Studies concerning enzymatic hydrolysis of energy crops

Received for publication, February 15, 2007
Accepted, April 20, 2007

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Abstract

A lot of attention has been focused on the use of maize whole crop as substrate for renewable energy production (e.g. ethanol and biogas). Since physical and chemical pretreatments of this substrate present many disadvantages, the alternative would be the enzymatic hydrolysis. However, the lignin and hemicellulose complexes in the cell walls of maize hinder the accessibility of cellulase to cellulose, reducing therefore significantly the hydrolysis efficiency.

In the present study, we investigated the efficiency of several commercial enzymes to hydrolyze three substrates (namely maize straw and corn cob mix [cultivar: Gavott] and maize whole crop [cultivar:Vic] to fermentable sugars. For VIC whole crop, the reducing sugars concentration increased of 8.73 fold (when a mixture of 0.5 % Denilte II S + 0.5% MethaPlus L 100 was added) and of 13.14 fold (when a mixture of 1 % Denilte II S + 1 % MethaPlus L 100 was added), reported relatively to the untreated sample. In the same conditions, for the straw fraction of Gavott the reducing sugars concentrations were lower. The reducing sugars accumulation depends on: the cultivar, the maturity level, and the growth conditions. For the corn cob mix of Gavott, the highest result was of 53.47% when we added 0.5 % Liquozyme 120L.

Keywords: *energy crops, enzymes, bioconversion, hydrolysis, sugars, lignocellulosic material*

Introduction

For some years now, growing efforts has been devoted to the bio-conversion of agricultural biomass (especially energy crops) in order to produce bio-fuels (ethanol, biogas, etc) as alternative to fossil energy sources.

Lignocellulosic biomass consists mainly of cellulose, hemicellulose and lignin. Cellulose is the most abundant renewable biopolymer [7, 8, 9]. As most lignocellulosic substrates also contain hemicellulose, a system of enzyme somewhat analogue to the cellulase complex is required for the hydrolysis of xylan, the major constituent of hemicellulose [4, 8]. Hydrolysis of agricultural biomass to fermentable sugars (glucose, xylose, arabinose etc.) is required prior to energy conversion process [1, 4].

Cellulose crystallinity, accessible surface, as well as the presence of lignin and hemicellulose, determine the resistance of biomass to enzymatic hydrolysis [9].

Hydrolysis can be performed chemically or enzymatically, following the appropriate pretreatment steps [6, 12]. The hydrolysis processes used in the past were essentially chemical, but the costs and the formation of toxic by-products made them noncompetitive [4, 8] and sometimes unsuitable. Enzymatic processes, which hold several advantages, are now substituting the chemical ones. The efficiency of enzymatic process is quite high and the mild process conditions require neither expensive materials nor high process energy [8, 9, 12].

The use of cellulase complex (containing endoglucanase, exoglucanase and β-glucosidase) from different microorganisms or a mixture of cellulases with other enzymes (xylanases, amylases, laccase, pectinases etc.) for hydrolysis of lignocellulosic substrates into fermentable sugars gains more and more interest [2, 3, 7, 10, 11].

Since the extraction of relevant amounts of fermentable sugars from lignocellulosic materials requires intensive treatement using several enzymes to break down recalcitrant lignin and hemicellulose matrix. An alternative approach for biofuel production is the use of biomass with a low lignocellulosic content (e.g. corn cob mix). In this way, high hydrolysis rates could be reached at lower cost.

The objective of the present research was to optimize the enzymatic hydrolysis by controlling to increase conversion of recalcitrant molecules (lignin, cellulose, hemicelluloses) to reducing sugars.

In this paper, the hydrolysis behaviors of two crop substrates were compared using different strategies of enzymes application:

Materials and Methods

Substrates:

Two different substrates were used, namely maize whole crop of the cultivar Vic (Fao Index 240) and the corn cob mix from maize cultivar Gavott (Fao index 280)..

The materials were dried and ground (1 mm sieve diameter) as a standard routine for laboratory analysis.

Enzymes preparation

Enzymatic hydrolysis was performed with commercial enzymes in different concentrations 0.1%-1% w/w referred to the substrate dry matter content. The following commercial enzyme preparations were used:

MethaPlus L 100 (β-glucanase, cellulase, xylanase) produced by BIOPRACT GmbH, Germany.

