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Isolation of Glucose Isomerase Hyperproducing Strain, *Streptomyces* sp. SM 805 and Its Enzymatic Properties

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***Streptomyces* sp. No.8, which produced glucose isomerase was isolated from soil samples. The isolated strain, No.8, was identified as belonging to the Genus *Streptomyces*. A mutant strain, SM 805, showed the greatest ability to produce glucose isomerase. It was developed from the strain, No.8, by mutagenesis induced by NTG and UV treatment. The mutant strain, SM 805, produced about 7 times more glucose isomerase than the parental strain, No.8. This enzyme catalyzed the isomerization of D-xylose, D-glucose and D-ribose. It was inactive in the absence of metal ions, but was activated by the addition of Mg^{2+} or Co^{2+}. The optimum temperature and pH for enzyme activity were 80°C and pH 8.5, respectively. The enzyme was stable in a pH range of 6.0 to 10.0, and it was highly thermostable. There was no activity loss below 80°C, and even above 90°C about 45% of its activity was retained. The reaction equilibrium was reached when about 53% fructose was present in the reaction mixture. Whole cells containing glucose isomerase from *Streptomyces* sp. SM 805 were immobilized by glutaraldehyde treatment. The resultant immobilized enzyme pellets showed a relatively long stability during the isomerizing reaction. The half-life of the immobilized enzyme during the operating was 45 days in the presence of 10 mM Mg^{2+}.**

Glucose isomerase (D-Xylose isomerase E.C.5.3.1.5) catalyzes the reversible isomerization of glucose to fructose (17). Almost every known glucose isomerase is an intracellular enzyme which also isomerizes xylose. This enzyme is used for the commercial production of high fructose syrup. Producers of starch syrups have attempted to increase the sweetness of their syrups to use as sugar substitutes. Since Marshall and Kooi (11) reported the ability of *Pseudomonas hydrophila* to convert D-xylose to D-fructose, a large number of microorganisms that are capable of producing glucose isomerase have been found. Among these, various species of *Streptomyces* have been the most extensively studied and used as a source of the enzyme. These include *Streptomyces phaeochromogenes* (8), *S. albus* (15), *S. cinnamonensis* (6), *S. flavovirens* (19), *S. fradiae* (10) and unidentified *Streptomyces* sp. (3).

Glucose isomerase is usually prepared in the form of heat treated whole cells and immobilized enzymes for the isomerization of glucose. Several techniques of the whole cell immobilization of glucose isomerase have been reported. These whole cell glucose isomerases were immobilized by direct crosslinking of whole cells with glutaraldehyde, or by entrapment of the enzyme support matrices, such as gelatin (12) and collagen membrane (20) crosslinked with glutaraldehyde.

In production of the enzyme for practical use the most important problem is that the selection of a microbial source which produces a commercially suitable high quality glucose isomerase. Therefore, in this study we attempted to isolate a microorganism which produced glucose isomerase. As a result of screening, we obtained *Streptomyces* sp. strain SM 805, which produced an effective glucose isomerase.

This paper deals with the characteristics of a microorganism isolated from soil and some properties of the glucose isomerase produced by the microorganism.

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Key words: *Streptomyces* sp. SM 805, glucose isomerase

MATERIALS AND METHODS

Isolation of Microorganisms with Ability to Produce Glucose Isomerase

Soil samples were collected from several sites in Korea. These soil samples were dispersed in sterile water and plated onto a medium containing 1% xylose, 0.03% vitamin-free casamino acid (Difco), 0.2% KNO_3, 0.2% K_2HPO_4, 0.2% NaCl, 0.05% $MgSO_47H_2O$, 0.02% $CaCO_3$, 0.001% $FeSO_47H_2O$ and 1.8% agar (8). The pH of the medium was adjusted to 7.0. The medium also contained 50 μg of cycloheximide per ml of medium (2). The plates were incubated at 30℃ for 3 days and the colonies which developed were transferred to a nutrient rich medium composed of 1% xylose, 1% polypeptone, 1% yeast extract, 0.1% $MgSO_47H_2O$, 0.3% K_2HPO_4, and 1.8% agar, at a pH of 7.0. This procedure was repeated until a pure culture was obtained. Colonies which formed were innoculated into a liquid rich medium and incubated for 2 days with shaking at 30℃, after which the glucose isomerase activities of the culture fluids were measured and compared. The microbiological properties of the isolated microorganism were investigated according to the method described Nomomura (13) and Williams (21).

