BUDAPEST UNIVERSITY OF TECHNOLOGY AND ECONOMICS
DEPARTMENT OF AGRICULTURAL CHEMICAL TECHNOLOGY

Ph.D. thesis booklet

BIOETHANOL PRODUCTION:
PRETREATMENT AND ENZYATIC HYDROLYSIS OF CORN STOVER

AUTHOR:
ENIKŐ VARGA

SUPERVISOR:
DR. KATALIN RÉCZEY

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1. INTRODUCTION AND AIM OF THE DISSERTATION

The dramatic increase in energy demand and parallel the more serious problems caused by greenhouse effect have turned the world’s interest to the alternative energy sources. The European Union (EU) ratified Kyoto protocol, established in 1997, by limiting the global net emission of carbon dioxide (CO\(_2\)) on the level of the year 1990. One way of reducing both environmental effects and dependence on fossil fuels is to use renewable fuels, like bioethanol. The European Commission has decided to increase the market share of alternative fuels to minimum 5% until 2010, and introduced the objective of 20% substitution by renewable fuels in transportation by the year 2020.

The rate of renewable energy in the total energy consumption in Hungary was only 3% in 2001, mainly low-efficiency direct burning, but the using of alternative fuels in the transport sector was nearly negligible. In half a year, Hungary will be a member of the EU, which underlines these problems and pushes to decide to overcome these deficiencies in the near future.

The climatic and geographical conditions of Hungary offer good possibilities in more intensive use of biomass. One economical way to production biofuel or fuel additives is the utilisation of agricultural or forest residues and by-products. Corn stover is the most abundant agricultural residue in Hungary, produced 10-11 million tons annually. Less, then 10% of this amount has been used as an animal feet. Because of the complex structure of the lignocellulose it is resistant to the degradation, the major portion of corn stover has traditionally been removed from the field by the practice of open-field burning. In defend of the air quality a new law was brought on 1\(^{st}\) July 2001, which prohibit the open air burning in the fields in Hungary. Thus 90% of corn stover are simple turned in the ground.

Corn stover and herbaceous straws in general consist of three main components: 30-40% cellulose, 25-35% hemicellulose, 15-20% lignin. Cellulose is associated with hemicellulose and other structural polysaccharides, surrounded by a lignin sheath. The lignin, which is a complex three-dimensional polyaromatic matrix, is partly covalently associated with hemicellulose, thus
preventing hydrolytic enzymes. The highly ordered, crystalline structure of cellulose itself poses another obstacle to hydrolysis. To enhance the enzymatic susceptibility of the cellulose, applying specific pre-treatment process is essential. The goal of the pretreatment is to disrupt the lignocellulosic matrix to make the substrate more accessible to the enzymes. The scheme of the ethanol production from lignocellulosic material has three main steps: (1) pre-treatment, (2) enzymatic hydrolysis of cellulose to glucose, (3) glucose fermentation to ethanol.

At Budapest University of Technology and Economics, at the Department of Agricultural Chemical Technology one of the most important topic for several years is the investigation of bioethanol production in Hungary in an economical way. My work was a minor part of these research program.

The aims of my present dissertation was to obtain a solid cellulose-rich fraction accessible for enzyme treatment to hydrolysis to glucose, without producing inhibitors, which obstacle the enzymatic hydrolysis and/or the fermentation from glucose to ethanol. Several different pre-treated methods, i.e. acid and alkaline pre-hydrolysis, supercritical CO₂ extraction, wet oxidation (with and without added alkaline or acid addition), and steaming, were studied. All kind of pre-treatment must meet the following requirements:

- Fractionation of the raw material into high quality feedstock for further processing/conversion,
- Avoid the degradation or loss of carbohydrate,
- Avoid the formation of by-products inhibitory to the subsequent hydrolysis and fermentation processes,
- Have a minimum consumption of energy and chemicals,
- Be cost-effective with low operating and capital cost.

2. APPLIED METHODS

Corn stover, which is the corn stalk and the leaves without root and corncob was applied as a feedstock in the investigations presented in this thesis. Corn stover was grown in South Hungary, and harvested in autumn of 1999, 2000 and 2001
following the harvest of the mature crop. The selected and washed corn stover was air-dried to an average 90% dry matter (DM) content and ground to approximately to 3 mm particle size. The dried and ground straw was stored in the dark in paper bags at room temperature.

In this study chemical and physico-chemical pre-treatments were applied. For chemical pre-treatments various bases (0.5-10% NaOH, 1-2% Ca(OH)\textsubscript{2}), and inorganic acids (0.5-5% H\textsubscript{2}SO\textsubscript{4}, 1-5% HCl) were used separately and also consequently under relative mild conditions (120°C, 30-120 min).