Liquozyme 120L (containing α-amylase) produced by Novozymes, Denmark.

Denilite II S (containing laccase) produced by Novozymes, Denmark.

Enzymatic hydrolysis

The enzymatic treatment was performed either in one or two steps process. In the one-step process, MethaPlus L 100 or Liquozyme 120L were used. In the two-steps process, laccase (Denilite II S) was first used for a 20 h incubation period to remove lignin. After the incubation period laccase was inactivated by heating the sample at 95°C for 10 minutes. In the second step MethaPlus L 100 and/or Liquozyme 120L were used for an additional hydrolysis period of 20 h. The hydrolysis incubation took place at 55^0C, pH = 5-5.5 on a rotary shaker at 200 rpm. 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 dinitrosalycilic acid reagent at optical density 640 nm, by the modified method described by Peterson and Porath [5].

Results and Discussions

The efficiency of the enzymatic treatment was evaluated by measuring reducing sugars concentration after the hydrolysis steps.

Figure 1 shows the results of enzymatic hydrolysis for Maize whole crop Vic cultivar using various treatments.

As expected, the reducing sugars concentration in the control is lower than in the treated samples.

The release of phenolic compounds in the medium through Laccase action on lignin inhibited cellulase activity. Therefore the samples were heated at 95°C for 10 minutes. The action was deemed to inactivate laccase and remove phenolic compounds from the medium before further enzymatic hydrolysis. The cellulase activity was significantly increased after the first enzymatic attack using laccase.

By using solely 1% MethaPlus L 100, the percentage of reducing sugars was 4.95 % w/w in reference to the substrate dry matter content. At the same total enzyme concentration, the use of 0.5% Denilite II S together with 0.5% MethaPlus L 100 increases the yield of reducing sugars to 10.74 %. This represents 117 % increase. The influence of Liquozyme 120L in the mixture was noticeable. In fact, the addition of 0.5 % Liquozyme 120L to the mixture, enhanced the yield of reducing sugars of 34.87 %.

The treatment involving 1% Denilite II S together with 1 % MethaPlus L 100 yielded only 15.77 % of reducing sugars, representing 4.37 % lower yield than the variant with Liquozyme 120L though the total enzymes concentration was higher (2 % vs. 1.5 % in the former case).

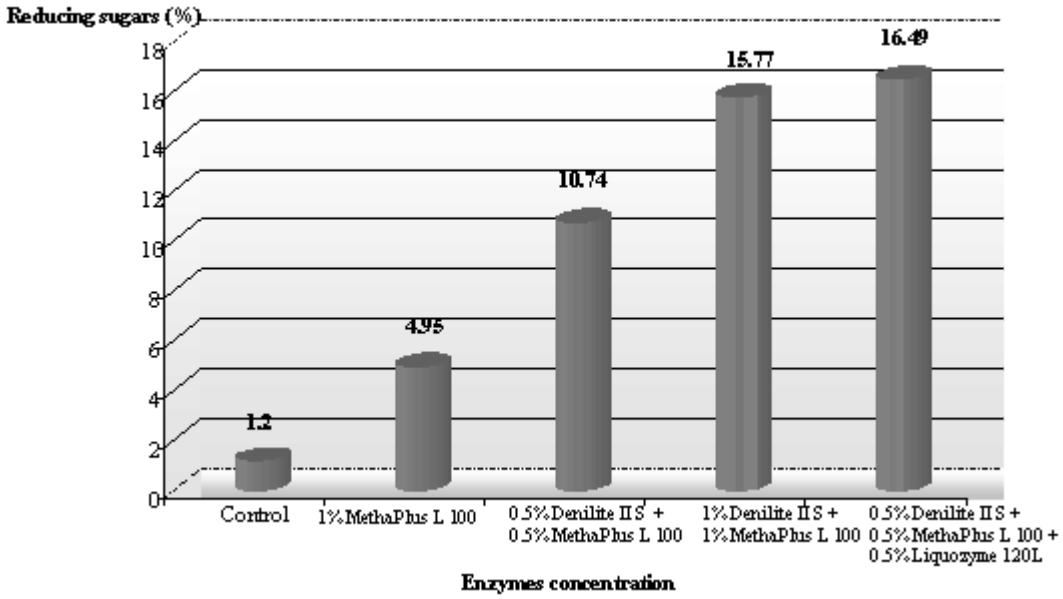

Figure 1. Reducing sugar yield of Maize whole crop 2004 (Vic sort) after enzymatic hydrolysis

