HYDROLYSIS OF AGRICULTURAL BIOMASS BY COMBINED PRETREATMENT AND ENZYMATIC METHODS IN ORDER TO PRODUCE BIOFUELS (ETHANOL, BIOGAS)

HIDROLIZA ENZIMATICA CU PRETRATAMENT A PLANTELOR ENERGETICE AGRICOLE IN SCOPUL OBTINERII DE BIOCOMBUSTIBILI (ETANOL, BIOGAZ)

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The use of energy crops (maize straw, wheat straw, barley straw etc.) as substrate for renewable energy production (e.g. biogas) is more efficient when it is degraded by different hydrolysis methods. However, fibers contained inside energy crops (e.g. cellulose and hemicellulose) are only hardly and slowly degraded by anaerobic bacteria. The slow degradation of these substances can decrease the methane yields of agricultural biogas plants.In the present study, we investigated the efficiency of combined pretreatment (different concentrations H_2SO_4 + 30 minutes at 121^{0}C) followed to enzymatic hydrolysis. Testing different concentration of H_2SO_4, good results were obtained for maize whole crop when we used combined pretreatment (3% H_2SO_4 + 30 minutes at 121^{0}C) followed to enzymatic hydrolysis (3.9 fold higher) and for Gavott Maize Straw when we used combined pretreatment (2% H_2SO_4 + 30 minutes at 121^{0}C) followed to enzymatic hydrolysis (3.6 fold higher) comparing with untreated samples.

Keywords: physical pretreatment, enzymatic hydrolysis, biomass degradation

Introduction

Recently, several studies have been focused on using agricultural biomass (especially energy crops) in order to produce bio-fuels (ethanol, biogas etc.), as alternative to fossil energy sources.

Cellulose crystallinity, accessible surface, as well as the presence of lignin and hemicellulose, determines the resistance of biomass to enzymatic hydrolysis (KANUF, 2004).

The goal of any pre-treatment is to alter or remove structural and compositional impediments in order to improve the rate of enzyme hydrolysis and increase yields of fermentable sugars from cellulose or hemicellulose.

Studies in this field separate the different types of pretreatment into mechanical (cutting, milling, extrusion), chemical (phosphoric acid, hydrochloric acid, sulphuric acid, acetic acid, ammonia, sodium hydroxide, sulphur dioxide, Fe^{2+}/hydrogen peroxide), physical (steaming, pulping, radiation, freezing/thawing) and biological (white rot fungi) (ISRAEL-ROMING, 2005).

Acid pretreatment with H_2SO_4 was of interest to our experiments, because it relies on the difference in resistance to acid hydrolysis of cellulose and hemicellulose, (BADGER, 2002; KANUF, 2004) is frequently used in order to obtain recovery cellulose ethanol (MUHAMMED, 2004; SUN, 2002).

Introducing enzymatic pretreatment with efficient enzyme mixtures of cellulases or the mixture of cellulases with other enzymes (xylanases, hemicellulase, pectinases and oxidative enzymes) like intermediary step in biodegradation lignocellulosic residues, accelerate the degradation of the polysaccharides and thereby increase the methane yields of energy crops, gaining more and more interest (BERLIN, 2006a and b; CHAVEZ, 2006; WEN, 2004).

The aim of this work is to test several pretreatment methods (physical, chemical) in combination with enzymatic hydrolysis stage regarding their efficiency on cellulose and hemicellulose degradation to reducing sugars from maize straw under controlled conditions.

Materials and Methods

Substrates

Maize material consisted in Gavott Maize Whole Crop and Gavott Maize straw was used as substrates for our experiments. The maize plant was dried and ground (fibre length <1 mm) as a standard routine for laboratory analysis and frozen until required for tests.

Enzymes

Enzymatic hydrolysis was performed with MethaPlus L 100 (β-glucanase, cellulase, xylanase) produced by BIOPRACT GmbH, Germany. This enzyme was used in accordance with the manufacturer's information.

Combined pretreatment (chemical and physical)

Lignocellulosic material was treated with 1%, 2% and 3% sulfuric acid. The pretreatment was carried out at 121^0C for 30 minutes.