Mutagenesis and Selection of Mutant Strains

Spores were collected by centrifugation from a 5 day old slant washed twice with sterilized distilled water. The spores were resuspended in sterilized distilled water and transferred to a 0.2% solution of N-methyl-N'-nitro-N'-nitrosoguanidine (NTG). The solution was agitated for 30 minutes. Spores were collected by centrifugation and washed twice with sterile 0.8% saline solution then suspended in a sterile 0.8% saline solution. Spores from the saline solution were transferred to a petri dish containing on agar medium as described above. The spores were allowed to germinate and resporulate. Spores from a single colony were suspended in a sterile saline solution in a petri dish. The suspension was radiated for 1 hour with ultraviolet light at a distance of 25 cm above the petri dish. Spores were collected from the saline solution and plated onto petri dish containing an agar medium containing D-xylose as the sole carbon source as described above. Spores were handled and microorganism was cultured in a liquid rich medium, then tested for glucose isomerase activity.

Assay of Glucose Isomerase

The assay of glucose was carried out follows: The reaction mixture was prepared by adding 0.5 ml of 0.2 M phosphate buffer solution (pH 7.2), 0.2 ml of 1 M glucose solution and 0.1 ml of 0.1 M $MgSO_47H_2O$ solution to 0.2 ml of the enzyme solution. The final volume was brought up to 2 ml with distilled water. After incubation for one hour at 70℃ the reaction was stopped by the addition of 2 ml of 0.5 M perchloric acid. The D-fructose produced was measured by the systein-carbazole method (4). One unit of the enzyme was defined as the amount of the enzyme that produced one mg of D-fructose under the assay condition described above.

Cultivation in a 30 l Jar Fermentor and Preparation of the Crude Enzyme

Streptomyces sp. strain, SM 805, was inoculated from a slant into 200 ml of a liquid rich medium in a 1 l Sakaguch flask, and cultivated at 30℃ for 36 hours with shaking. It was then used as seed when 800 ml of this seed culture was inoculated into a production medium containing 2% xylose, 0.8% glucose, 2% corn steep liquor, 0.05% $MgSO_47H_2O$, 0.1% K_2HPO_4, and 0.02% $CoCl_26H_2O$ in a 30 l jar fermenter (Marubish, MSJ-U). Culture conditions were a temperature at 30℃, an agitation speed at 350 rpm, and an air flow rate of 0.8 vvm. At the end of cultivation the broth was steam-heated at 70℃ for 30 minutes then cooled rapidly. Cells were harvested by centrifugation. The whole cell enzyme paste obtained was used for immobilization. The crude enzyme solution was prepared as follows: The cell suspension was sonicated at a frequency of 10 KC for 20 minutes, cooled with ice water and then centrifuged at 10,000 rpm for 10 minutes. The supernatant was fractionated with ammonium sulfate and the precipitate formed between 35% and 70% saturation was collected, dissolved in 0.02 M phospate buffer at pH 7.5, and dialyzed overnight against the same buffer containing 0.02 M $MgSO_4 \cdot 7H_2O$. The dialyzed enzyme solution was freeze dried and used as the crude enzyme. The enzyme preparation was stored at 4℃.

Immobilization of Whole-cells Containing Glucose Isomerase

Cell paste, obtained from culture broth, was suspended in distilled water and immobilized by a crosslinking system composed of 0.2% polyethyleneimine, 0.05% chitosan, and 0.2% glutaraldehyde. After reaction for one hour at 25℃ the cell mass was collected on a Buchner funnel, washed with water, and the cell cake extruded through a 1 mm die opening then dried overnight at room temperature. The dried product was ground and sieved to obtain the desired size of 20 to 40 mesh.

Continuous Operation of the Immobilized Enzyme

The immobilized enzyme pellets were packed in a glass column (2.5 cm internal diameter, 25 cm height) with a jacket for temperature control. The column was operated at 60℃ with a 45% glucose solution containing 10 mM Mg^{2+} at a pH 8.0. The substrate solution was passed through the column at a flow rate which was

regulated to give the degree of glucose conversion desired.

