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THE BIOCONVERSION OF STARCH HYDROLIZATES TO GLUCONIC ACID AND ITS SALTS ON MICROBIOLOGICAL WAY

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Key words: *Aspergillus niger*, starch hydrolizate, gluconic acid, glucose oxidase, glucoamylase

Starch hydrolizates were used to obtain sodium gluconate and calcium gluconate. In the process of bioconversion *Aspergillus niger* mutant J-0-12 was used. The medium containing starch hydrolizate was treated by glucoamylase and parallel *A. niger* was cultivated in this medium. *A. niger* converted formed glucose to gluconic acid. In the best experiments of bioconversion, obtained yields were close to stoichiometric values.

INTRODUCTION

Different sources enumerate some microorganisms able to convert glucose to gluconic acid [2, 13, 15]. These are bacteria from *Acetobacter, Gluconobacter, Pseudomonas* and filamentous fungi from *Aspergillus, Penicillium,* and *Endomycopsis*. Particularly some *A. niger* strains [10, 11] or their mutants [8, 9] are used in gluconic acid production. It has been found out [1, 3, 15] that in fungi from *Aspergillus* glucose oxidase (β-D-Glucose: oxygen oxidoreductase) is the main enzyme responsible for glucose oxidation to gluconic acid [5]. Besides that enzyme, these fungi have, necessary in this bioconversion, catalase as well as considerable amylolytic activity. The raw material used in gluconic acid and its salts production is pure glucose. The main aim of that work is to investigate the possibilities of substitution of pure glucose by starch hydrolizates which could greatly decrease the costs of production.

MATERIALS AND METHODS

In our experiments we used *A. niger* mutant J-O-12 from the collection of Research Laboratory, Citric Acid Factory in Zgierz [8]. Starch hydrolizates used in our experiments were produced in Potato and Starch Processing Factory in Luboń from potato starch by acid hydrolysis. Starch hydrolizate contained 80% d.w. and glucose coefficient DE = 40. In the process of bioconversion, we used extract of glucoamylase AMG 300 with the activity of 300 AGU/cm^3, produced by Novo Industri.

METHOD OF FERMENTATION

The process of bioconversion was carried on in the laboratory fermenter of working capacity 35 dm^3 and in industrial fermenters of working capacity 8000 dm^3, in 30-32°C with continuous agitation (250 rpm) with the aeration rate 0.4-0.6 $dm^3/dm^3/min$. pH of the medium was kept on the level 5.0-6.0 using 25% NaOH or $CaCO_3$. Soybean oil was used as antifoam agent.

Composition of the medium

Starch hydrolizate	160-200 g
$MgSO_4 \times 7H_2O$	0.2 g
KH_2PO_4	0.2 g
NH_4OH 25%	0.8 cm^3
H_3PO_4	1.0 cm^3
Tap water	1.0 dm^3

After sterilization (30 min, 110°C) the medium was cooled down to 60°C and enzymatic solution (AMG 300) was added in amount of 0.02-0.045%. It was kept in the same temperature for 4 hours and then cooled down to 35°C and inoculated with conidia suspension of *A. niger* J-O-12. In case of industral fermenters, medial fermenter-inoculator of working capacity 800 dm^3 was used.

ANALYTICAL METHODS

Bioconversion was controlled by quantitative analysis of gluconic acid, and by chromatographic analysis of organic acids as well as by glucose determination in the medium. The amount of gluconic acid was directly calculated from the capacity of NaOH used for neutralization during bioconversion process or by cation-exchangig resin method [7] in which gluconic acid salt was changed into the acid and was determined by titrating with 0.1 n NaOH. Chromatographic analysis was carried out according to Lugg and Overell [6] using Whatman paper 1, and butanol, formic acid, water (4:1:5 v/v) with bromocresole blue as a solvent. After drying chromatograms in room temperature, yellow spots of acids were visible on the blue background. The Fehling method for determination of reduced sugars with Soczyński modification [16] was used for analysis of glucose in the medium.

