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# GLUCOAMYLASE SYNTHESIS BY *ASPERGILLUS AWAMORI* NRRL 3112 IN THE MICROTECHNICAL SCALE

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Key words: glucoamylase, microbial synthesis, yield-effecting factors.

*Aspergillus awamori* NRRL 3112 was grown using the submerged technique in 10-liter laboratory fermenters during 7 days with continuous agitation and aeration of the nutrient. The activities of glucoamylase (GA) and $\alpha$-amylase as well as changes in sugar and protein concentrations and pH were determined. Corn-bran fortified with potato starch proved to be the most favorable nutrient for glucoamylase synthesis.

Fungi of the genera *Aspergillus, Rhizopus* and *Endomycopsis* are commonly used in industrial glucoamylase production [13]. The growth conditions of mycelium such as nutrient medium constituents, pH, temperature, aeration, agitation and time of growth as well as the genetically determined properties of strain are of determining importance for the enzyme yield [1, 8]. Glucoamylase as an inductive enzyme is synthesized in the presence of appropriate inducers, mainly the products of starch degradation. Starch, from wheat bran or corn meal, is the basic component of the nutrient medium, sometimes it is added as potato starch [2, 19]. The nitrogen source in this nutrient is supplemented by an addition of ammonium sulphate [17], while in other processes malt roott as well as corn and yeast extracts are used [2, 6]. A higher yield of glucoamylase may be obtained when there is a steady control of the nutrient pH throughout the growth of mycelium [3]. Similar results are observed when the culture is grown at higher temperatures but in the latter case intensified synthesis of trans-

glucosylase may take place [9], which has and adverse effect on the quality of the preparation. A proper choice of agents for mycelium cultivation enhances the fungus metabolic processes and consequently contributes to a shortening of the cultivation period.

## MATERIALS AND METHODS

*Aspergillus awamori* NRRL 3112, a strain originating from the USA [15, 16] was used. It was stored on Czapek-Dox nutrient [4] in a slits form, which were gelified using 2% agar, and inoculated every three months. The mother cultures for the fermenter were prepared by inoculating the fresh nutrient slits with fungal spores and incubating them for at least 7 days at 30°C. The cultures were used to make spore suspension in order to inoculate the fermenter nutrients. The base corn-bran nutrient (No. 4) mentioned before [12], containing 6.85% of starch and 0.38% of total N was used in the fermenter submerged cultures of the fungus. The exact composition of the nutrient is reserved. Corn-bran nutrients (No. 4) with ad addition of 2% and 3% potato starch were also used, as well as an original corn nutrient (20% of corn slurry) recommended by Cadmus et al. [2] for this strain.

The fermenter cultures were grown in 10-liter fermenters, produced by the Institute of Physics, University of Lublin, containing 4 l of nutrient. The fermenters with the nutrient were sterilised for 3 h at 1 atm overpressure and then inoculated using 5% of spore suspension with about $2\times10^6$ spores/ml. The incubation was carried out for 7 days at 30°C or 35°C. During this period the nutrient medium was continuously agitated at the speed of 560 rpm and aerated by sterile air, using 0.83 v(v)min (standard) or 1.24 v(v)min during the first two days and 1.66 v(v)min (standard) or 2.49 v(v)min on the following days. The air before being supplied to the fermenter was humidified in sterile conditions. This prevented excessive evaporation and concentration of nutrient liquid.

## ANALYTICAL METHODS

In cultivation liquids the glucoamylase activity was determined according to Kujawski and Zając [7]. One unit of the enzyme activity (GA) was defined as 1 mg of glucose which may be obtained from starch in 1 h at standard conditions. The α-amylase activity was determined using SKB method [14] with the mineral standard [5]. The content of reducing sugars was estimated according to Somogyi and Nelson [18], and proteins by Lowry et al. [10] using bovine albumine as standard. Everyday the pH of the samples was measured.