RESULTS AND DISCUSSION

Screening of Microorganisms for Glucose Isomerase Producing Capacity

More than 350 colonies of *Actinomyces* were isolated from soil samples and each was tested for its capacity to produce glucose isomerase. Among these, strain No.8 was selected because it had greatest capacity to produce glucose isomerase. After mutagenesis of strain No.8 induced by NTG and UV treatment, mutant strains were tested for glucose isomerase production capacity. We selected *Streptomyces* sp. strain, SM 805, which showed the highest glucose production capacity. The selected mutant strain, SM 805, produced about 7 times more glucose isomerase (315 Units/ml) than the parental strain No.8. This result is similar to that of Baruara and William (1). The selected strain, SM 805, was used for detail study. Taxonomic study of isolated strain No.8 was carried out according to the method described by Nomomura (13) and Williams (21).

The isolated strain grew in the form of a many branched mycellium with a typical aerial mycellium, and had the smell of damp soil characteristic of *Sreptomyces*. The isolate also had the LL-type of diaminopimelic acid, in the acid hydrolyzate of the cells, indicating that it belongs to cell type I of Lechevalier and Lechevalier (9). These results indicate that the isolated strain No.8 belongs to the genus *Streptomyces*. Morphological and physiological characteristics of a strain No.8 are shown in Table 1 and Fig. 1.

Enzyme Production in a 30 *l* Jar Fermentor

The time course of cultivation of strain SM 805 is illustrated in Fig. 2. Cell growth reached to a maximum at around 38 hours of cultivation, and the production of glucose isomerase reached a maximum at about 45 hours of cultivation.

Table 1. The taxonomic characteristics of strain No.8.

1. Aerial mass color on oatmeal agar	Grey
2. Reverse side pigment	Dark brown
3. Melanoid pigment	Variable
4. Spore chain morphology	Rectiflexibles (RF)
5. Spore size	0.6~0.8 μm
6. Diaminopimelic acid	LL-type
7. Carbon utilization Glucose, Xylose, Fructose, Mannitol, Arabinose, Rhamonose, Sucrose	Positive (+)
Raffinose	Doubtful (±)
Salicin	Negative (−)

Thereafter, the enzyme activity of the culture broth gradually decreased. Based on the volume of culture broth, the maximum yield was 13g per liter of broth. The pH profile showed a decrease to a low value of 5.7 after 21 hours of cultivation, followed by an increase thereafter. The decrease in the yield of the enzyme after reaching a maximum value at 45 hours was probably due to autolysis of cells and deactivation of glucose isomerase by proteolytic enzymes during the death phase. Glucose isomerase in the cells of a strain of *Streptomyces* exhibited high heat-stability at an optimum temperature of about 80℃ (7, 15, 16, 18), whereas enzymes

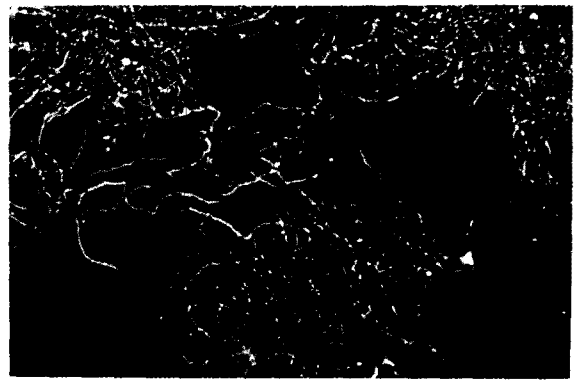

Fig. 1. Photomicrograph of mycelia of *Streptomyces* sp. No.8.

Streptomyces sp. No.8 cultured in liquid rich medium for 2 days at 30℃.

*One scale division equals 1 μm

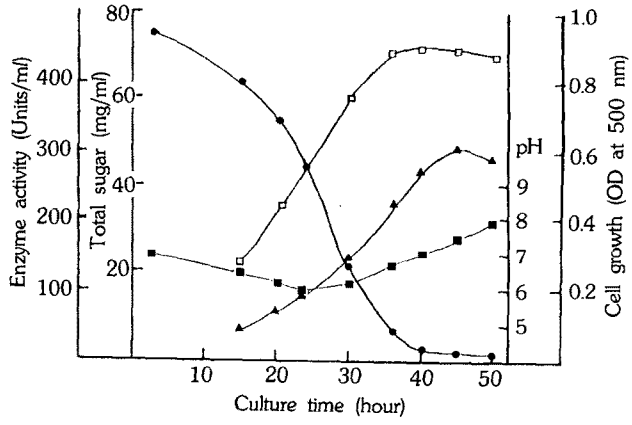

Fig. 2. Time course of cultivation of strain SM 805 in a 30 *l* jar fermentor.