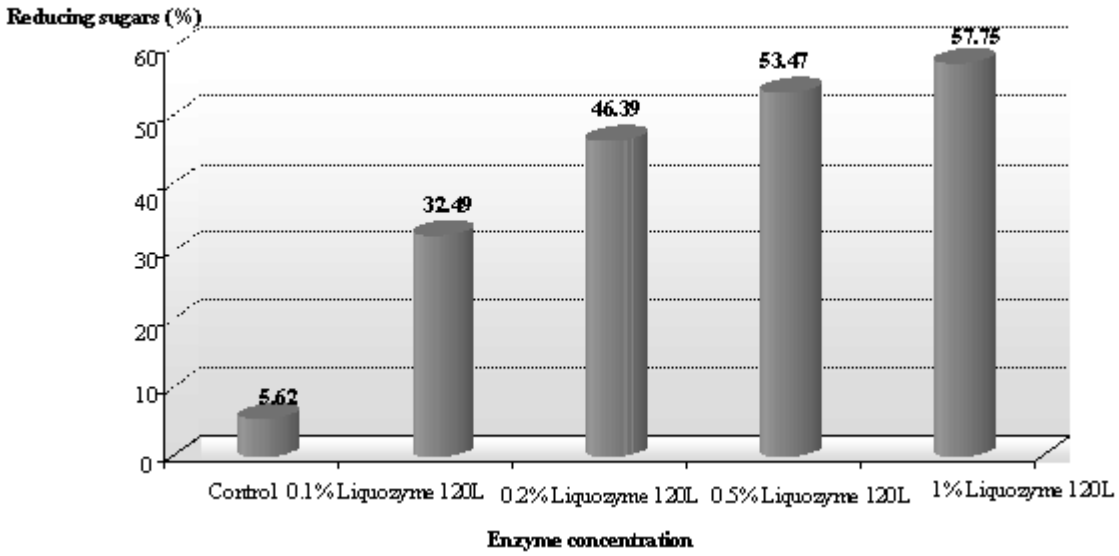

Figure 2. *Reducing sugar yield of Corncob 2006 (Gavott sort) after enzymatic hydrolysis*

Complementary to Maize whole crop (Cultivar: Vic) which has a high fiber content, corn cob (Gavott sort), which is a more easily degradable material, was studied. Corn cob contains a high concentration of starch, therefore we studied the influence of Liquozyme 120L (containing α-amylase) at different concentrations on the conversion of starch to fermentable sugars (figure 3).

According to figure 3, the reducing sugars concentration after 20 hours was higher with the increase in enzyme loading, being of approximately 58 % when we added 1 % Liquozyme 120L. Nevertheless, an increase from 0.5 % to 1 % of enzyme concentration is not economically relevant, because it determined an increase with only 8 % of reducing sugars concentration.

Conclusions

The goal of this research was to investigate the influence of different commercial enzymes on the conversion of energy crops to fermentable sugars.

Good results were obtained for maize whole crop when we used a mixture of Denilite II S, MethaPlus L 100 and Liquozyme 120 L, the reducing sugars concentration being of 13.74 fold higher compared to the control

(no enzyme addition).

In case of maize straw, in the same conditions the increase was only of 1.36 fold, than the control. For maize straw, reducing sugars accumulation was little bit higher being of 27.23 %, when we used a mixture 1% Denilite II S and 1% MethaPlus L 100.

For corncob, due to the higher content of starch, we observed that the reducing sugars concentration was higher with the increase in enzyme loading being with approximate 90 % fold higher when 0.5 % Liquozyme 120L was added, reported to the control.

In the future, the efforts should be focused on the optimizing the enzymatic treatment, including the use of different chemical mediators for improving the efficiency of laccase or other enzymes used for the enhancement of hydrolysis yields.

Acknowledgments

This research has been financially supported by Romanian National Program CEEX (No.72/01.08.2006). We would like to thank Annette Buschmann, technical staff at the State Institute of Farm Machinery and Farm Structures, for her help in realising the trials.

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