## RESULTS AND DISCUSSION

Already in earlier experiments [12] it was found that *Aspergillus awamori* NRRL 3112 was not only able to produce large quantities of glucoamylase but it met other requirements of a glucoamylase producer as well. Some experiments described in this paper were made in order to enhance the cultivation process and to increase the glucoamylase yield. The attempts aimed at intesifying metabolic processes were based on a fortification of the nutrient with potato starch (2% and 3%), increasing the cultivation temperature (35°C) as well as aeration. Figs. 1, 2 and 3 present the dynamics of glucoamylase, α-amylase, sugars, protein concentration and pH changes for the fungus grown at 30°C in standard aeration conditions. Fig. 1 presents the results obtained on the original corn nutrient according to Smiley [2], Fig. 2 the corn-bran nutrient, and Fig. 3 shows the same nutrient supplemented with 2% of potato starch.

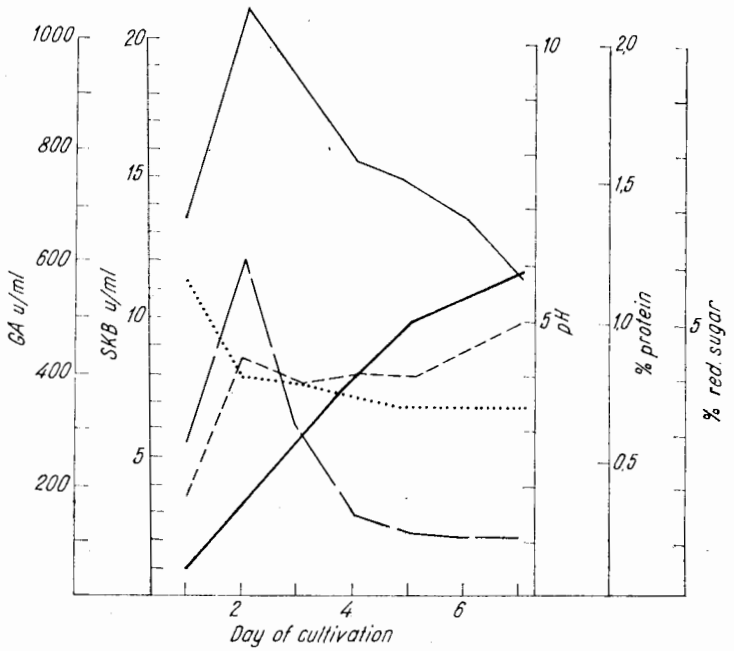


Fig. 1-7. Glucoamylase synthesis and dynamics of constituent changes during the fermenters cultivation of *Aspergillus awamori* NRRL 3112
Nutrient: corn/acc. to Smiley [3]
Conditions: temp. 30°C, aeration 1-2 day 0,83 v/v/min and 3-7 day 1,66v/v/min, agitation 560 rpm
Description:
——— glucoamylase activity in GA u/ml
— — — α-amylase activity in SKB u/ml
——— % of reducing sugars
– – – – – % of protein
..... pH

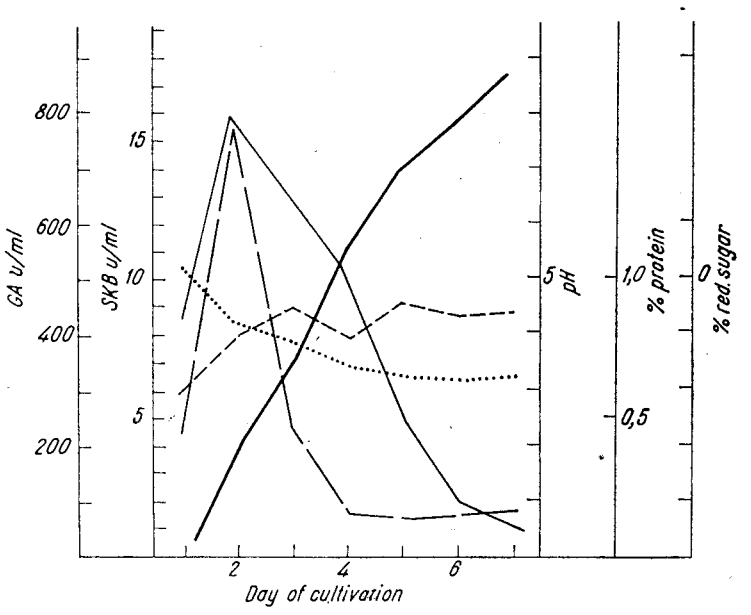


Fig. 2. Nutrient: corn-bran No 4
Conditions and description as in Fig. 1.

