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Modern technologies for the production of glucoamylase enzyme products

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INTRODUCTION & AIM

The modern global market for enzyme products is characterised by a wide range of products used at various stages of technological processes in the food industry. However, this does not prevent the intensification of fundamental and applied research aimed at identifying promising producers, developing prolonged-action enzyme compositions, improving purification methods and increasing the stability of enzyme preparations.

There are a number of enterprises in Ukraine that specialise in the production of enzyme preparations: LLC "Enzym Company", JSC "VITAMINY" and "Technolog" Private JSC. However, the existing volumes of glucoamylase production are insufficient to fully meet the needs of various sectors of the national economy.

In modern biotechnological practice, there is a growing trend towards the use of thermostable enzymes, due to their effectiveness in intensive industrial processes. Among the variety of microbial producers of thermostable glucoamylases, special attention is paid to bacteria of the genus *Bacillus* (*Bacillus licheniformis, B. subtilis, B. amyloliquefaciens*), which have gained significant industrial importance due to their biotechnological characteristics.

The aim of our research was to analyse the technological aspects of the process of obtaining the enzyme product of glucoamylase.

METHOD

Within the scope of this study, strains No. 1, 2, 3, 4 and 5 of *Bacillus subtilis* bacteria were selected to assess amylolytic activity. Quantitative determination of amylolytic activity was performed using a modified iodometric method. The optical density of the iodine-starch complex was recorded spectrophotometrically using a Specol 211 (Germany) at a λ=600 nm.

Table 1

Amylolytic activity of *Bacillus subtilis* strains in deep culture

Sample number	Amylolytic activity, units/ml	Glucosamylolytic activity, units/ml
1	49.2	18.3
2	15.3	10.2
3	86.1	21.7
4	45.6	15.2
5	**87.6**	**25.3**

Glucosidase activity was determined in a 0.5 ml reaction mixture containing 0.25 ml of a 1% starch solution and 50 mM phosphate buffer (pH 7.4). Total protein concentration was determined by the Bradford spectrophotometric method, with bovine serum albumin as a standard for constructing the calibration curve.

RESULTS & DISCUSSION

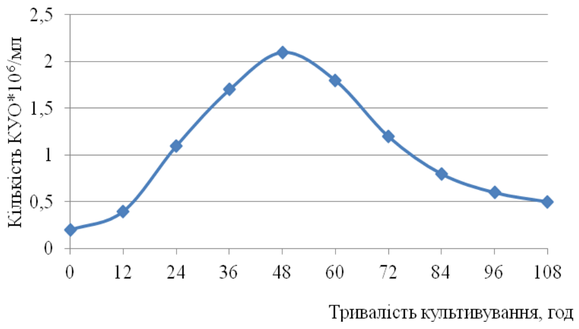

Fig. 1. Growth of *B. subtilis-5* in a standard nutrient medium

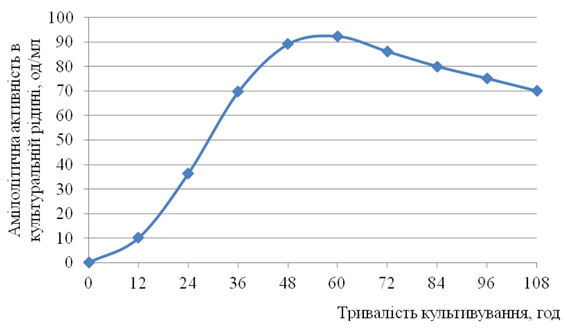

Fig. 2. Activity of B. subtilis-5 glucoamylase in a standard nutrient medium

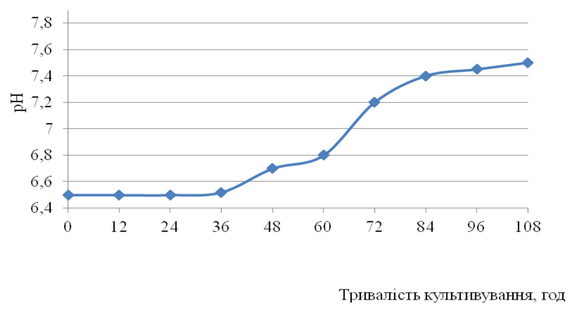

Fig. 3 Change in pH of the standart nutrient medium *B. subtilis-5*

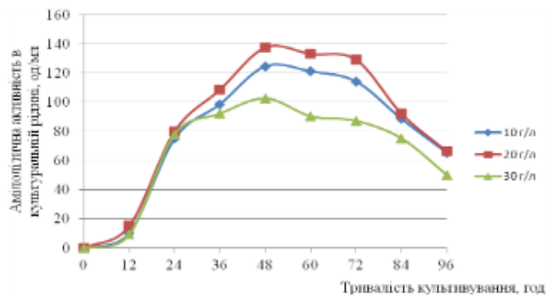

Fig. 4 Dynamics of glucoamylase activity of *B. subtilis-5* on a nutrient medium with different concentrations of brewer's grains

The results of the study showed that strains No. 1, 5 and 3 were characterised by the highest glucoamylase activity, which peaked on the third day of cultivation. At the same time, bacterial strains 2 and 4 showed significantly lower levels of amylolytic activity.

The determination of the growth dynamics of *B. subtilis* strain-5 (Fig. 1-4) on media with different concentrations of brewer's grains showed that the maximum growth rate of the culture was observed after 36 hours of incubation on a nutrient medium containing 30.0 g/l of brewer's grains. Under these conditions, the number of viable cells reached 4 × 10^6 CFU/ml.

The data obtained indicate a concentration-dependent effect of brewer's grains on the production of glucoamylase by bacteria.

An increase in the concentration of brewer's grains to 20 g/l led to stimulation of amylolytic activity, while a further increase in its content in the culture medium caused an inhibitory effect.

CONCLUSION

The results obtained indicate the possibility of stimulating enzyme biosynthesis by including molasses and brewer's grains in the culture medium. Maximum amylolytic activity was observed at a molasses concentration of 10 g/l and a brewer's grains concentration of 20 g/l. These cultivation conditions led to a twofold increase in *B. subtilis* biomass and a 1.5-fold increase in amylolytic activity compared to the standard medium.

Thus, research in the field of modern technologies for obtaining glucoamylase enzyme products is timely, relevant and has significant scientific and practical potential.

REFERENCES

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