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# IMMOBILIZATION OF CELLS WITH GLUCOSE ISOMERASE ON COLLAGEN. CHARACTERISTIC OF THE COMPLEX

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Key words: *Actinoplanes missouriensis*, glucose isomerase, collagen, isomerisation of glucose.

*Actinoplanes missouriensis* cells containing glucose isomerase were immobilized on collagen by the method of macromolecular complexation. The properties of the obtained complex were compared with those of the enzyme in free cells. Glucose was isomerized by the continuous method in a reactor.

Various enzymes, including also glucose isomerase, are being immobilized with the use of inorganic carriers such as silicon compounds, ferric oxides, graphite or glass [1, 7, 13, 17, 20] or organic carriers, e.g. DEAE-cellulose, keratin, alginate and agar gels, polyacrylamides [2, 3, 5, 6, 8, 15, 16]. Noteworthy among the organic carriers is collagen. It is cheap to produce, its permeability and mechanical strength are good, and it may be used to immobilize microorganism cells. The advantages of collagen as carrier for immobilizing enzymes and microorganism cells were discussed elsewhere [10].

One of the techniques of immobilizing enzymes and cells on collagen is macromolecular complexation. The basic chemical reagent in this method is glutaraldehyde which, however, must be applied in a strictly defined concentration in view of its strong enzyme-inactivating properties. In addition to causing intramolecular enzyme-carrier cross bonding, glutaraldehyde polymerizes the collagen which becomes elastic, flexible and insoluble in water.

To determine optimum conditions of catalytic processes in an enzymatic reactor with the use of immobilized glucose isomerase it is necessary to establish optima of temperature and pH of the reaction as well as thermostability which may differ considerably from the respective parameters of free enzyme. Such discrepancies in other enzymes were reported by numerous authors [6, 9, 19, 21], but no information was found in the literature about glucose isomerase immobilized on collagen. It is also

important to determine parameters characterizing the operation of the enzymatic reactor such as residence time of substrate molecule in the reactor (T), degree of substrate conversion into the product (X) and productivity of the enzymatic reactor (Pr) [22].

The aim of this research was to determine optimum parameters of immobilization of microorganism cells containing glucose isomerase on collagen, to characterize the enzyme in immobilized and free cells, and to determine the relations between the basic parameters affecting isomerization of glucose to fructose in an enzymatic reactor operating continuously.

## METHODS

1. Strain and conditions of culture. The glucose isomerase-synthesizing microorganism *Actinoplanes missouriensis* was used. It was obtained in culture on a medium composed by the author [11, 12]. Following culture the cells were centrifuged, the obtained deposit was washed with distilled water, centrifuged once again and dried.

2. Collagen. Prepared collagen from ox tendons in the form of 10-20 mm fibres was obtained from Eastern Regional Research Laboratories, Philadelphia, Pa., U.S.A.

3. Substrate. The isomerized substrate was a 1 M solution of commercial glucose containing 0.01 M $MgSO_4 \cdot 7H_2O$ and 0.001 M $CoCl_2 \cdot 6H_2O$; the pH of the substrate was adjusted to 7.8 with sodium sulfite.

4. Cells immobilization and preparation of the reactor. Immobilization was performed with the macromolecular complexation technique [22]. The optimum concentration of glutaraldehyde was determined experimentally by adding 0.05-1.3% doses of this substance to the mixture of collagen and microorganism cells. The collagen with immobilized cells was shaped into a membrane and left to dry at room temperature. The dry collagen membrane was cut into 5 × 5 mm squares, 10 g of which were then placed in the enzymatic reactor.

5. Parameters characterizing properties of glucose isomerase:

— optimum temperature of the process was found by determining the activity of glucose isomerase in immobilized and free cells in the temperature range 50-90°C; results of determinations are given in per cent activity;

— optimum pH of the process was found by determining the activity of glucose isomerase in immobilized and free cells in the pH range 6.5-8.5; results of determinations are given in per cent activity;

— thermostability was investigated by determining activity of glucose isomerase in immobilized and free cells during 1 h in the temperature range 50-90°C; results of determinations are given in per cent activity;

— the stability of the activity of glucose isomerase in immobilized and free cells was studied by storing microorganism cells and collagen membrane with immobilized cells for five months at 4 and 25°C; samples of the membrane and of the cells were taken at one-month intervals for enzymatic activity determinations.