RESULTS AND DISCUSSION

In initial bioconversion experiments in which glucose was substituted by starch hydrolizate, we found out that the strain *A. niger* J-O-12 except the activity of glucose oxidase, was characterized also by amylolytic activity (Fig. 1, curve 1). Our observations agree with earlier reports. Amylolytic activity of *Aspergillus* has been described by many authors [14]. However the amylolytic activity of our strain is not very high which results in very slow substrate hydrolysis and it limits

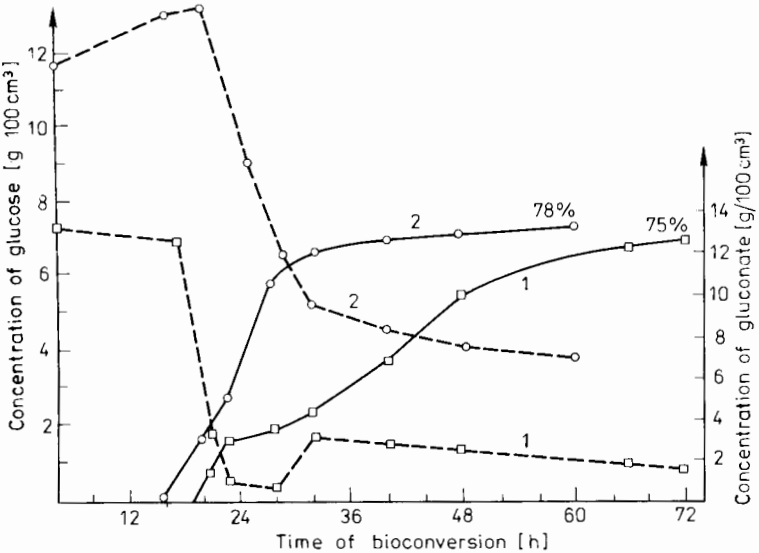

Fig. 1. Bioconversion of starch hydrolizytze to sodium gluconate without glucoamylase (1), with glucoamylase (2); --- glucose, — - gluconate

bioconversion of glucose to gluconic acid. In 72 hours of the process 12.5 g/100 cm^3 of gluconate was obtained that was only 75% of theoretical yield. In further experiments glucoamylase enzymatic solution was added to the medium, which accelerated the substrate hydrolysis. The main idea was to use glucoamylase parallel to bioconversion of glucose to gluconate. Under these conditions the process was shortened to 34–40 hours with the yield of gluconate close to stoichiometric (98%). As it is shown in Fig. 2, the increase of enzyme 50% influenced neither the dynamics of bioconversion nor the final gluconate yield. In both cases the substrate hydrolysis proceeded to the end and formed glucose was oxidized to gluconic acid. In these experiments antifoam agent was added during bioconversion that was very important for a proper process. In the case when the antifoam agent is not used, mycelium gathers in foam layer that decreases the contact surface of mycelium with the substrate that results in slower bioconversion and even stopping the whole process. This experiment has been shown in Fig. 1, curve 2.

In experiments carried on in industral fermenters haring similar conditions to those in laboratory, except that the initial concentration of the substrate was a little lower. As it is shown in Fig. 3 when the concentration of the starch hydrolizate was 175 kg/m^3 the process lasted 50 hours and did not finish (curve 1). When the concentration of the substrate was 160 kg/m^3 the process of bioconversion ended in 38 hour with the yield of 80% as compared with theoretical (curve 2). We tried to obtain calcium gluconate using starch hydrolizate as a substrate in further laboratory experiments. In these experiments, the neutralization of gluconic acid was carried out not by NaOH but