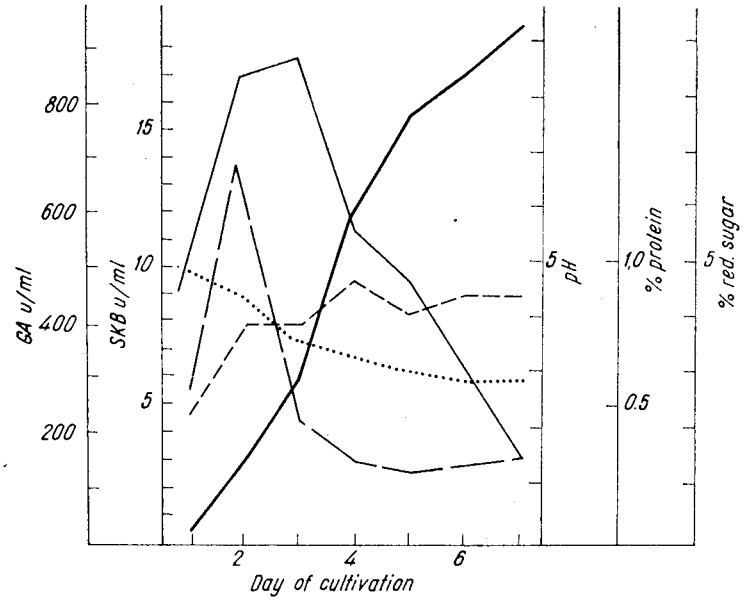


Fig. 3. Nutrient: corn-bran No 4+2% of potato starch
Conditions and description as in Fig. 1.

It can be seen from the figs that the corn nutrient gave worse results (Fig. 1) because the yield of glucoamylase reached only 580 GA u./ml after 7 days of cultivation. A large amount of sugar residue resulted in a high content of dry substance (8.5%) in the cultivation liquid and it reduced to a great extent the possibility of liquid concentration. The mycelium grew more dynamically and produced more glucoamylase in both corn--bran nutrients (Fig. 2 and 3) than in the case of the previously mentioned medium. A difference in favour of the fortified nutrient with 2% starch can be noticed. The mycelium growing in this nutrient displayed especially at 5 and 6 days, a 1-day advance with respect to glucoamylase synthesis. This may be very important in the production of this enzyme on an industrial scale. The period of a more intensive enzyme synthesis seemed to terminate on the 5 day of cultivation, and after 7 days the yield amounted to 850 GA u./ml for the base nutrient and 910 GA u./ml for the fortified nutrient. During the growth of mycelium the nutrient components were subjected to certain regular changes. Maximum activity of $\alpha$-amylase was noticed at 2 days cultivation and at 3 days its rapid decrease was observed in all experiments. It may have been related to nutrient acidifying and inactivation of the acid-labile $\alpha$-amylase. It is noteworthy that the values for pH of the liquids changed from 5.-5.0 on the first day to 3.5-3.0 at 7 days of cultivation as a result of mycelium metabolism. On the second day of the process the nutrient acidity increased most rapidly and pH reached a critical level for $\alpha$-amylase. These changes had no influence on the stability of glucoamylase.