Streptomyces sp. SM 805 was cultivated in submerged culture medium at 30℃ using 30 *l* fermentor. The pH, enzyme activity, cell growth and total sugar of culture broth during cultivation were measured.

Total sugar; ●—●, cell growth; □—□, pH; ■—■, enzyme activity; ▲—▲

which participate in autolysis are inactivated upon heat-treatment for about 10 minutes above 60℃. In this study, the culture broth was heat-treated at 70℃ for 30 minutes resulting in increased enzyme stability. Thus, heat-treatment of the culture broth is considered to be very effective.

Enzymatic Properties of Glucose Isomerase

Substrate Specificity

As shown in Table 2 the enzyme showed isomerizing activity for D-xylose, D-glucose and D-ribose, but not for D-arabinose, D-galactose, D-manose, L-xylose, and L-glucose. Its affinity for D-xylose was much higher than its affinity for D-glucose and D-ribose. The substrate specificity of the enzyme was similar to that of an enzyme from *Streptomyces griseofuscus* (7).

Effect of Metal Ions on the Activity of the Enzyme

Table 2. Substrate specificity of the enzyme.

Substrate	Activity (%)
D-Glucose	100
D-Xylose	126
D-Ribose	37
D-Galactose	0
D-Arabinose	0
D-Sorbitol	0
D-Mannose	0
D-Mannitol	0
L-Glucose	0
L-Xylose	0
L-Arabinose	0

After incubation at 70℃ for 1 hr using 0.2 M sugar or sugar alcohol as substrate, ketose was assayed by the standard assay method and expressed as fructose, xylulose or ribulose.

Table 3. Metal requirement for enzyme activity.

Metal salt (1 mM)	Activity (%)
None	0
$FeSO_4$	15
$MgSO_4$	100
$CaCl_2$	0
$CuSO_4$	0
$NiSO_4$	3
$MnCl_2$	13
$CoCl_2$	72
$ZnSO_4$	0

The enzyme was dialyzed against 0.01 M EDTA for 24 hrs then dialyzed against demineralized distilled water for 24 hrs to remove metal ions. The activity was assayed by the standard assay method, omitting $MgSO_4$.

As shown in Table 3 the enzyme was activated by addition of metal ions, such as Mg^{2+} and Co^{2+}, and showed no activity in the absence of Mg^{2+} and Co^{2+}, Fe^{2+}, Mn^{2+} and Ni^{2+} were slightly effective and others were ineffective. The reguirement of metal ions for enzyme activity is similar to an enzyme produced by *Streptomyces phaeochromogenes* (17) and *S. griseofuscus* (7).

Effect of Temperature and pH on the Activity of the Enzyme

Fig. 3 shows the effect of temperature on glucose isomerase activity. The optimum temperature was 80℃, which is in agreement with other studies (7, 15, 16, 18). As shown in Fig. 3 the enzyme activity at 80℃ was two times higher than at 60℃. However, degradation of keto-sugars, as shown by pronounced discoloration of an aqueous sugar solution, occurs at higher temperature. Therefore, continuous column operation was performed at 60℃.

When glucose isomerase activity was examined in the pH range of 5.0 to 11.0 a sharp increase in enzyme activity occurred between pH 6.0 and 8.0, as shown in Fig. 3. The optimum pH appears to be between pH 8.0 and 8.5. This result is similar to that reported by Chou *et al* (3). The pH optimum for activity of the enzyme was relatively lower than the value of pH 9.5 reported by Tsumura and Sato (18). Under alkaline conditions a non-metabolizable sugar, psicose, is produced in hot glucose and fructose solutions (14). Therefore, a low pH optimum is an attractive property for enzyme application because the use of neutral or lower pH in the isomerization of glucose prevents the formation of psi-

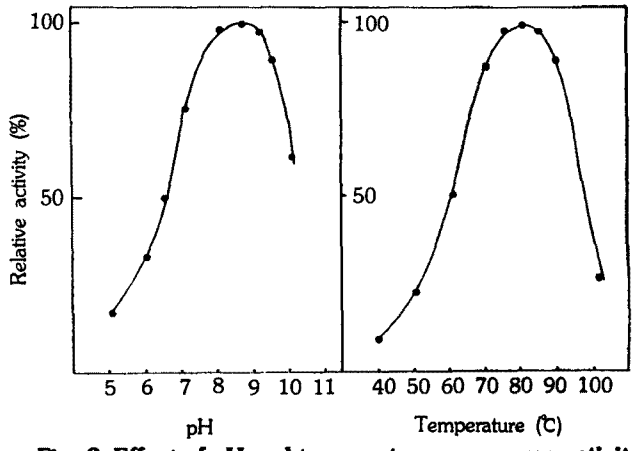

Fig. 3. Effect of pH and temperature on enzyme activity.
Temperature and pH values were varied as indicated. A potassium phosphate buffer (0.05 M) was used between pH values of 5.0 to 8.5 and a carbonated-bicarbonated buffer (0.05 M) was used between pH values of 8.5 to 11.0.

cose.

