ANALELE UNIVERSITATII DIN ORADEA
FASCICULA: ECOTOXICOLOGIE, ZOOTEHNIE SI TEHNOLOGII DE INDUSTRIE ALIMENTARA, VOL. VII, ANUL 7, 2008

THE TEMPERATURE'S INFLUENCE ON THE ENZYMS FROM THE FERMENTATIVE STAGE OF BIOTECHNOLOGICAL PROCESS OF OBTAINING ETHANOL

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Abstract

Technology of ethanol manufacture is a complex technological process which can be influenced in certain phases of direct and indirect factors that can modify the normal process. In this direction fall and the experiences presented in this paper respectively the effect of the change of temperature on enzymes from the liquefaction and saccharification phase of process technology.

Key words: enzyms, α-amylase, Dyazim X5, Termamyl SC

INTRODUCTION

Alcoholic fermentation in industrial conditions uses substrates rich in natural sugar fermentescibil and speed of fermentation and processing of glucidelor in primary and secondary products is dependent on many factors which can be divided into two broad categories: biological factors dependent on fermentation's microagents, and physical - chemical factors dependent on the composition of the environment under fermentation and circumambience conditions.

The increase process of microorganisms is the result of interaction between individual cell and the culture medium. Applying the laws of thermodynamics, kinetics, the transfer of mass, momentum and energy shows that the culture medium, through composition, temperature, pressure and limiting concentrations of substrate, directly affect the growth of microorganisms and the performance of elaboration of useful products.

To emphasize the action of enzymes in the two phases of the biotechnology process of obtaining ethanol, respectively: liquefaction and saccharification, aims to establish some physical-chemical and biological values to which their actions to have a maximum efficiency on the obtained results.

The determination of these physical values such as temperature and time, combined with the chemical (pH) and biological (as the starch content in the used substrate) to produce ethanol which through a proper selection and storage of physical-chemical and biological parameters go to a maximum action of enzymes on the substrate and implicitly to the increase

of economic efficiency by getting into a shorter period of time as and lower costs with basic materials and energy.

MATERIAL AND METHOD

The main material used in the alcohol industry is represented by maize grain. From a physical point of view, the beans should be whole without breaches and must not show traces of mold. To be used in alcohol industry the corn must fulfil the following characteristics: to have a content of dry matter of 85%, 56% of starch and 10% of crude protein.

In the alcohol industry are used more enzymes depending on the phase of technology process. So the enzyme used for liquefaction is termamil SC which is a liquid enzyme preparation containing an alpha-amylase produced by a strain of Bacillus genetically modified.

Termamyl SC is a remarkable liquefaction enzyme that can act at PH and concentrations of calcium ions in less than conventional α-amylase. Termamyl SC is a brown liquid with a density of 1,20-1,25 g / ml. The enzyme is an endo-amylase which hydrolyse links 1.4-α glucosidic of amylose and amylopectin.

The enzyme used to saccharification is Dyazim X5 is a amiloglucozidază produced by submerged fermentation of a strain of Aspergillus niger genetically modified. The main component Dyazim X5 is a amiloglucozidază which hydrolyses both links alpha - 1.4, as well as links α - 1.6 of gelanitizat starch and dextrin. Dyazim X5 contains significant amounts of acid alpha amylase which hydrolyses links α-1, 4 from amylose and amylopectin. Dyazim X5 is a brown liquid with a density of about 1.15 g / ml.

Dyazim X5 is used in the alcohol industry for saccharification of sourdough containing liquefied starch.

To emphasize the maximum efficiency of enzymes used in stage and liquefaction and saccharification of technological process to obtain ethanol was take into account the following parameters: speed drainage, reduce sugar for liquefaction and reduce sugar for saccharification.

In this paper we will emphasize one of the physical factors that influence the contents of enzymes in the stage of liquefaction and saccharification from technological process of obtaining ethanol, namely the temperature.

In the first experiment it is show the influence of temperature on the rate of leakage from liquefaction and the quantity of the reducer sugar from saccharification.

There were taken 6 samples each containing 200 grams of maize flour and mixed with 600 ml of warm water, adjust pH to 6.2 and added

0.35 ml enzyme Termamyl SC (equivalent to 0.35 l / t starch) liquefaction time was an hour.