The protein content in the liquids after the period of its growth (2-3 days) unerwent slight and irregular changes. As expected, the mycelium responded to higher cultivation temperature (35°) with a more intensive metabolism. The whole process of glucoamylase synthesis and some changes in the remainig components of the nutrient during cultivation at 35°C and in standard aeration are shown in Figs 4 and 5; on the base nutrient No. 4 (Fig. 4) and on the latter nutrient with 2% starch addition (Fig. 5) respectively. In both processes discussed a faster production of glucoamylase during 1-4 days of growth than in the analogous processes at 30°C was observed. Moreover, after seven days of cultivation the glucoamylase activities in the culture liquids were similar to those obtained at 30°C. They amounted to 760 GA u./ml for the base medium and 930 GA u./ml for the fortified one 2% of potato starch. It was observed that at 30°C cultivation (Figs. 2 and 3) as well as at 35°C (Figs. 4 and 5) the growth of mycelium on the nutrient medium with 2% starch addition was better with respect to the rate of glucoamylase accumulation and the total yield of the enzyme. A small intensity of sugar consumption during cultivation (Fig. 5) is justifiable considering the more intensive character of this nutrient. The metabolism of other medium constituents which were determined, corresponded to the above described observations. Further expe-

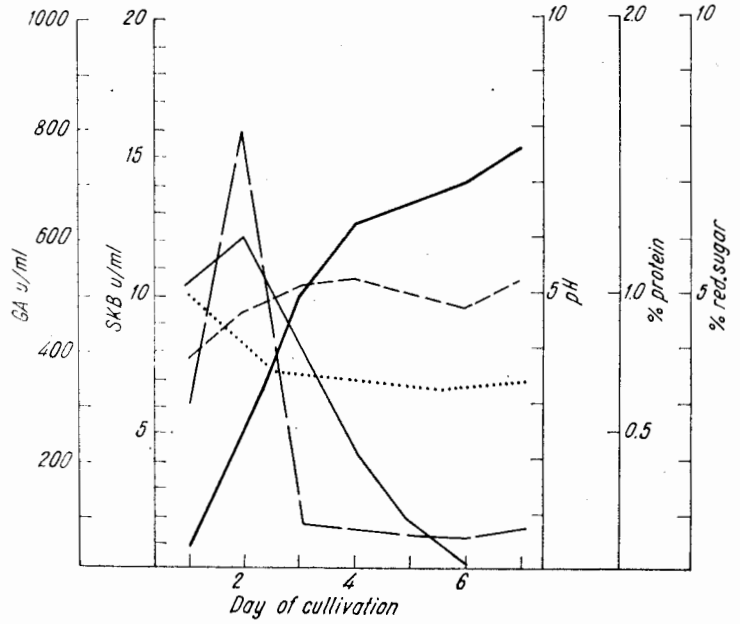


Fig. 4. Nutrient: corn-bran No 4
temp. 35°C
Other conditions and description as in Fig. 1.

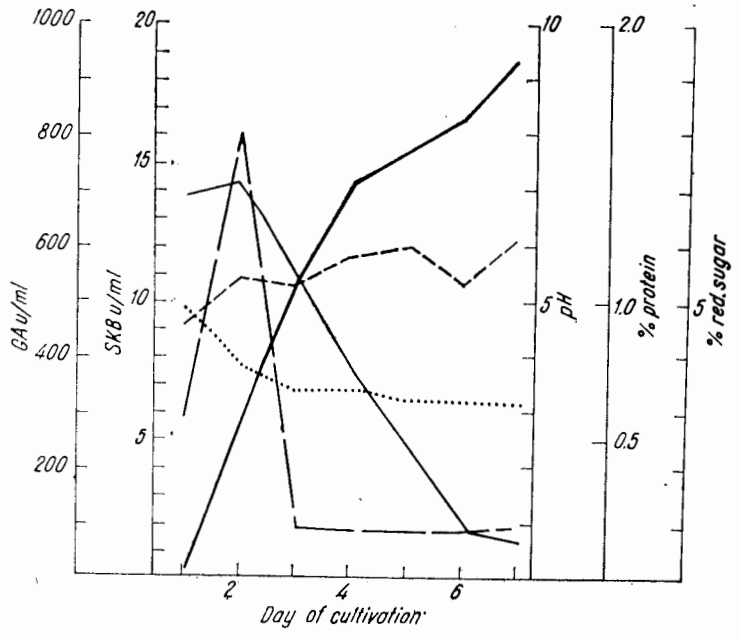


Fig. 5. Nutrient: corn-bran No 4+2% of potato starch
temp. 35°C
Other conditions and description as in Fig. 1.