Enzymatic hydrolysis

The pretreated material was enzymatic hydrolyzed with 1% MethaPlus L 100 referred to the substrate dry matter content, to determine the cellulose conversion. Hydrolysis was performed at 55^0C, pH = 5-5.5 on a rotary shaker at 200 rpm, for 20 hours. All the experiments were performed in triplicate and the results are

presented as mean values. For each trial, control samples (without enzyme addition) were prepared.

Determination of reducing sugar concentrations

The degree of cellulose degradation was estimated by quantifying the amount of reducing sugars formed during enzymatic hydrolysis. Reducing sugars were determined as glucose by using 3,5 dinitrosalycilic acid reagent (DNS) according to the modified method described by Miller (1959). 2 mL from filtrated sample was added to 3 mL DNS in a test tube, heated 15 minutes at 100^0C and the absorbance of the samples was measured at 640 nm.

Results and Discussion

Taking into account the literature data (KANUF, 2004; VARGA, 2004), in a first trial, the substrates were pretreated with different concentrations 1%, 2% and 3% H_2SO_4, followed by physical pretreatment at 121^0C for 30 minutes.

As seen in Figures 1 and 2, the best results were obtained when the substrates were pretreated chemical and physical (with H_2SO_4 + 30 minutes at 121^0C). The reducing sugars concentration was approximately 3.5 times higher to compare to the control (only physical pretreated - 30 minutes at 121^0C).

However, an increase from 2% to 3 % of H_2SO_4 is not determined by a significant increase of reducing sugars concentration.

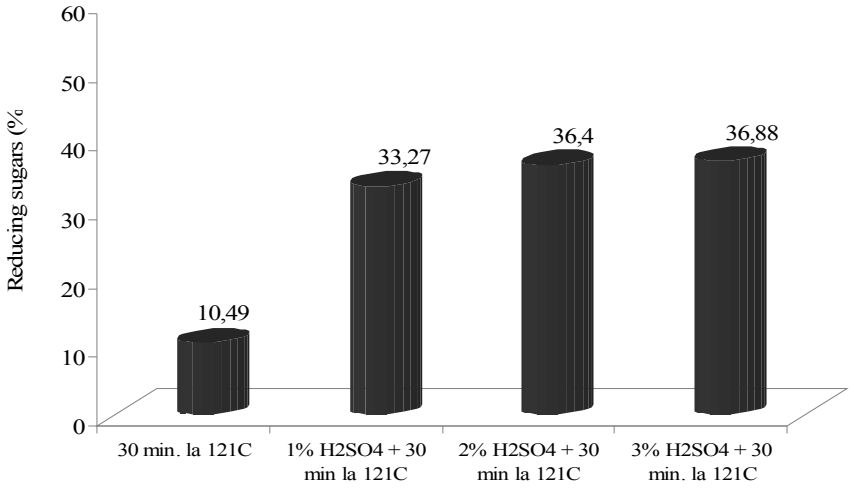

Figure 1 *Glucose accumulation for Gavott Maize Whole Crop chemical and physical pretrated*

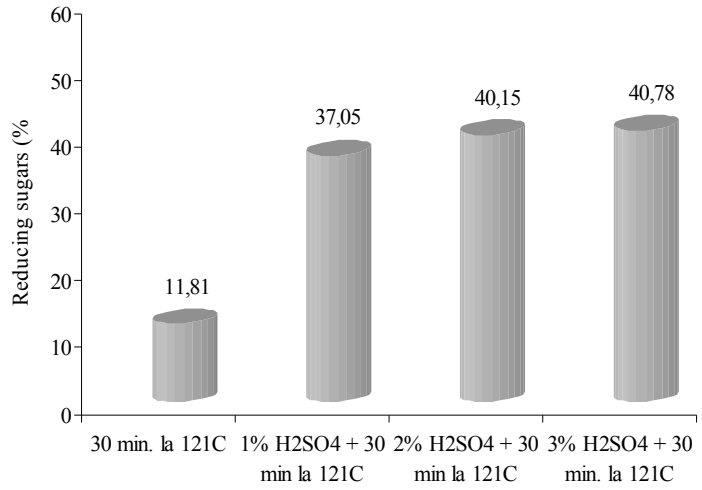

Figure 2 *Glucose accumulation for Gavott Maize Straw chemical and physical pretrated*

Further, substrates pretreated chemically and physically were enzymatic hydrolyzed (figures 3 and 4).

