

## USE OF ENZYMES IN HYDROLYSIS OF MAIZE STALKS

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Lignocellulosic biomass is the most abundant organic raw material in the world. Cellulose and hemicellulose from plants and other biomass can be hydrolyzed to produce sugars. Native lignocellulosic biomass provides limited accessibility to cellulase enzymes due to structural features. The investigations were carried out with waste lignocellulosic raw material, consisting of maize stalks and cobs. Enzyme hydrolysis was performed after acid hydrolysis with a cellulosic product. It was established that the enzyme stage, as a first treatment phase, was inefficient. It was found that cellulase activity was considerably improved after acid hydrolysis of a crushed mass. A two-stage process with acidic and then enzyme hydrolysis method was most efficient and promising for obtaining sugars for ethanol production.

*Keywords:* Lignocellulosic biomass; Enzyme hydrolysis; Cellulase

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### INTRODUCTION

Lignocellulosic biomass is among the most promising alternative energy sources because it is inexpensive, renewable, widely available, and environmentally friendly. The need for producing fuels and chemicals from renewable biomass has become abundantly clear over the past decade (Xie et al. 2005). However, the cost of producing fermentable sugars from biomass hydrolyzate using existing technology is relatively high and has been a major obstacle.

Acid hydrolysis is perhaps currently seen as the most technologically mature method of sugar release from biomass. Acid (sulfuric or hydrochloric) can serve both for disruption and hydrolysis of the cellulosic polymers. Strong acid allows complete breakdown of the components in the biomass to sugars, but also requires large volumes of concentrated sulfuric acid and can result in the production of furfural, an inhibitory byproduct. Dilute acid allows reduced acid concentrations, but requires higher temperatures, and again gives furfural (Goldstein and Easter 1992; Ladisch and Swartzkopf 1991). Hydrolysis of cellulose is significantly more difficult than for starches because cellulose is in a crystalline form with hydrogen bonding. Acid hydrolysis of hemicellulose occurs under less harsh conditions than cellulose because hemicellulose is an amorphous polymer (Quintero-Ramirez 2008).

Enzymes such as cellulase,  $\beta$ -glucosidase, and hemicellulase are used in the hydrolysis of lignocellulosic materials in bioethanol production. Efficient hydrolysis of cell-wall polysaccharides is important in this process, and it is known that enzymatic hydrolysis of lignocellulosic biomass is affected by the structural properties of cell-wall

components (Grethlein 1984; McMillan 1994; Chang and Holtzapple 2000; Mosier et al. 2005).

The degree of cellulose crystallinity is a major factor affecting enzymatic hydrolysis of the substrate. It has been reported that a decrease in cellulose crystallinity especially influences the initial rate of cellulose hydrolysis by cellulose (Quintero-Ramirez 2008; Grethlein 1984; McMillan 1994; Chang and Holtzapple 2000; Mosier et al. 2005).

Enzymatic hydrolysis is slower than acid or caustic chemical hydrolysis, but does not require the large volumes of chemicals, chemical recovery or neutralization, or the plant to be constructed of special acid/caustic compatible materials (Dale and Moelhman 2000).

The enzymatic hydrolysis of lignocellulose is affected by many factors. The limiting factors have been traditionally divided into two groups, those related to lignocellulose structural features and those related to the mechanism and interactions of the cellulolytic enzymes. However, the heterogeneous nature of lignocellulose and the multiplicity of enzymes make it difficult to fully understand the interactions between enzyme and lignocellulose; furthermore, the interactions change as hydrolysis proceeds. Therefore, it is apparent that the rate and extent of biomass hydrolysis are inextricably linked to biomass structural characteristics (Zhu 2005).

The aim of this paper is to establish the efficiency and to determine the conditions of a two-stage acid and enzyme hydrolysis of maize stalk agricultural waste to sugars.

## EXPERIMENTAL

The investigations were performed on waste lignocellulosic vegetable raw material in maize production – maize stalks and cobs.

The content of cellulose in the raw material was determined after Kuerschner's method, of pentosans – after the bromide-bromate method, of lignin – after the Koenig-Komarov method, and of water-soluble substances (Obolenskaya et al. 1965).

The mechanical impact – raw material crushing, was performed in a Yokro crusher at a hydromodulus of 1:9 for 60 minutes.