6. Enzymatic reactor. Glucose was isomerized by a singlestage continuous method in a glass enzymatic reactor (360 mm long with 22 mm internal diameter). The reactor was equipped with an external heating mantle maintaining temperature at 73°C by means of water circulation. The isomerization process is shown schematically in Fig. 1.

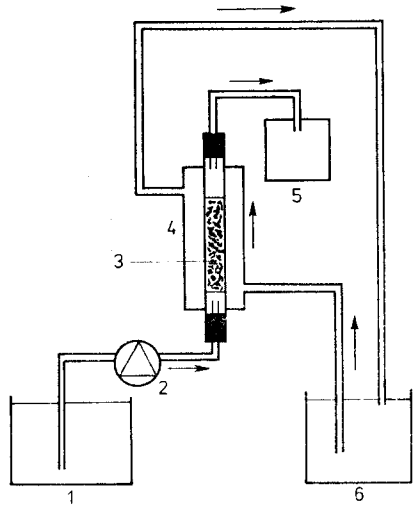


Fig. 1. Diagram of isomerization in enzymatic reactor; 1 — tank with substrate, 2 — pump supplying substrate, 3 — mass of immobilized enzyme, 4 — enzymatic reactor, 5 — tank with product, 6 — ultrathermostat with heating water

7. The parameters characterizing the operation of the reactor were calculated from formulas given in [22]:

— residence time of substrate molecule in the reactor (in min) was calculated from

$$T = \frac{V}{Q} \qquad (I)$$

where V — working capacity of the reactor (ml) and Q — substrate flow rate (ml/min);

— degree of glucose isomerization (in per cent) was calculated from

$$X = \frac{S_0 - S}{S_0} \times 100 \qquad (II)$$

where $S_0$ — concentration of substrate placed in the reactor (mg/l) and S — concentration of substrate removed from the reactor (mg/l);

— reactor productivity (in mg fructose $l^{-1}min^{-1}$) was calculated from the formula

$$Pr = \frac{X \times S_0}{T} \qquad (III)$$

where X — degree of glucose isomerization, $S_0$ — concentration of substrate placed in the reactor, T — residence time of substrate molecule in the reactor (min).

8. Determination of microorganism cells mass after culture. The cell mass was dried in an air flow (room temperature) and weighed.

9. Glucose isomerase activity was determined with thiobarbituric acid and expressed as J/g microorganism cells or as g complex. The glucose isomerase activity unit is the amount of enzyme producing 1 mg of fructose from 1 M glucose solution during 1 min at 70°C and pH 7.0 [14].

## RESULTS AND DISCUSSION

As can be seen in Fig. 2 there was a strong dependence between glutaraldehyde concentration in the collagen-enzyme mixture and immobilized enzyme activity. The lowest activity losses of about 4% occurred when the reagent concentration was 0.05-0.30%; when this concentration was 1.3% the activity decrease was as much as 80% of the initial value.

