, thermostability, and pH stability of the xylose/glucose isomerase of the aforementioned and some other microorganisms [3–6, 15, 16, 18].

The xylose/glucose isomerase activity of intact *Arthrobacter* sp. cells is stable at 70°C and pH 8.0–9.0

within 1 h and declines by more than 80% in the case of incubation at 80°C for 1 h [10]. The xylose/glucose isomerase activity of immobilized *Arthrobacter* sp. B-5 cells incubated at 80°C at pH 7.0–8.0 is stable over a period of at least 1 h. At 70°C, the enzymatic activity is stable within a wider pH range (7.0–10.0) (Fig. 5a). At pH 8.0, the immobilized xylose/glucose isomerase retained most of its activity within 1 h at 80°C and at least within 5 h at 70°C (Fig. 5b).

One of the important characteristics of enzyme preparations is their stability during storage. The xylose/glucose isomerase of immobilized *Arthrobacter* sp. B-5 cells stored at room temperature (≤25°C) completely retained its activity over a period of 2 years (Fig. 6). For comparison, the immobilized xylose/glucose isomerase of *Streptomyces* sp. stored at 10°C retained its activity over a period of about 1 year [19]. The activity of the *Actinomyces olivocinereus* 154 xylose/glucose isomerase decreased by 20% after storage at 0–4°C for one year and by 50% after storage at room temperature for 3 months [3, 20].

Thus, the immobilization of the xylose/glucose isomerase–containing *Arthrobacter* sp. B-5 cells in cobalt hydroxide gel enhances the affinity of the enzyme for D-xylose and D-glucose, its thermo- and pH-optima of action and stability, and makes the addition of stabilizing cobalt ions into the isomerization medium unnecessary. These data show the feasibility of using this xylose/glucose isomerase preparation for the production of glucose–fructose syrup.

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