The acid hydrolysis of the vegetable biomass was performed in 1 L stainless steel laboratory autoclaves rotated at constant rate in a polyglycol bath under the following conditions:

- Hydromodulus – 1:10
- Sulphuric acid consumption – 1%
- Initial temperature – 80°C
- Maximum temperature – 140°C
- Time to reach the maximum temperature – 60 min.
- Retention time at maximum temperature – 60 min.

The solid residue was washed with water and the obtained hydrolysate was filtered and separated from the solid residue.

The enzymic hydrolysis was performed with the Novozymes AS products Pulpzyme HC – xylanase, and NS-51081 – a cellulosic product, under the following conditions:

- Mass concentration – 10%
- Temperature – 60°C
- Treatment duration – 120 min. (20 to 300 min.)
- Enzyme consumption – 0.1 to 5%
- pH 8 for Pulpzyme HC and pH 4.5 for the cellulosic product NS-51081.

The content of reducing substances of the hydrolysates obtained was determined after the dinitrosalicylic acid (DNS) method (Miller 1959).

The sugars that were contained in the hydrolysates from the various raw materials were characterised by means of Fourier transform infrared spectroscopy (FTIR). The spectra were obtained on a PerkinElmer System 2000 apparatus.

## RESULTS AND DISCUSSION

The chemical analysis of the analyzed maize stalks and cobs, as presented in Table 1, shows relatively high polysaccharides content (71.0-77.6%), which makes them potentially suitable for treatment through hydrolysis to sugars. The content of the easily hydrolysable polysaccharides that are basically pentosic is high. The amount of the water-soluble reducing substances may through raw material crushing be doubled to 8.3%.

**Table 1.** Chemical Composition of Raw Material

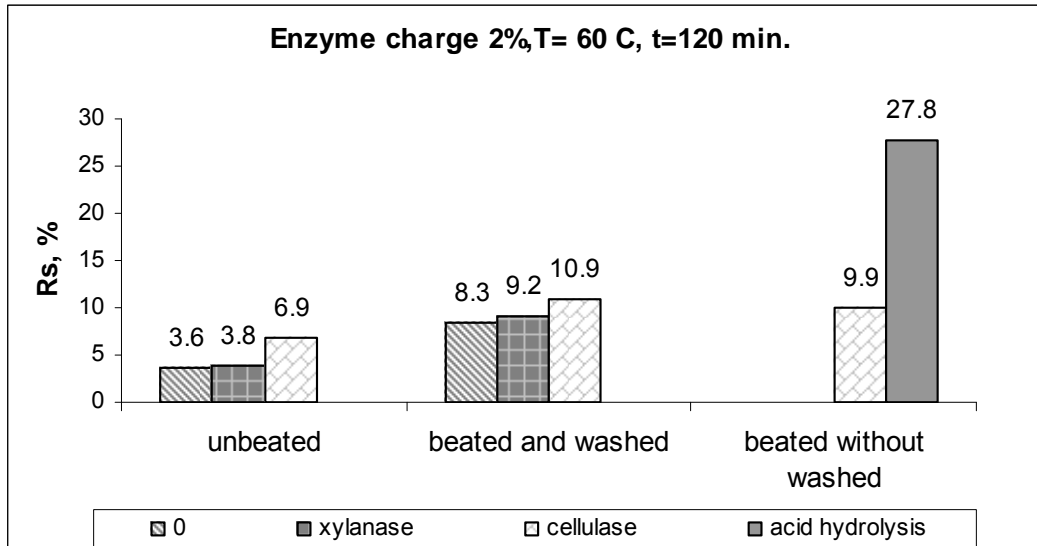
Chemical composition	maize stalks	cobs
lignin (%)	19.35	21.02
Cellulose (%)	40.28	35.05
pentosans (%)	35.06	33.05
EHS(%)	37.30	35.95
water soluble RS (%)	3.6	
after beating RS (%)	8.3	
total polysaccharides (%)	77.58	71.0

The efficiency of the enzyme treatment, as a first stage of hydrolysis of cornstalks (Fig. 1), was examined. As seen from the figure, the hydrolytic action of enzymes on uncrushed raw material was weak, with the xylanase practically not reacting with xylan chains. Better results were obtained for the cellulase product.