The temperature of bath's heating was set from 80 to 104 º C after which the temperature was lowered to 60 ° C pH was adjusted to 4.3 and was introduced by the saccharification enzyme Diazyme x4 0.8 l / tonne of starch for 4 hours. The leakage speed is measured by draining funnel œ 6 and reducer sugar is measured in grams / l.

In the second experiment it was taken the same number of samples keeping the quantity of basic materials as in the experiment No. 1.

The temperature of liquefaction has been 94 º C for one hour then the 6 samples were placed in the heating bath from 45 ° C to 70 ° C for 4 hours. I mention that Ph and the amount of enzyme was retained as in the first experiment.

RESULTS AND DISCUSSION

The paper emphasize the maximum efficiency of enzymes used in biotechnology process to obtain ethanol the liquefaction and saccharification phase under the influence of the physical factor, temperature.

In the first experiment it was shown the influence of temperature on the process of liquefaction and saccharification keeping constantly others parameters: pH 6.2 for liquefaction, pH 4.3 to saccharification, dry substance respectively starch, the amount of enzyme 0.35 l / tonne of starch for liquefaction and 0.8 l / tonne of starch for saccharification.

In the first table for the liquefaction are presented the results from the first experience, where it appears that at the temperature 94 º C the report between the leakage time and the amount of reducer sugar in liquefaction and saccharification is the most efficient obtaining a better rate of leakage, meaning a liquefaction with a maximum efficiency reportedly to the amount of consumed energy and the content of reducer sugar from saccharification.

Table 1

The influence of the temperature on the leakage rate from liquefaction and the reducer sugar from saccharification

Temperature (ºC)	80ºC	86ºC	92ºC	94ºC	98ºC	104ºC
Leakage rate	8''	6''	5''	5''	4''	6''
Reducer sugar liquefaction	23,72	28,7	35,57	37,18	34,2	29,9
Reducer sugar saccharification	110,6	121,4	138,68	139,2	137,6	136,3

This can be observed through the graphical representation of these results when you can see an increase in the quantity of reducer sugar for liquefaction and a decrease in the rate of leakage, the best report being around 94 º C where the quantity of reducer sugar that enter in saccharification and the rate of leakage for liquefaction, so a good conversion of starch from basic materials.

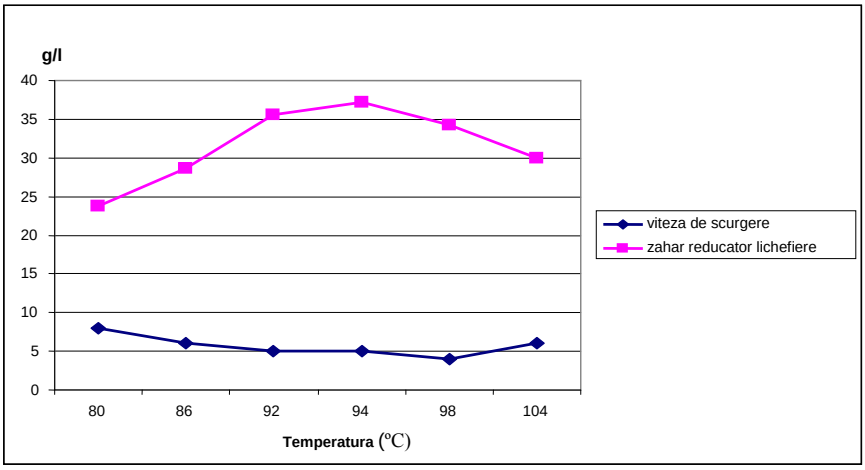

Fig. 1 Graphical representation of the influence of temperature on the rate of leakage from liquefaction and reducer sugar in saccharification

In the second table are presented the results obtained in the second experiment where the temperature of liquefaction has been 94ºC, the other parameters have remained constant, adjusting only the temperature of saccharification at 45ºC - 75°C where it was found that temperature of 60ºC represents the optimum temperature and the amount of reducer sugar obtained from saccharification.