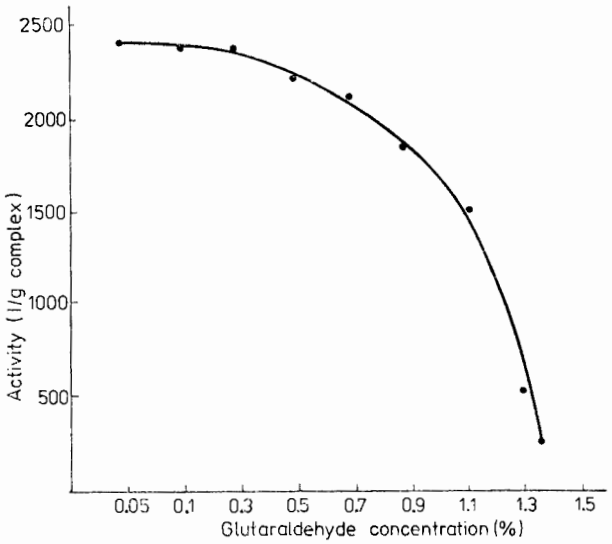


Fig. 2. Effect of glutaraldehyde concentration on glucose isomerase activity

Glutaraldehyde concentrations of 0.05, 0.1 and 0.3% were not high enough to polymerize the collagen. It was found that the right concentration is 0.5% at which the membrane remained stable and showed no signs of dissolution after 80 h of incubation in water at 80°C (Tab. 1). At this concentration the losses of activity of the immobilized enzyme were also small (Fig. 2).

Table 1. Effect of glutaraldehyde concentration on stability of collagen membrane

| Incubation of collagen in water at 80°C (h) | Glutaraldehyde concentration (%) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0.05 | 0.1 | 0.3 | 0.5 | 0.7 | 0.9 | 1.1 | 1.3 |
| 10 | + | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| 20 | − | + | ++ | ++ | ++ | ++ | ++ | ++ |
| 30 | − | + | ++ | ++ | ++ | ++ | ++ | ++ |
| 40 | − | − | ++ | ++ | ++ | ++ | ++ | ++ |
| 50 | − | − | + | ++ | ++ | ++ | ++ | ++ |
| 60 | − | − | + | ++ | ++ | ++ | ++ | ++ |
| 70 | − | − | − | ++ | ++ | ++ | ++ | ++ |
| 80 | − | − | − | ++ | ++ | ++ | ++ | ++ |

++ — undissolved collagen, + — partiellly dissolved collagen, − — dissolved collagen

Immobilization of cells with glucose isomerase shifted the enzyme's activity optimum to higher temperatures: in immobilized cells the maximum activity of the enzyme was at 73°C while in free cells this maximum was at 65°C (Fig. 3).

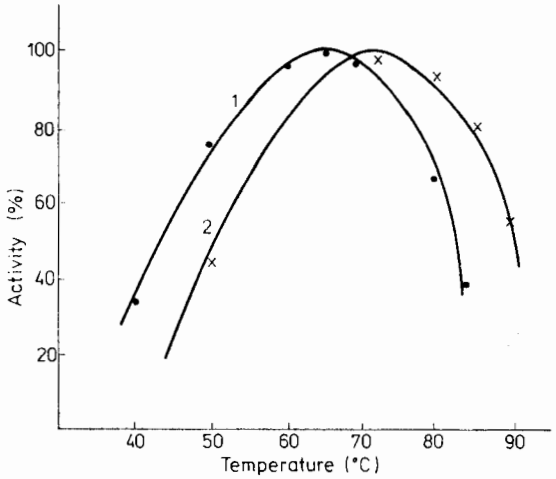


Fig. 3. Optimum temperature for glucose isomerase in free and immoblized *Actinoplanes missouriensis* cells; 1 — isomerase in free cells, 2 — isomerase in immoblized cells

The activity optima of both kinds of enzymes, i.e. in free and immobilized cells, were very close to one another — at pH 7.5 and 7.8, respectively. As the alkaline reaction of the medium increased, the enzyme in immobilized cells was inactivated to a lesser degree (Fig. 4).

The enzyme in immobilized cells was clearly more resistent to elevated temperatures than the enzyme in free cells (Fig. 5). At 90°C the activity of the former decreased by 22% while that of the latter was 55% lower.