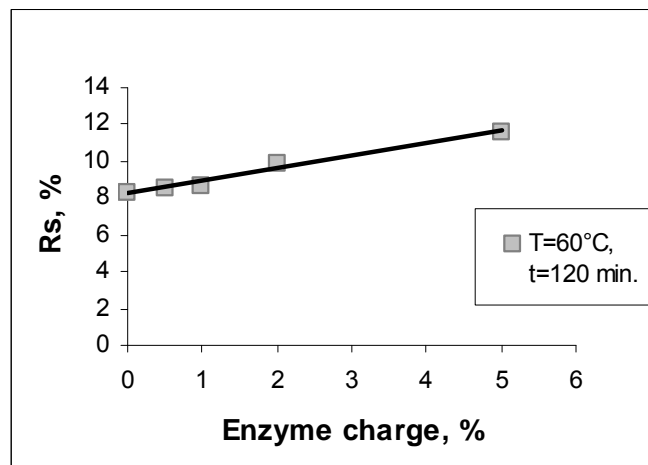
The preliminary raw material crushing did not influence the efficiency of the treatment with enzymes. In spite of the increased active surface of the vegetable material, their action was too restricted. Probably, the lignin impedes the access of the enzyme molecules to the amorphous sections of the carbohydrate chains.

When examining the effect of the cellulase product, the low reactive capacity of the enzyme towards the preliminarily crushed raw material at the first stage, which is not improved even at high dosages (Fig. 2), was confirmed.

When performing acid hydrolysis of crushed maize stalks (Fig. 1), the yield of reducing substances was 27.8% of the raw material. The result was considerably higher than the one obtained in enzyme hydrolysis. The process took place in the depth of the cellular structure and affected all easily hydrolysable polysaccharides.

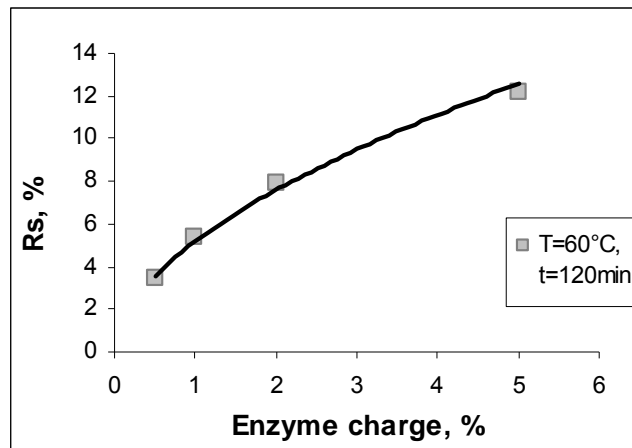


**Figure 1.** Effect of enzyme treatment as a first stage of maize stalks hydrolysis



**Figure 2.** Reducing substances dependence on cellulose charge in first stage of maize stalks hydrolysis

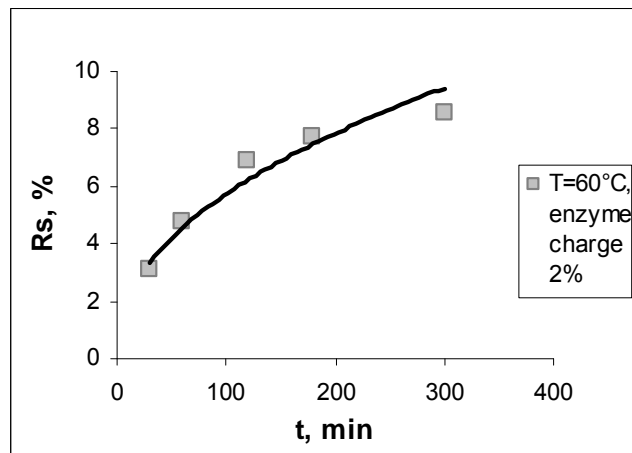
The process of enzyme hydrolysis as a second degree of treatment was investigated, and the effect of the enzyme consumption on the amount of released reducing substances was determined (Fig. 3). An increase of the effect of enzyme action by about 30% as a result of preliminary beating of the raw material was established.