riments were carried out at 35°C on fortified corn-bran nutrient (supplemented with 2% starch) with standard aeration 0.83/1.66 v(v)min and with higher aeration up to 1.24/2.48 v(v)min. The results of these experiments fully confirmed our assumptions, because higher aeration not only intensified glucoamylase synthesis, but also resulted in a considerable increase in enzyme yield (Fig. 6). A positive effect of higher aeration was found especially at 4 and subsequent days of cultivation. After 7-day cultivation in an intensely aerated medium the activity of glucoamylase reached nearly 1000 GA units/ml. The dynamics of other nutrient constituents was found to be typical. The use in these more intensive temperature and aeration conditions of a nutrient with a still higher quantity of starch did not markedly improve the results (Fig. 7).

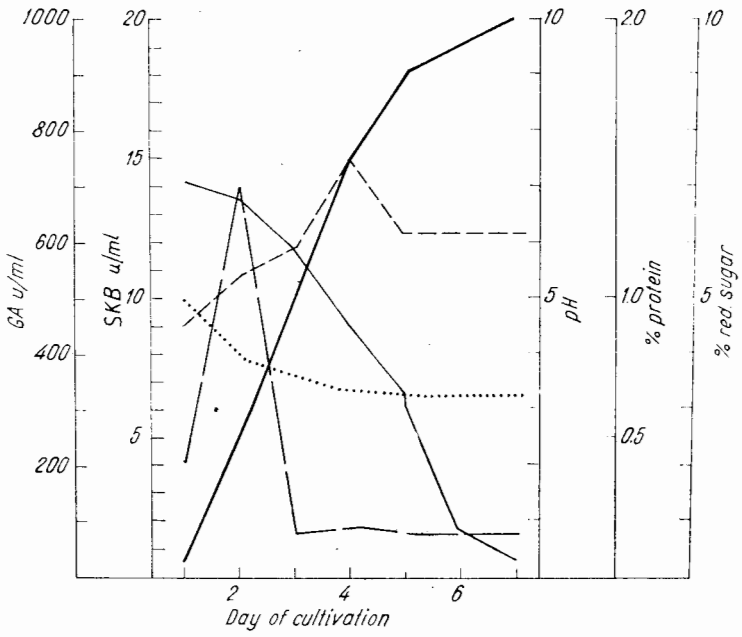


Fig. 6. Nutrient: corn-bran No 4+2% of potato starch
Conditions: temp. 35°C, aeration 1-2 day 1.24 v/v/min and 3-7 day 2.49 v/v/min, agitation 560 rpm
Description as in Fig. 1.

A comparison of nutrient No. 4 supplemented with 2% starch (Fig. 6) with that supplemented with 3% starch (Fig. 7) shows a similar kind of glucoamylase synthesis and analogous changes of sugar concentration in the nutrients. A higher temperature and aeration as well as nutrient fortification with starch may not have a visible effect on the α-amylase synthesis, nutrient acidity and changes in the soluble protein level.