During storage of free and immobilized cells in various temperatures

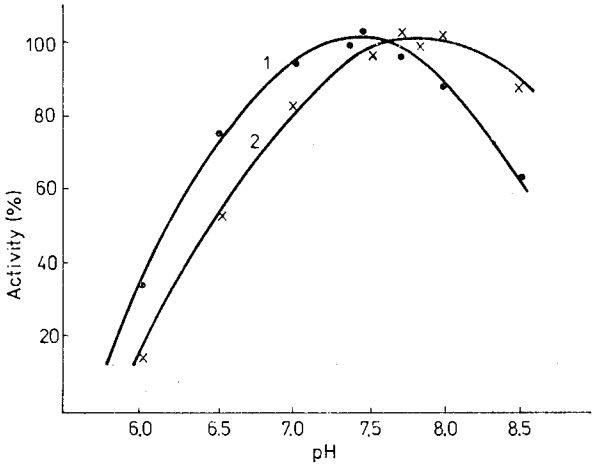


Fig. 4. Optimum pH for glucose isomerase in free and immobilized *Actinoplanes missouriensis* cells; 1 — isomerase in free cells, 2 — isomerase in immobilized cells

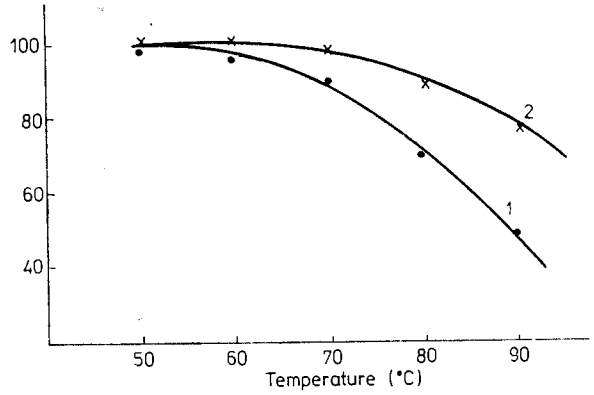


Fig. 5. Thermostability of glucose isomerase in free and immobilized *Actinoplanes missouriensis* cells; 1 — isomerase in free cells, 2 — isomerase in immobilized cells

(Table 2) enzyme inactivation was slower at 4°C. It is worth noting that when both free and immobilized cells were stored at an identical temperature, greater losses of enzyme activity were observed in the latter.

Residence time T (Fig. 6) had a considerable effect on the degree of glucose isomerization and on reactor productivity. The extreme "residence times" of 0.6 and 16 min had an adverse effect on the parameters of enzymatic reactor operation. The most effective "residence time" was 1 min: the degree of glucose isomerization and reactor productivity were optimal. Tsumura and Ishikawa [18] studied the effect of various "residence times" on glucose isomerization with glucose isomerase immobilized on DEAE-cellulose and found that in the residence time range of 2-25 min the degrees of glucose isomerization varied by up to 30%.

Table 2. Losses of glucose isomerase activity in free and immobilized *Actinoplanes missouriensi* cells during storage

| Cell state | Initial activity (J/g) | Storage temperature (°C) | Glucose isomerase activity in J/g after indicated number of months | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 2 | % | 3 | % | 4 | % | 5 | % |
| Free | 4700 | 25 | 4500 | 4.3 | 4350 | 7.5 | 4200 | 10.6 | 4000 | 14.9 |
| Immobilized | 2200 | 35 | 2080 | 5.5 | 2000 | 9.1 | 1940 | 11.8 | 1760 | 20.0 |
| Free | 4700 | 4 | 4600 | 2.2 | 4550 | 3.2 | 4470 | 5.0 | 4330 | 8.0 |
| Immobilized | 2200 | 4 | 2130 | 3.2 | 2090 | 5.0 | 1990 | 9.5 | 1930 | 12.3 |

During 400 h of reactor operation the losses of enzyme activity amounted to about 23%, the glucose isomerization degree was about 20%, while productivity was about 32 mg fructose $l^{-1}min^{-1}$ (Fig. 7).

The effect of partial filling of the reactor with air on glucose isomerase activity is illustrated in Fig. 8. According to the obtained results, the air which entered the reactor together with the substrate and formed numerous bubbles on the enzymatic mass decreased isomerase activity down to 30% after about 60 h of the process. Removal of air from the reactor led to an immediate increase of enzyme activity. Dinelli and Morisi [4] isomerized glucose in a column reactor and observed that the presence of air bubbles inside the reactor led to inactivation of glucose isomerase.