**Figure 3.** Reducing substances dependence on enzyme charge

With the increase of the enzyme consumption, the amount of individual reducing substances rose considerably, reaching 12% of the lignocellulosic mass. The established higher efficiency of cellulase in hydrolysis at the second stage shows that the residual mass after the acid hydrolysis possessed more cellulose chains accessible for the enzyme.

The performed temperature-temporal examination of the cellulosic hydrolysis as a second stage of the raw material treatment confirms the conclusion drawn (Fig. 4). It was established that, at an optimum enzyme temperature of 60°C and a process duration of 5 hours, a yield of reducing substances above 8%, or nearly 70% of the maximum established level of 12.83% in case of a 24 hour treatment, was obtained.



**Figure 4.** Reducing substances dependence on reaction time

Based on these results, the performance of a two-stage acid and enzyme hydrolysis of a crushed raw material under moderate technological conditions may provide passage into solution of up to 50% of all polysaccharides assigned to reducing substances, which makes the combined method a promising one for obtaining sugars for ethanol production.

## CONCLUSIONS

1. The investigations performed showed the potentialities of two-stage acid and enzyme hydrolysis of maize stalk agricultural raw materials to sugars.
2. It has been established that the enzyme stage, as a first treatment phase, can be inefficient irrespective of the preliminary raw material crushing, probably because of impeded access of the enzyme molecules to the amorphous sections of the carbohydrate chains.
3. The cellulase activity was considerably improved after acid hydrolysis of a crushed mass.
4. It turned out that the following multistage process for production of sugars was most efficient: beating; acid hydrolysis with dilute acid, and enzyme hydrolysis with cellulase products.

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## REFERENCES CITED

- Chang, V. S., and Holtzapple, M. T. (2000). "Fundamental factors affecting enzymatic reactivity," *Appl. Biochem. Biotechnol.* 84–86, 5–37.
- Dale, M. C., and Moelhman, M. (2000). "Enzymatic simultaneous saccharification and fermentation (SSF) of biomass to ethanol in a pilot 130 liter multistage continuous reactor separator," *Ninth Biennial BioEnergy Conference* New York, ([www.nrbp.org/papers/049.pdf](http://www.nrbp.org/papers/049.pdf)).
- Goldstein, I., and Easter, J. (1992). "An improved process for converting cellulose to ethanol," *Tappi J.* 28, 135-140.
- Grethlein, H. E. (1984). "Pretreatment for enhanced hydrolysis of cellulosic biomass," *Biotechnol. Adv.* 2, 43–62.
- Ladisch, M., and Svartzkopf, J. (1991). "Ethanol production and the cost of fermentable sugars from biomass," *Bioresour. Technol.* 36, 83–95.
- McMillan, D. J. (1994). "Pretreatment of lignocellulosic biomass," In "Enzymatic Conversion of Biomass for Fuels Production," Himmel, M. E., Baker, J. O., and Overend, R. P. (eds.), American Chemical Society, Washington, DC. 292–324.
- Miller, G. (1959). "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Anal. Chem.* 31, 426-428.
- Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holzapple, M., and Ladisch, M., (2005). "Features of promising technologies for pretreatment of lignocellulosic biomass," *Bioresour. Technol.* 96, 673–686.
- Obolenskaya, A. V., Stegolev, V. P., Akim, G. L., Akim, E. L., Kossovich, N. L., and Emelianova, I. Z. (1965). "Prakticheskie rabotai po himii drevesinae i cellulozae," *Lesnaya promishlenost, Moskva* (Russian).

- Quintero-Ramirez, R. (2008). "Hydrolysis of lignocellulosic biomass," ([www.apta.sp.gov.br/cana/anexos/paper\\_quintero\\_Brasil.pdf](http://www.apta.sp.gov.br/cana/anexos/paper_quintero_Brasil.pdf)).
- Xie, Y., Phelps, D., Lee, C.-H., Sedlak, M., Ho, N., and Wang, N.-H. L. (2005). "Comparison of two adsorbents for sugar recovery from biomass hydrolyzate," *Ind. Eng. Chem. Res.* 44, 6816-6823.
- Zhu, L. (2005). "Fundamental study of structural features affecting enzymatic hydrolysis of lignocellulosic biomass," A dissertation submitted to the office of graduate studies of Texas A&M University.

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