Under the physico-chemical pre-treatments, two processes: the wet oxidation and the steam pre-treatment were applied for corn stover. Both reactions were carried out at elevated temperature (200±10°C) and pressure.

For steam pre-treatment sulphuric acid (0.5-2%) were added, while wet oxidation was carried out both under alkaline (2 g Na\textsubscript{2}CO\textsubscript{3}/L) and acidic (0.5 mL 96% H\textsubscript{2}SO\textsubscript{4}/L) conditions. Following steam pre-treatment the pressure is swiftly reduced (1-2 sec), which makes the materials undergo an explosive decompression, but after wet oxidation the pressure decreases slightly (15-20 min). The applied reaction time was also longer (5-15 min) during wet oxidation, while it was only 2-5 min during steam explosion. The major difference between wet oxidation and steam explosion pre-treatment is the presence of oxygen during wet oxidation, which resulted, that the wet oxidation reaction is more complete.

During these the high temperature pre-treatments the presence of moisture initiates an auto-hydrolysis reaction catalysed by organic acids, which are initially formed from acetyl groups, liberated mainly from hemicellulose fraction of the biomass. Limitations of these processes include destruction of portion of the xylan fraction, incomplete disruption of the lignin-carbohydrate matrix, and generation of compounds that may be inhibitory to microorganisms used in enzymatic hydrolysis or fermentation. Compared to other pre-treatment processes, wet oxidation has the advantages that organic molecules, including lignin, decompose to CO\textsubscript{2}, H\textsubscript{2}O and simpler and more oxidised organic compounds, mainly to low-molecular-weight carboxylic acids, thus this method appears to produce fewer inhibitory by-products.
After pre-treatment the material was separated into a solid and a liquid fraction and the pre-treated solid fraction was washed carefully with distilled water. The composition both of the untreated and pre-treated corn stover and also the liquid fraction were analysed. To evaluate the efficiency of the pre-treatment, the convertibility of the solid cellulose fraction to fermentable glucose was determined using a mixture of two commercially available enzyme solutions: the cellulase (Celluclast 1.5) and the β-glucosidase (Novozym 188). The enzyme loading was 5-25 FPU/g Dry matter (DM). The activity of cellulase was determined in filter paper unit according Ghose method and the activity of β-glucosidase in CBU according Berghem method.

Before hydrolysis the pre-treated, washed, solid materials were diluted to 5% (DM) using 0.05 M acetate buffer (pH 4.8). The reaction time was 72 hours to determine the time required for total hydrolysis, and samples were taken under regular intervals. The percentage of cellulose enzymatically converted to glucose (ECC) was calculated as a quotient of liberated glucose (g) during the hydrolysis and weight of cellulose (g) before enzymatic hydrolysis. The ECC value based on the glucose concentration measured by HPLC was calculated as:

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\text{ECC} = \frac{c \cdot V}{m \cdot 1.11} \cdot 100\%
\]

where “c” is the concentration of D-glucose after enzymatic hydrolysis (g/L), “V” is the total volume (L), and “m” the weight of cellulose before enzymatic hydrolysis (g). The 1.11 factor converts the cellulose concentration to the equivalent glucose concentration.

High cellulose conversion requires high enzyme loading, which has strong negative effects on the process economy. Thus, methods to increase enzyme effectiveness are important for reduction of enzyme consumption. In my preliminary experiments the effect of surfactant addition (Tween 80) to the enzymatic hydrolysis and the effect of the ultrasonic waves - treatment were investigated.

Ethanol production depends not only on the sugar yield, but also on the fermentability of the solution. To investigate the fermentability of the pre-treated corn stover SSF (simultaneous saccharification and fermentation) was
performed with baker’s yeast. In the first step of SSF the pre-treated corn stover was pre-hydrolysed at 50°C for 24 hours with 5-10 FPU/g DM cellulase enzyme loading. Than cellulase was added again - loading 5-20 FPU/g DM cellulase - simultaneously with dried Baker’s yeast (1 g/L) at 30°C.

3. NEW RESULTS OF THE DISSERTATION

3.1. Effect of ultrasonic waves and addition of surfactant on the enzymatic hydrolysis of corn stover

Ultrasonic waves and Tween 80 surfactant were used during enzymatic hydrolysis of native corn stover, to test whether it helps the desorption of cellulase from the cellulose and thus enhance the efficiency of the enzymatic hydrolysis.

Based on the results of these experiments it was found, that:

- No positive effect could be observed after the ultrasonic treatment.
- The achieved conversion of ground corn stover with Tween 80 was 20±0.5% compared to 18±0.5%, without addition of surfactant. The conversion of cellulose to glucose increased by 8-12% after 24 h hydrolysis by the addition of Tween. However this value was still quite poor and showed that the pre-treatment is essential for the sufficient enzymatic hydrolysis.