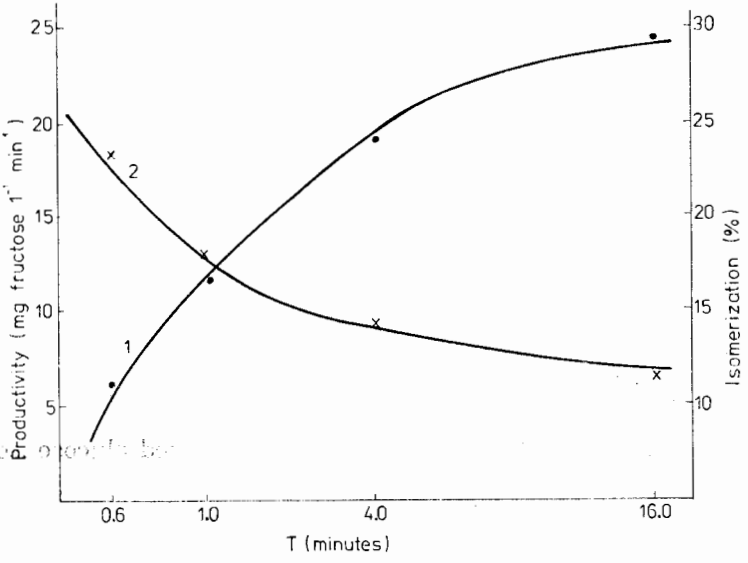


Fig. 6. Effect of various T values on reactor productivity and glucose isomerization; 1 — productivity, 2 — isomerization

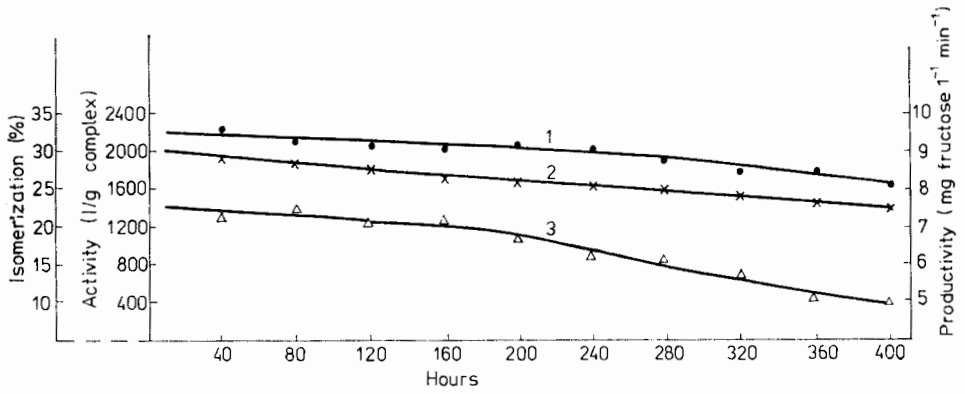


Fig. 7. Continuous glucose isomerization in the enzymatic reactor; 1 — activity, 2 — isomerization, 3 — productivity

