Scientific Bulletin. Series F. Biotechnologies, Vol. XXI, 2017
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

PRELIMINARY RESEARCH ON ENERGETIC CAPITALIZATION OF LIGNOCELLULOSIC MATERIALS IN FORM OF BIOETHANOL

Diana GROPOȘILĂ-CONSTANTINESCU, Gabriela MARGĂRIT, Radu-Cristian TOMA, Dana BARBA, Luminița VIȘAN, Marius HANGAN

University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Biotechnologies, 59 Mărăști Blvd., District 1, Bucharest, Romania

Corresponding author email: bth.diana@yahoo.com

Abstract

The huge amount of biomass composed of plant residues considered "waste" is a potential source of useful products. Works of this research were based on the study of the bioconversion potential in bioethanol of different types of wastes of lignocellulosic nature, obtained from the harvesting and processing of cereals (straw, cobs). Efficient capitalization of plant biomass is only possible when effective methods of delignification and decrystallization of the lignocellulosic complex are applied. Our research focused on the following general objectives: selection and characterization of lignocellulosic waste that can produce bioethanol, selection and adaptation of highly-productive microorganisms that ensures high conversion yields of bioethanol from the substrates obtained from lignocellulosic waste, experimentation of the technology at micropilot level. The raw materials used, wheat straw, barley straw and corn cobs, have been subjected to thermal pretreatment (autoclaving at 121^{0}C for 30 minutes), enzymatic pretreatment (laccase), chemical pretreatment (NOH) and enzymatic hydroyisis (MethaPlus), in order to make available the polysaccharide substrates for the subsequent enzymatic hydrolysis. In case of alkaline pretreatment applied to all three types of lignocellulosic materials, best results were obtained when a solution of NaOH 4% was used. Anaerobic digestion of lignocellulosic materials led to ethanol concentrations of 4,9%, in case of corn cobs, 3,2% for wheat straw and 3.9, for barley straw.

Key words: *bioethanol, biomass, lignocellulosic materials.*

INTRODUCTION

Continues decrease of the amount of vegetation through natural desertification phenomena, fire or irrational exploitation of lignocellulosic waste, determines the production of excess CO_2, generating, along with the industrial activities, the well-known greenhouse effect, which heats slow but continuous the Earth, becoming an imminent threat to humanity (Malherbe et al., 2003; Levine et al., 1996). On the other hand, it is known that more than half of vegetal biomass from agriculture and forestry, consisting largely of lignocellulose compounds represents waste or by-products, that are thrown or partially used, thus losing a significant amount of raw materials used for the development of sustainable processes, which would help to conserve natural resources (Huang et al., 2011).

Currently, worldwide, the recovery and reintroduction in the economic circuit of the material resources are dealt as components of strategies for harmonizing the relationships between economic growth, consumption of natural resources and environmental protection (Huang et al., 2011).

On a global scale, are obvious a series of restrictions regarding material resources, which has made recycling to become an objective necessity. In this context, interest in capitalizing lignocellulosic biomass has increased considerably, knowing that they are the most abundant organic matter sources. The huge amount of biomass formed from plant residues considered "waste" represent a potential source of useful byproducts (Malherbe et al., 2003). Thus, many worldwide researches that are currently performing are justified for the use of lignocellulosic waste in developing sustainable processes and products. In many European countries, lignocellulosic plant waste mixed with animal manure is used, with promising results for bio fuel production (Sun et al., 2002). Therefore, one of the current directions

of international research is focused on improving the digestibility of lignocellulosic waste by applying combined physicochemical and enzymatic treatments in order to release the recalcitrant carbohydrate substrates by a controlled hydrolysis (Jorgensen et al., 2003; Arora et al., 2002).
Experiments performed in this paper were aimed at determining the influence of physical and chemical pretreatment of lignocellulosic materials on their susceptibility to further enzymatic hydrolysis, determining the degree of hydrolysis of pretreated lignocellulosic materials, depending on their nature and testing the ability to produce bioethanol from pretreated lignocellulosic materials, depending on their nature (Lisov et al., 2004; Ruggeri et al., 2003).

MATERIALS AND METHODS

Lignocellulosic materials and the enzymes used for their degradation are shown in the following tables.

Table 1. Lignocellulosic materials

Nº	Lignocellulosic material	Dry substance content (%)	Cellulose content (%)	Lignin content (%)
1.	Wheat straw	85	39,4	15
2.	Barley straw	87	37-42	18
3.	Corn cobs	85	50,2	20,4

Before use, straw and cobs were physically pretreatment by shredding to sizes between 1-10 mm.

Table 2. Enzymes

Nº	Enzyme	Producer	Composition
1.	Methaplus 100L	BIOPRACT GmbH, Ger.	β-glucanase, cellulase, xylanase
2.	Denilite 2S	Novozymes, Den.	laccase

Table 3. Pretreatments

Thermal pretreatment	Autoclaving, 121^{0}C, 30 min
Enzymatic pretreatment	Laccase 10% reported to the weight of dried vegetal material. Samples were incubated at 55^{0}C, 20 h, 200 rpm, pH 5.5.
Alkaline pretreatment	NaOH solutions: 0,5%, 1%, 2%, 4%.Samples were incubated at 50^{0}C, 200 rpm, 2 h.