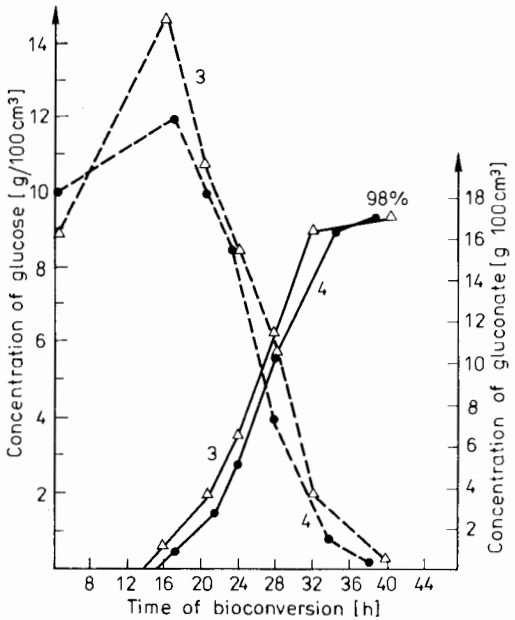

Fig. 2. Bioconversion of starch hydrolizate to sodium gluconate with addition glucoamylase 0.03 g/100 cm³ (3), 0.045 g/100 cm³ (4); antifoam agent was given

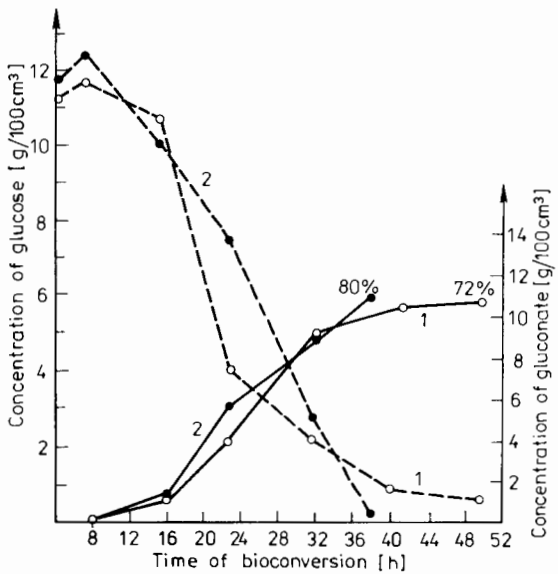

Fig. 3. Bioconversion of starch hydrolizate to sodium gluconate in industrial fermenter; 1–175 kg of hydrolizate in 1m³ of medium, 2 — 160 kg/m³

by water suspension of $CaCO_3$ that was added during bioconversion. The process has been shown in Fig. 4. It lasted 30 hours. At that time glucose was totally oxidized to gluconate that was obtained in the amount of 16.5 g/100 cm³. The yield was 93% as compared with theoretical. After bioconversion all solutions were chromatographically analysed (Fig. 5). We found big spots of gluconic acid in all samples. Additionally faint citric acid spots were noticed only in industrial samples. It proves great homogenity of the process.

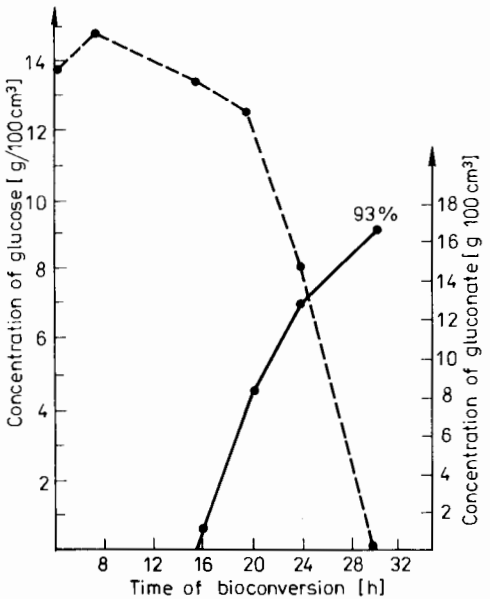

Fig. 4. Bioconversion of starch hydrolizate to calcium gluconate with addition glucoamylase 0.02 g/100 cm³

CONCLUSIONS

1. Starch hydrolizates can be used for sodium and calcium gluconates production. From the economic point of view it is more useful to help substrate hydrolysis by adding glucoamylase than to carry on the process only with the use of microorganisms.