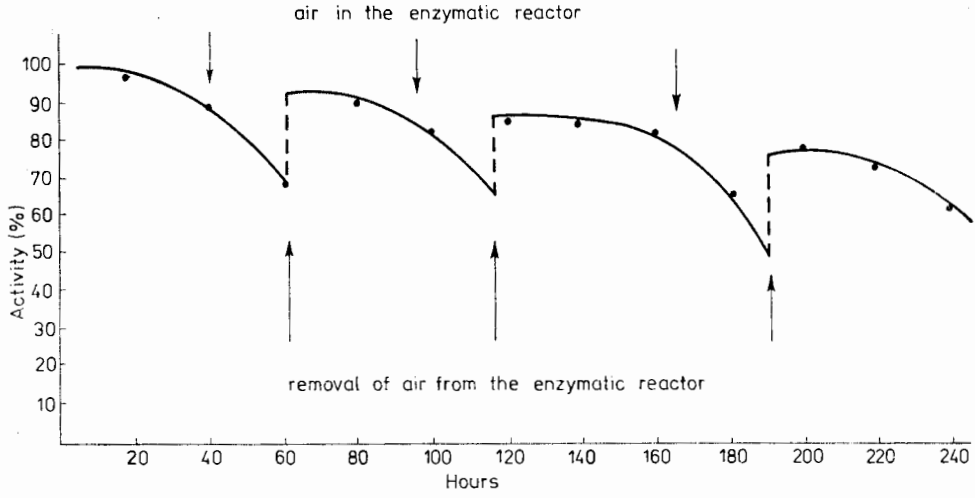


Fig. 8. Effect of air in the enzymatic reactor on glucose isomerase activity

## CONCLUSIONS

1. Macromolecular complexation as a technique of immobilizing glucose isomerase-containing cells on collagen ensures binding of the enzyme to the carrier.

2. Glutaraldehyde concentration affects immobilized glucose isomerase activity and degree of collagen membrane polimerization.

3. Immobilization on collagen of microorganism cells with glucose isomerase altered optimum temperature of activity and pH of the enzyme and increased its thermal stability in comparison to the same enzyme unbound with the carrier.

4. The time of residence of the substrate molecule in the (T) reactor had an effect on the degree of glucose isomerization and on reactor productivity.

5. Continuous isomerization of glucose in the enzymatic reactor changed the activity of the enzyme in immobilized microorganism cells.

6. The presence of air in the mass filling the enzymatic reactor adversely affected enzymatic catalysis.

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UNIERUCHAMIANIE NA KOLAGENIE KOMÓREK ZAWIERAJĄCYCH IZOMERAZĘ GLUKOZOWĄ I CHARAKTERYSTYKA KOMPLEKSU

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Streszczenie

Unieruchamiano na kolagenie komórki mikroorganizmu *Actinoplanes missouriensis* metodą makromolekularnej kompleksacji zawierające izomerazę glukozową.

Określano właściwości uzyskanego kompleksu w stosunku do enzymu zawartego w komórkach wolnych oraz prowadzono izomeryzację glukozy w reaktorze w układzie ciągłym.

Stwierdzono, że makromolekularna kompleksacja zapewniała wiązanie komórek z nośnikiem. Optymalne stężenie aldehydu glutarowego podczas unieruchamiania powinno wynosić 0,5% w stosunku do użytego nośnika (rys. 2, tab. 1). Izomeraza glukozowa zawarta w unieruchomionych komórkach wskazywała optimum działania w temp. 73°C zaś w wolnych w 65°C (rys. 3). Optymalne pH działania izomerazy glukozowej zawartej w unieruchomionych komórkach wystąpowało przy 7,8 zaś w wolnych przy 7,5 (rys. 4). Inkubacja unieruchomionego w komórkach enzymu, w temp. 90°C powodowała 22% straty aktywności w porównaniu z 55% strat aktywności, enzymu w komórkach wolnych (rys. 5). Temperatura przechowywania w 4°C powodowała niższe o ok. 10% straty aktywności izomerazy glukozowej w komórkach wolnych i unieruchomionych w porównaniu z przechowywanymi w temp. 25°C. Preparaty unieruchomione miały średnio o ok. 20% wyższe straty aktywności enzymatycznej w porównaniu z wolnymi przechowywanymi w analogicznych warunkach (tab. 2). Czas pobytu „T" odgrywał dużą rolę w kształtowaniu produktywności i stopniu izomeryzacji glukozy i jego optymalna wartość wynosiła 1,0 min (rys. 6). Podczas 400 h prowadzenia ciągłej izomeryzacji glukozy w reaktorze enzymatycznym, straty aktywności enzymu wynosiły ok. 23%, stopień fruktozy $1^{-1}min^{-1}$ (rys. 7). Powietrze dostające się do złoża wypełniającego reaktor wywierało ujemny wpływ na aktywność izomerazy glukozowej (rys. 8).