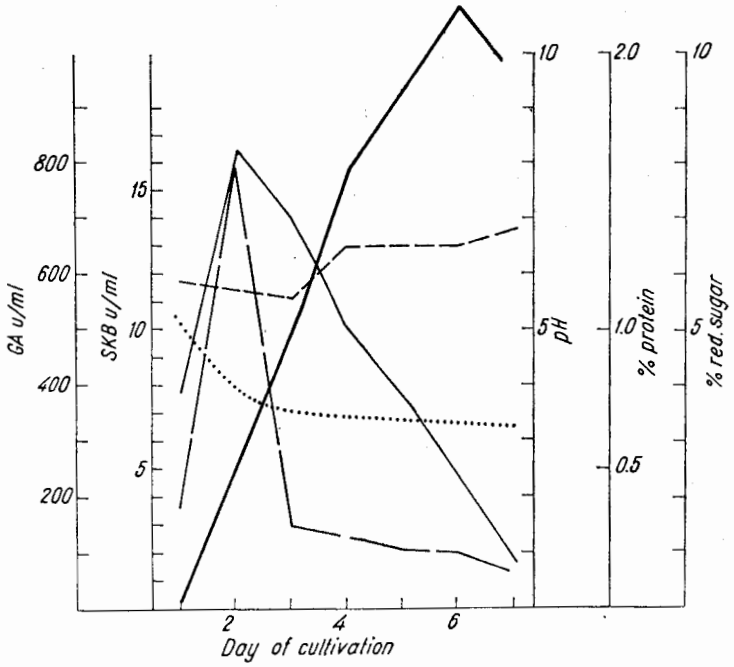


Fig. 7. Nutrient: corn-bran No 4+3% of potato starch
Conditions as in Fig. 6, description as in Fig. 1.

## CONCLUSIONS

1. The corn-bran nutrient proved to be the most favourable for glucoamylase production by *Asp. awamori* NRRL 3112.

2. The nutrient supplemented with 2% potato starch accelerated the enzyme accumulation — what may be important for industry — but did not affect the yield of glucoamylase.

3. The nutrient fortification with starch with a simultaneous increase of the temperature and aeration enhanced the glucoamylase dynamics and resulted in an increase in the yield of the enzyme.

4. The high yield of glucoamylase (over 900 GA u./ml) obtained in our experiments seems to promise good results in the semi-technical scale under specific conditions.

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SYNTEZA GLUKOAMYLAZY PRZEZ *ASPERGILLUS AWAMORI* NRRL 3112 W SKALI MIKROTECHNICZNEJ

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STRESZCZENIE

Szczep *Aspergillus awamori* NRRL 3112 hodowano metodą wgłębną w fermentorach laboratoryjnych o pojemności 10 l, zawierających 4 l pożywki, przez 7 dni w temperaturze 30 lub 35°C stale mieszając (560 obr./min) i napowietrzając (0,83 l/l/min lub 1,24 l/l/min przez pierwsze 2 dni i 1,66 l/l/min oraz 2,49 l/l/min w następnych dniach hodowli).

Stosowano podstawową pożywkę otrębowo-kukurydzaną (nr 4) o zawartości 6,85% skrobi i 0,38% N ogólnego, pożywkę nr 4 wzbogaconą 2 lub 3% krochmalu ziemniaczanego oraz zalecaną dla tego szczepu pożywkę zawierającą 20% śruty kukurydzanej.

Synteza glukoamylazy (GA) przebiegała szybko na pożywce otrębowo-kukurydzanej, natomiast oryginalna pożywka kukurydzana dała gorsze wyniki. Okres intensywnego gromadzenia enzymu przypadał na pierwsze 4-5 dni wzrostu. W drugim dniu hodowli obserwowano natomiast maksymalne nagromadzenie $\alpha$-amylazy i cukrów, po czym obie te wartości szybko malały. pH płynów fermentacyjnych zmieniało się w zakresie od 5,5 do 3,5, a nawet 3,0 po 7 dniach hodowli. Zmiany te nie miały wpływu na stabilność GA.

W podwyższonej temperaturze (35°C) w ciągu 1-4 dnia grzybnia szybciej wytwarzała GA, aniżeli w temperaturze 30°C, jednakże po 7 dniach hodowli uzyskane wartości nie różniły się zasadniczo. Zwiększone napowietrzenie (150%) nie tylko przyspieszyło syntezę GA, lecz spowodowało wydatny wzrost ilości wytworzonego enzymu. Korzystny wpływ ujawnił się szczególnie w czwartym i dalszych dniach hodowli. W intensywniejszych warunkach hodowli dodatek skrobi do pożywki nr 4 korzystnie wpłynął na syntezę GA.

Podwyższona temperatura, zwiększone napowietrzanie i wzbogacenie pożywki wydają się nie mieć wpływu na syntezę $\alpha$-amylazy, pH pożywki i zmiany w poziomie rozpuszczonego białka.

Wykonane badania stanowią podstawę do zaproponowania tego procesu i optymalnych warunków syntezy glukoamylazy do technologii w skali przemysłowej.