Table 2

The influence of the temperature on the reducer sugar from the saccharification

Saccharification temperature	45ºC	50ºC	55ºC	60ºC	65ºC	70ºC
Reducer sugar g / L	88,4	93,5	105,8	133,3	128,4	100,9

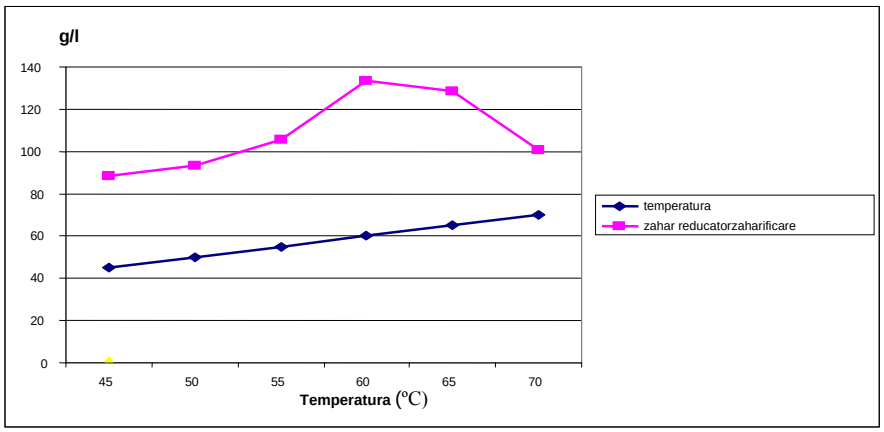

Fig. 2 Graphical representation of temperature influence on the reducer sugar at saccharification

The data from the second experiment presented above are represented in figure 2 where it can be seen that at a temperature of 60°C at saccharification and temperature 94ºC of conversion of starch from liquefaction it obtain the largest amount of reducer sugar.

CONCLUSIONS

The paper has shown that for the proper conduct of biotechnology process of obtaining ethanol, the action of the temperature and enzymes with the major role in liquefaction and saccharification of the substrate for fermentation own words has a major involvement in constant keeping of optimal physical parameters, chemical and biological to obtain the best possible economic efficiency both as consumption of energy and basic materials, and as period of time until obtaining ethanol.

REFERENCES

1. Albert L.Lehninger “1987” Biochimie Editura Tehnică Bucureşti;
2. Anghel L., “1993” Biologia şi tehnologia drojdiilor, Editura tehnică, Bucureşti;
3. Banu C. “2006” Bioalcoolul-combustibilul viitorului, Editura AGIR, Bucureşti;
4. Banu C. “2005” Dicţionar explicativ pentru ştiinţele exacte. Industrie alimentară IAL 6 – Aditivi, Editura Academiei, Editura AGIR, Bucureşti;
5. Banu C. “2002” Manualul inginerului de industrie alimentară, Vol II, Editura Tehnică, Bucureşti
6. Banu C., “2000” Aditivi şi ingredienţi pentru industria alimentară, Editura Tehnică Bucureşti.
7. Cornea C.P., P. Niculiţă “2005” Bazele microbiologice ale biotehnologiilor, Editura printed, Bucureşti;
8. Cojocaru C., L. Cojocaru “1969” Procedee tehnologice in industria fermentativă, Editura Tehnică, Bucureşti;
9. Iordăchescu D. “1974” Enzime structură şi mecanisme de acţiune Editura Medicală, Bucureşti;
10. Felszeghy E.; A.Abraham, “1972” Biochimie, Editura Didactică şi Pedagogică, Bucureşti;
11. Hopulele T., “1980” Tehnologia berii, spirtului şi a drojdiei Vol II;
12. Pele M.; P.Niculiţă, “ 2005” Biotehnologie, Editura Ceres, Bucureşti;
13. Vasilescu I., “1963” Tehnologia şi aplicaţiile industriale ale enzimelor, Editura Tehnică, Bucureşti;
14. Lynd L.R. “1996” A likely features and costs of mature biomass ethanol technology. Applied Biochemistry and Biotechnology. Vol. 57/58. The Humana Press;
15. Mihali C.; G.Oprea, “2003” Tehnologie generală în industria alimentară, Editura Risoprint, Cluj-Napoca;
16. Neniţescu C.D., “1968” Chimia organică, vol II, Editura a IV-a, Editura Didactică şi Pedagogică, Bucureşti;
17. Petculescu E. “1979” Tehnologii în industria alimentară, Editura Didactică şi Pedagogică, Bucureşti;
18. Rotaru V., “1992” Tehnologia spirtului şi drojdiei de panificaţie, Editura Didactică şi Pedagogică, Bucureşti;
19. Wyman Ch. E, “1996” Handbook on bioethanol production and utilisation. Eds. Taylor and Francis Ltd. Washington DC;
20. W. M. Ingledew, “1987” Applied microbiology & food science department, University of Saskatchewan, Saskatoon, Canada;