To determine the concentration of reducing sugars resulted from each step of the experimental protocol a spectrophotometric method was used based on the color reaction of reducing sugars with dinitrosalicylic acid.
Ethanol production was tested with *Sacharomyces cerevisiae*, at an inoculation ratio of 5% (Shin Sato et al., 2007).

RESULTS AND DISCUSSIONS

Experiments performed in this paper were aimed at: 1) determining the influence of physical and chemical pretreatment of lignocellulosic materials on their susceptibility to further enzymatic hydrolysis; 2) determining the degree of hydrolysis of pretreated lignocellulosic materials, depending on their nature; 3) testing the ability to produce ethanol from pretreated lignocellulosic materials, depending on their nature.
In a first set of experiments we focused on emphasizing the changes induced to the susceptibility of lignocellulosic materials to the enzymatic attack of cellulases and hemicellulases complex by performing thermal and chemical treatments combined with laccase treatment.

Table 4. The influence of thermal pretreatment on the enzymatic hydrolysis of lignocellulosic materials

Type of lignocellulosic material	Initial mg/ml	Hydrolysis 2 h mg/ml	Hydrolysis 4 h mg/ml	Hydrolysis 20 h mg/ml
Wheat straw Blank	0,47	0,91	1,32	3,32
Wheat straw	0,62	1,18	3,20	7,07
Barley straw Blank	0,59	0,99	1,90	3,02
Barley straw	0,65	1,28	3,31	7,28
Corn cobs Martor	0,74	1,14	1,95	3,30
Corn cobs	0,82	1,41	3,45	7,48

For each sample was carried out a blank that was incubated at room temperature for 30 minutes at 220 rpm, pretreated with laccase, and then hydrolyzed with Methaplus.
From the examination of the results shown in the table above it can be concluded that the application of thermal pretreatment combined to the laccase pretreatment, increases the amount of reducing sugars in the supernatant, compared with blanks, with the following

percentages: wheat straw, with 213%; barley straw, with 241%; corn cobs, with 226%.
The way which the concentration of NaOH solution influence the results of enzymatic attack further applied to lignocellulosic materials it was seen in the experiences carried out according to the following protocol:

Variant 1: 5 g vegetal material+80 ml DW
Variant 2: 5 g vegetal material+50 ml NaOH 0,5%
Variant 3: 5 g vegetal material+50 ml NaOH 1,0%
Variant 4: 5 g vegetal material+50 ml NaOH 2,0%
Variant 5: 5 g vegetal material+50 ml NaOH 4,0%

Each alkaline hydrolysis was preceded by a treatment with laccase.
The results are shown in the following table:

Table 5. The influence of alkaline pretreatment on the enzymatic hydrolysis of wheat straw

Variant	Initial mg/ml	Hydrolysis 2 h	Hydrolysis 4 h	Hydrolysis 20 h
		mg/ml	mg/ml	mg/ml
1.	0,47	1,83	3,47	9,41
2.	0,51	2,12	8,38	16,92
3.	0,83	2,57	8,88	21,04
4.	0,71	2,67	9,01	24,60
5.	0,65	2,73	9,07	26,35

After 20 hours of enzymatic hydrolysis, using a 4% NaOH solution (variant 5), was obtained an increase in the concentration of reducing sugars by 2.87 times, compared with Variant 1.

Table 6. The influence of alkaline pretreatment on the enzymatic hydrolysis of barley straw

Variant	Initial mg/ml	Hydrolysis 2 h	Hydrolysis 4 h	Hydrolysis 20 h
		mg/ml	mg/ml	mg/ml
1.	0,615	1,86	4,71	9,56
2.	0,665	2,25	8,64	18,20
3.	0,721	2,62	8,95	21,30
4.	0,651	2,65	9,09	24,99
5.	0,627	2,76	9,17	27,56

As shown from the data presented in Table 6, if after 2 hours of hydrolysis differences in the concentrations of reducing sugars accumulated to the untreated variant were between 27% and 49%, after 20 hours it was between 96% and 301%.
The greater the time of hydrolysis, become increasingly more and more obvious the differences induced by the use of different concentrations of NaOH, reaching after 20 hours, between 180% and 280% in reducing sugars, compared to the control experiment (Table 6).

Table 7. The influence of alkaline pretreatment on the enzymatic hydrolysis of corn cobs

Variant	Initial mg/ml	Hydrolysis 2 h	Hydrolysis 4 h	Hydrolysis 20 h
		mg/ml	mg/ml	mg/ml
1.	0,74	2,28	5,50	11,02
2.	0,77	2,52	9,58	20,36
3.	0,82	2,92	10,18	22,50
4.	0,84	3,19	10,40	25,86
5.	0,89	3,44	10,94	28,33

In the case of corn cobs, it was also found the efficiency of alkaline pretreatment, which allowed after 2 hours of enzymatic hydrolysis, depending on the concentration of the alkaline solution, the release of reducing sugars of 10% to 50.9%, greater then the control experiment.