Effect of Temperature and pH on the Stability of the Enzyme

The reaction mixture was incubated at various temperatures for 1 hour and the remaining activity was assayed. As shown in Fig. 4 the enzyme was highly thermostable. There was no loss in activity below 80℃ and even above at 90℃ about 45% of activity was retained. The effect of pH on stability of the enzyme is shown in Fig. 5. After overnight incubation at 30℃ at various pH values, the remaining activity was assayed. The enzyme was stable in a range of pH from 6.0 to 10.0, but stability decreased sharply outside this range.

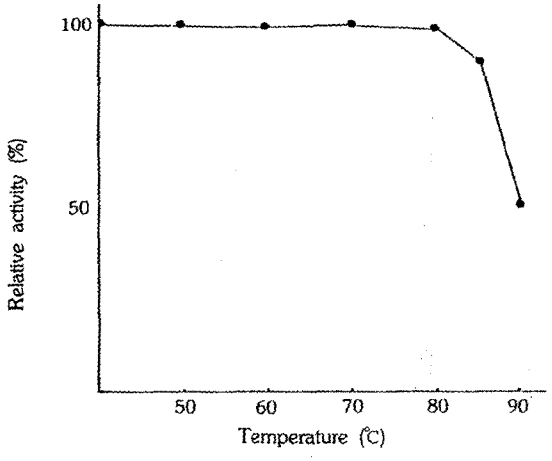

Fig. 4. Thermal stability of the enzyme.
After preincubation at each temperature for 1 hour, residual activity was determined by the standard assay method.

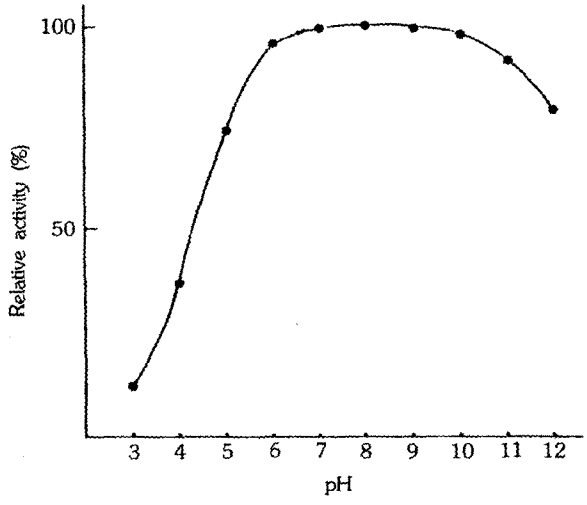

Fig. 5. Effect of pH on stability of the enzyme.
Residual activity was assayed by the standard assay method after preincubation of 0.1% enzyme solution at each indicated pH at 30℃ for 24 hrs.

Reaction Equilibrium

The interconversion of D-glucose and D-fructose was investigated starting with both sugar as a substrate. The enzyme preparation was incubated at 60℃ in a reaction mixture containing D-glucose or D-fructose as a substrate. At given time intervals the fructose content of the reaction mixture was determined. The results are shown in Fig. 6. Interconversion of D-glucose and D-fructose catalyzed by glucose isomerase proceeded until it reached an equilibrium with each sugar. At equilibrium the reaction mixture contained 53 percent D-glucose and 53 percent D-fructose, under the conditions described. The value of conversion rate of the enzyme was higher than 50 percent reported by Kasumi *et al.* (7) and 52 percent reported by Tsumura and Sato (17).