3.2. Chemical pre-treatments

The effect of low temperature, chemical pre-treatment both on the composition of corn stover and on the enzymatic hydrolysis were investigated. And the following conclusions could be stated:

- During acidic pre-treatment at low temperature (100-120°C) the major portion (>80%) of the hemicellulose fraction was solubilised, however the degradation of the lignin fraction was only around 25%. The achieved highest enzymatic conversion of the acidic pre-treated corn stover was
only 46%, thus the enzymatic digestibility of the pre-treated material increased only slightly.

- Alkaline pre-treatment at 100-120°C, increased the enzymatic hydrolysis of corn stover four times compared to untreated corn stover. The conversion increased from 18% to 72.4 and 79.4% using 5 and 10% NaOH, respectively. The original lignin content decreased by 90% and the solubilisation of the hemicellulose fraction was also high (>85%). Although the achieved high conversions, the pre-treatment with concentrated base is not proposed for pre-treatment of lignocellulosic material considering the environmental and economical views.

- Dilute acid combined with dilute base at 100-120°C, resulted nearly theoretical conversion (96%). Although the convertibility of the pre-treated cellulose is very important in characterisation of the pre-treatment, but a good fractionation method is also able to retain the cellulose in the pre-treated solid fraction. During this two-step chemical pre-treatment nearly half of the original cellulose and around 75% of the original hemicellulose was solved. The purification and/or utilisation of the solved sugars from the acidic and the alkaline solution and also from the washing-water is a difficult technical problem and makes a shock in process economy. The separation and the washing following both steps of this pre-treatment are necessary to remove the chemicals from the fibers, because it has a negative effect to the enzymes.

### 3.3. Physico-chemical pre-treatment

To make the pre-treatment more environmental friendly and find an economically feasible method, steam pre-treatment and wet oxidation process were tested for treating of corn stover and the following conclusions could be drawn from the results:

- Steam pre-treatment and wet oxidation showed several common features. Both processes increased the enzymatic conversion significantly, the highest conversions was 83.1 following wet oxidation and 83.6% after steam pre-treatment.
In comparison of the hemicellulose recovery of this two processes, the results were also the same around 60%, however cellulose recovery was 15% lower following steam pre-treatment. The wet oxidation was also much better in retaining cellulose in the solid fraction, approximately 90% of the original cellulose content remained in the solid, fiber fraction after wet oxidation, but only 78-80% after steam pre-treatment.

To obtain high ethanol yield, the efficient enzymatic hydrolysis is necessary, however the fermentability of the pre-treated material is also essential. The fermentability both of wet oxidised and steam pre-treated corn stover gave similar results. The achieved ethanol yield was above 80% and no inhibitory effect could be observed.

The highest ethanol yield of 85% of the theoretical was achieved, using acidic wet oxidised corn stover with an ethanol concentration of 52.3 g/L, which exceeds the technical and economical limit of the industrial-scale alcohol distillation.

Corn stover is a potential substrate for the ethanol production in Hungary, because of its huge amount and its high sugar content. The polysaccharides in this herbaceous material is highly convertible to monomeric sugars following pre-treatment at high temperature, such as wet oxidation or steam explosion. These pre-treatment processes used a minimal amount of chemicals (sulphuric acid or sodium-carbonate), but the achieved enzymatic cellulose conversions were generally four times higher compared to the untreated corn stover. Thanks to the sub-inhibitory levels of the potential fermentation inhibitors following pre-treatments, the hydrolysis released glucose was convertible to ethanol using baker’s yeast.
LIST OF PUBLICATIONS

Articles on the subject of the dissertation


Other related publications


Poster and oral presentations


KÁDÁR Zs., VARGA E., RÉCZEY K. **1999.** Possible substrates for ethanol fermentation. 3rd European Motor Biofuels Forum, Brussels, Belgium, 10-13 October.


VARGA E., RÉCZEY K. **2000.** Pre-treatment and enzymatic hydrolysis of corn stover. „Lippay János - Vas Károly” Scientific Conference, Budapest, 6-7 November.

VARGA E., RÉCZEY K. **2001.** Pre-treatment of corn stover to enhance the enzymatic digestibility. Scientific Conference (KÉKI), Budapest, 23 February


VARGA E., ÁDÁM J., SZENGYEL Zs., RÉCZEY K. **2002.** Chemical Pre-treatment of corn stover. 23th Symposium on Biotechnology for Fuels and Chemicals, Colorado, USA, 4-9 May.

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