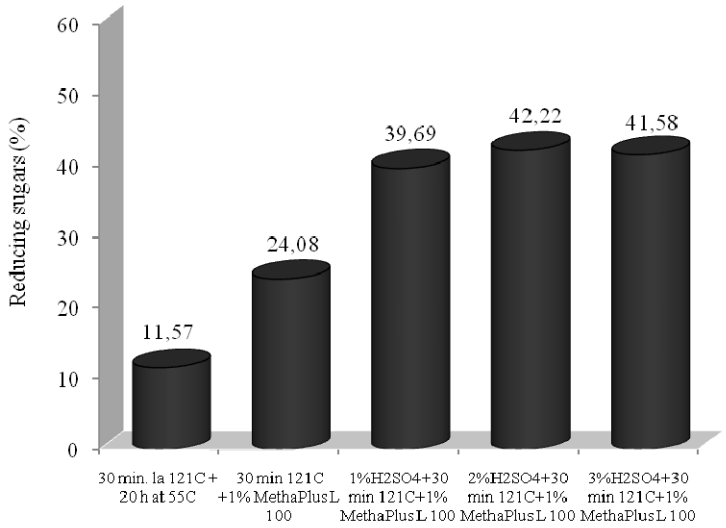

Figure 3 *Enzymatic hydrolysis of Gavott Maize Whole Crop chemical and physical pretrated*

The combined (chemical and physical) pretreatment determined an increased susceptibility of the studied materials to enzymatic hydrolysis with 74.48% for Gavott Maize Whole Crop comparing with untreated samples (figure 3).

Under the same conditions, for variant Gavott Maize Straw was obtained an increase of reducing sugars concentration with approximately 73% when the substrate was chemically and physically pretreated (2% H_2SO_4 + 30 minutes 121^0C) followed by enzymatic hydrolysis stage compared to control (30 min at 1210C + 20 h at 55^0C) (figure 4).

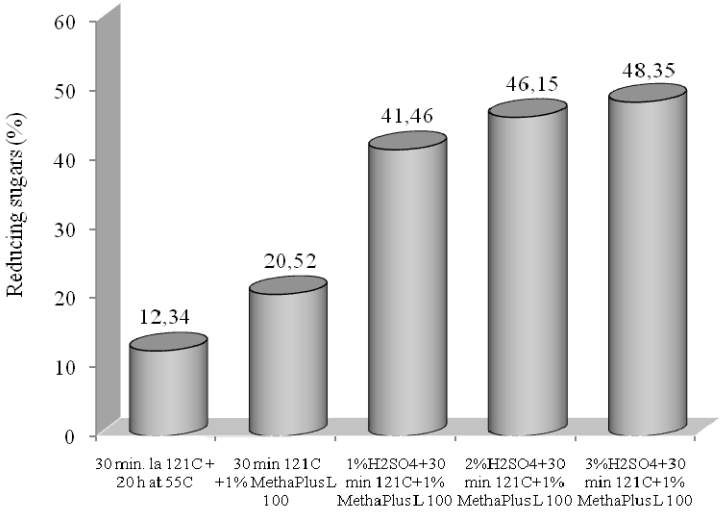

Figure 4 *Enzymatic hydrolysis of Gavott Maize Straw chemical and physical pretrated*

However, after application of enzymatic hydrolysis, the reducing sugars concentration increased with by approximately 14% for Gavott Whole Maize Crop (figures 1 and 3) and with approximately 16% for Gavott Maize Straw (figures 2 and 4) compared to only H_2SO_4 + 30 minutes 121^0C pretreatment.

Conclusions

The best results were obtained when the substrates were pretreated chemically and physically (different concentrations H_2SO_4 + 30 minutes at 121^0C). However, the increase from 2% to 3% of H_2SO_4 concentration is not caused by a significant increase of reducing sugars concentration. Good results were obtained for maize whole crop when we used combined pretreatment (3% H_2SO_4 + 30 minutes at 121^0C) followed to enzymatic hydrolysis (3.9 fold higher) and for Gavott Maize Straw when we used combined pretreatment (2% H_2SO_4 + 30 minutes at 121^0C) followed to enzymatic hydrolysis (3.6 fold higher) compared to untreated samples.

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