2. It is more economic to carry on substrate hydrolysis with parallel microorganisms cultivation and conversion of formed glucose to gluconate. Under these conditions the whole process can be finished in 30–40 hours, with the total uptake of glucose and the gluconate yield close to 100%.

3. The decrease of glucoamylase solution dose to 0.02 g/100 cm³ does not influence the dynamics of bioconversion and the final gluconate yield (Fig. 4).

4. Starch hydrolizates bioconversion to gluconate using *A. niger* mutant J-O-12 is characterized by high homogenity of the process. Except gluconic acid only small amounts of citric acid can be formed (Fig. 5).

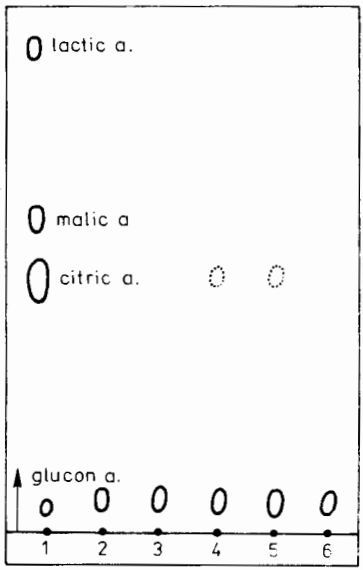

Fig. 5. Chromatogram of the medium after bioconversion; 1 — standard of acids, 2, 3 — laboratory exper., Na gluconate, 4, 5 — industrial exper., Na gluconate, 6 — Ca gluconate

5. It is necessary to use antifoam agent, e.g. soybean oil both for proper mycelium cultivation and protection of proper mycelium density in the whole volume of the medium in the fermenter.

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BIOKONWERSJA HYDROLIZATÓW SKROBIOWYCH DO KWASU GLUKONOWEGO I JEGO SOLI METODĄ MIKROBIOLOGICZNĄ

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Streszczenie

Zbadano możliwość zastąpienia czystej glukozy hydrolizatem skrobiowym w produkcji glukonianu sodu i wapnia. Proces prowadzono metodą mikrobiologiczną przy użyciu mutanta *A. niger* J-O-12 o wysokiej aktywności oksydazy glukozowej, w fermentorach o pojemności 35 dm^3 i 8 m^3. Aktywność amylolityczna użytego szczepu okazała się zbyt niska do przeprowadzenia dalszej hydrolizy substratu w dostatecznie krótkim czasie. Dlatego też w kolejnych doświadczeniach zastosowano glukoamylazę (AMG 300) w celu przyspieszenia hydrolizy użytego surowca. Wprowadzono skojarzony system działania glukoamylazy z jednoczesnym namnażaniem *A. niger* i konwersją tworzącej się glukozy do kwasu glukonowego. W takich warunkach czas biokonwersji wynosił 30–40 h przy całkowitym zużyciu glukozy i wydajności glukonianu bliskiej 100% wydajności teoretycznej. W warunkach przemysłowych osiągnięto wydajność 80%. Zastosowany szczep *A. niger* charakteryzuje się wysoką homogennością biokonwersji. W doświadczeniach laboratoryjnych nie stwierdzono powstawania innych kwasów organicznych, poza kw. glukonowym, jedynie w próbach przemysłowych powstawały dodatkowo niewielkie ilości kwasu cytrynowego. Przeprowadzone badania wskazują na pełną przydatność hydrolizatów skrobiowych w produkcji glukonianów skojarzoną metodą enzymatyczno-mikrobiologiczną.