Continuous Column Operation of Immobilized Enzyme

Continuous column operation of immobilized enzyme pellets was carried out at 60℃ and a pH of 8.0. As shown in Fig. 7, the required mean residence time (the value of an enzyme bed volume per substrate flow rate) was 30 minutes in order to attain 42% conversion of glucose in a column packed with 20g of the immobilized enzyme pellets. The operational stability of the immobilized enzyme during continuous column operation was examined. Table 4 shows activity with respect to time and yield as grams DS of 42% fructose per gram of enzyme. The activity decay profile indicates that the half life was about 45 days. After 45 days about 3,800 grams of DS (42% fructose) were produced per gram of immo-

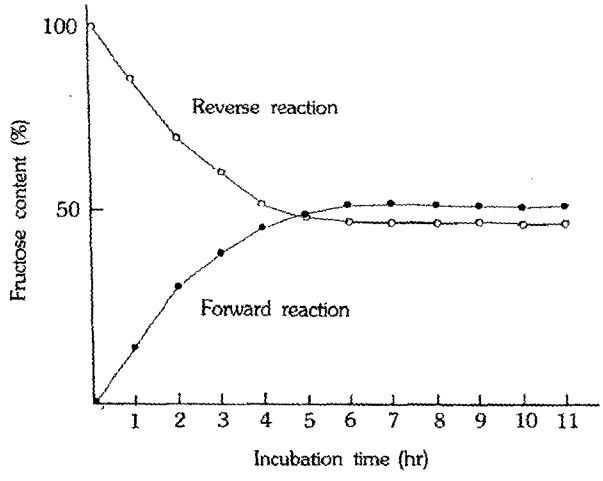

Fig. 6. Reaction equilibrium.
A reaction mixture composed of 0.5 M glucose (or fructose), a 0.1 M phosphate buffer (pH 7.2), 0.05 M Mg^{2+} and 300 μg/ml enzyme was incubated at 60℃. At th indicated time intervals, aliquots of the reaction mixtu were poured into 0.5 N perchloric acid and assayed f fructose content.

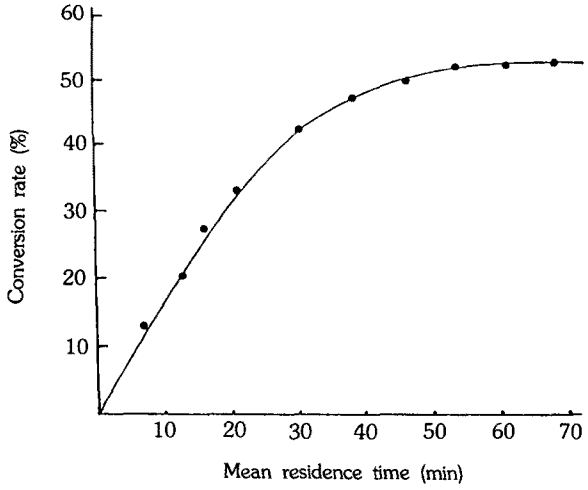

Fig. 7. Relationship between conversion rate and mean residence time of glucose feed solution during the column operation of immobilized enzyme.
The column (2.5 cm diameter 30 cm height) packed with 20g of immobilized enzyme pellets was operated at 60℃, with 45% glucose solution containing 10 mM Mg^{2+}, pH 8.0. The mean residence time was calculated using the value of enzyme bed volume per substrate flow rate.

Table 4. Stability and yield of immobilized glucose isomerase pellets

Time (days)	Relative activity (%)	Yield (g/g)
1.0	100	113
4.8	96.3	593
10.1	89.5	1,123
15.1	83.7	1,619
20.0	78.0	2,082
24.8	71.6	2,506
30.1	64.5	2,888
34.9	58.1	3,231
39.9	53.8	3,550
45.1	50.2	3,846

Continuous column operation of immobilized enzyme pellets was carried out at 60℃ and pH 8.0, using 45% glucose containing 10 mM Mg^{2+}. Glucose feed was perculated down through the bed at a flow rate sufficient to maintain a fructose conversion rate of 42%.
*Yield: DC of 42% fructose (g)/immobilized enzyme (g)

bilized enzyme. Huitron and Limon (5) found that the operational half-life of the immobilized enzyme was a function of the Mg^{2+} concentration and also depended on the geometry of the columns.

In evaluating an immobilized-enzyme system, the important factors to consider are the amount of enzyme loaded per unit of reactor column, the flow properties of the enzyme complex, and the stability of the enzyme complex. The operational stability of the immobilized enzyme employed in this study could be improved if the immobilized conditions and operational conditions were investigated in greater detail.

The glucose isomerase from *Streptomyces* sp. SM 805 reported here has several attractive properties, such as a relatively low pH optimum for activity and a high thermal stability, making it a potentially useful industrial enzyme. Further studies including optimal culture conditions and detailed conditions for immobilization and column operation are now in progress.

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