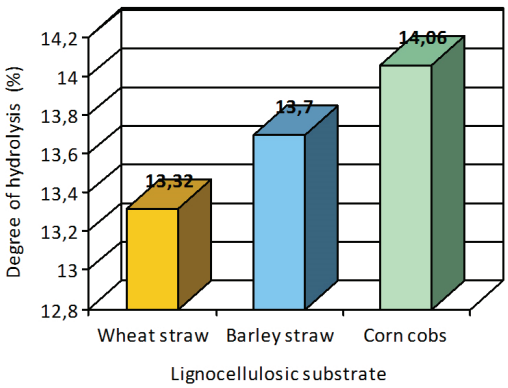

Figure 1. The degree of the enzymatic hydrolysis obtained after the thermal pretreatment of different lignocellulosic materials

The degree of the enzymatic hydrolysis of the three types of lignocellulosic substrates first subjected to autoclaving is expressed in percentage of reducing sugars on dry substance corresponding to the amount of processed lignocellulosic substrate.
The next figure illustrates the degree of enzymatic hydrolysis obtained after optimal alkaline pretreatment (4% NaOH solution) applied to the three types of lignocellulosic materials.
According to the data presented in Figure 3, enzymatically hydrolyzed and chemically pretreated corn cobs led to the best results regarding the content of bioethanol.

Anaerobic digestion of lignocellulosic materials led to ethanol concentrations of 3,2% in case of wheat straw, 3.9%, for barley straw and 4,9%, for corn cobs.

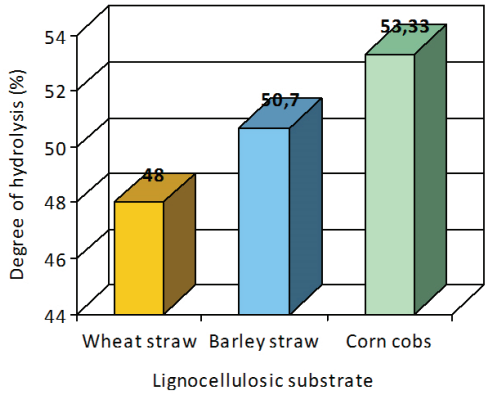

Figure 2. The degree of the enzymatic hydrolysis obtained after the alkaline pretreatment of different lignocellulosic materials

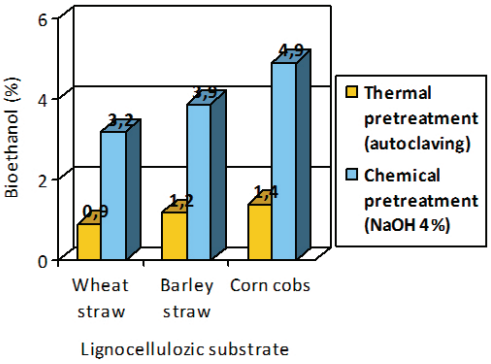

Figure 3. The productivity of processed lignocellulosic material in bioethanol

CONCLUSIONS

Thermal pretreatment consisting in autoclaving of substrates at 121^0C, for de 30 minutes, allows the obtaining of enzymatic hydrolysis degree which varies depending on the nature of the lignocellulosic material, as follows: wheat straw (13,32%) < barley straw (13,7%) < corn cobs (14,06%).

Alkaline pretreatment is much more effective in terms of availability of polysaccharide substrates for further enzymatic hydrolysis, compared to the thermal pretreatment, allowing higher degree of hydrolysis: wheat straw (48%) < barley straw (50,7%) < corn cobs (53,33%).

Anaerobic digestion of lignocellulosic materials led to ethanol concentrations of 3,2% in case of wheat straw, 3.9%, for barley straw and 4,9%, for corn cobs.

REFERENCES

Huang Renliang, Su Rongxin, Qi Wei, He Zhimin, 2011 Bioconversion of Lignocellulose into Bioethanol: Process Intensification and Mechanism Research, BioEnergy Research, 4(4), 225-245.

Malherbe S., Cloete T.E., 2003, *Environ. Sci. Biotechnol*., 1, 105-114.

Levine J.S, *Biomass burning and global change,* vol. 1, 1996,. Levine J.S (ed), The MIT Press, Cambridge, Massachusetts, USA.

Shin Sato, Feng Liu, Hasan Koc and Ming Tien, 2007, *Microbiology*, 153, 3023-3033.

Fang J. M., Sun R. C., Tomkinson J. Fowler P., 2000, *Carbohydrate Polymers* 41(4), 379-387.

Sun Y., Cheng J., 2002, *Biores. Technol*., 83, 1-11.

Lisov A., Leontievsky A., Golovleva L., Evans C., 2004, *Journal of Molecular Catalysis B: Enzymatic*, 31, 1-3

Arora D.S., Chander M., Gill P.K., 2002, *Int. Biodeterior. Biodegrad*. 50, 115-120.

Ruggeri B., Sassi G., 2003, *Process Biochem*., 10, 1-8.

Jorgensen H., Erriksson T., Borjesson J., 2003, *Enzyme Microb. Technol*., 32, 851-861.