Enzymatic hydrolysis and fermentation of wet oxidised corn stover

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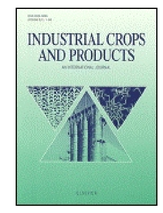

Abstract

With a production of 10 million tons/year, corn stover is the most abundant agricultural residue in Hungary. Due to its high cellulose content is a promising raw material for fuel ethanol production. However without pre-treatment it is resistant to hydrolysis, like most lignocellulosic materials. The wet oxidation (WO) process (water, oxygen, and elevated temperature) of corn stover (60g/L) at 195°C, 15 min, 12 bar O_2 and 2g/L Na_2CO_3 is an efficient method to increase the enzymatic digestibility of cellulose.

Enzymatic Hydrolysis

The efficiency of the wet oxidation was determined, both the separated residual fibrous fraction (FF) and the unseparated slurry (S) were enzymatically hydrolysed. (Fig. 1.)

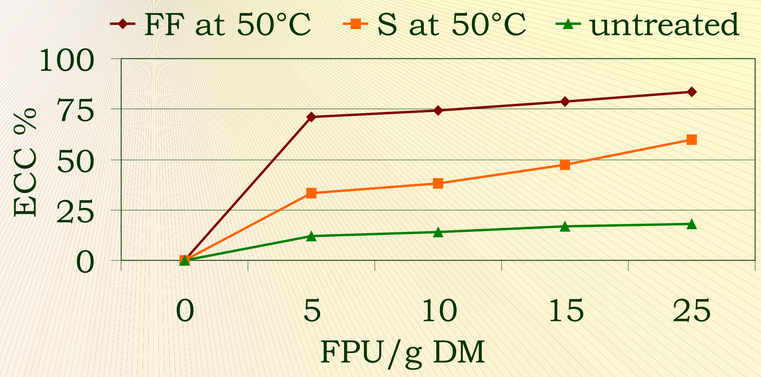

Fig.1. Enzymatic conversion from cellulose to glucose (ECC%) after hydrolysis of unseparated slurry (S), separated solid fraction (FF) and untreated corn stover at 50°C for 24h with enzyme loading of 5-25 FPU/g DM.

Hydrolysis with lower enzyme loading

The major cost of an ethanol process is the cost of the enzymes. For that reason, it is important to have high cellulose conversion at low enzyme loading.

* The highest enzymatic conversion of cellulose to glucose (ECC%) was achieved with the highest enzyme loading, however
* Decreasing the enzyme loading from 25 to 5 FPU/g DM, the ECC (%) decreased only with 15% in the case of separated residual fibrous fraction.
* Hydrolysis of slurry with decreased enzyme loading (five fold), decreased the ECC to the half. During the WO by-products were produced, which inhibit cellulases at low enzyme loading.
* The highest ECC of 84% was achieved in the case of separated residual fibrous fraction, with enzyme loading of 25 FPU/g DM, and this value was four times higher, than the ECC, of the untreated material (18%).

Simultaneous Saccharification and Fermentation

The highest ethanol concentration was 51 g/l at substrate concentration of 17% and 40 FPU/g DM (2*20) final enzyme loading. The highest ethanol yield calculated as a percentage of the theoretical yield from the consumed C_6 sugars was 96% in case of 12% substrate concentration and 40 FPU/g DM.
Decreasing enzyme loading from 40 to 10 FPU/g DM (5 FPU during the pre-hydrolysis and 5 FPU/g during the SSF) caused the decline of the ethanol yield to 78% of theoretical. (Fig. 2.)

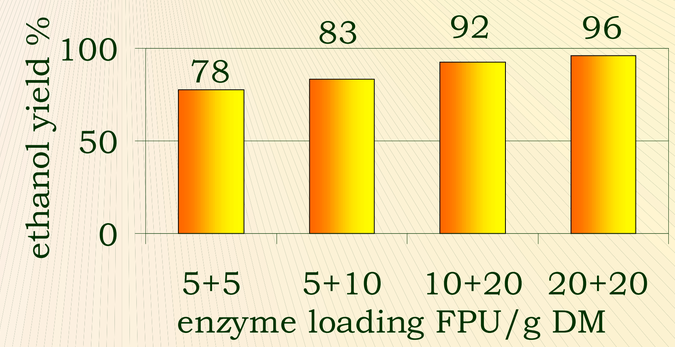

Fig.2. Ethanol yield after NSSF at 30°C for 120h, with different enzyme loading (5-20 FPU/g DM) during both pre-hydrolysis and SSF.

Material Balance from 100 g untreated cellulose

The amount of producible ethanol from 100g untreated substrate is one of the most important economical questions of ethanol production. From 100 g untreated cellulose 88% was recovered after WO and 41 % recovered as ethanol. (Fig.3.)

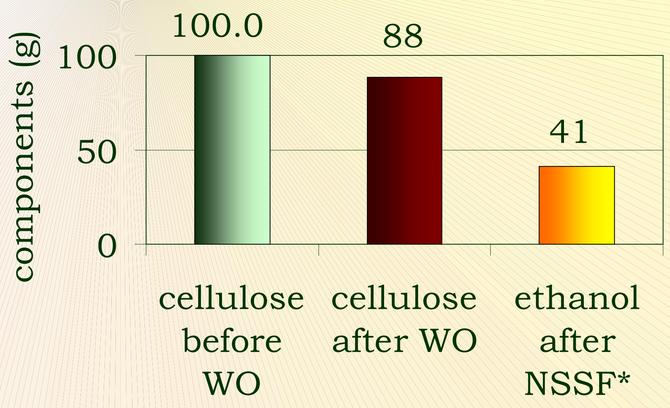

Fig.3. The amount of cellulose before and after WO and the amount of ethanol produced in NSSF, carried out at 12% substrate concentration and 40 FPU/g final enzyme loading at 30°C for 120h.

Materials & Methods

Wet Oxidation
60 g/L corn stover was treated at 195°C, 15 min, 12 bar O_2, 2 g/L Na_2CO_3

Enzymatic hydrolysis

Hydrolysis of FF
2% DM, pH=4.8, with 0.2 M citrate buffer,

Hydrolysis of S
2% DM pH=4.8, adjusted with 5M NaOH,
Temperature: 50°C,
Hydrolysis time: 24h
Enzymes: Celluclast 1.5L, Novozym 188
Enzyme loading: 5-25 FPU/g DM.

Analysis
sugars and ethanol were determined by HPLC, using Aminex HPX-87H column at 63°C, with 4 mM H_2SO_4 eluent at a flow rate of 0.6 ml/min, with detection by RI.

NSSF (non-isothermal simultaneous saccharification and fermentation)

To determine the real efficiency of the pre-treatment in ethanol production the wet oxidized corn stover was tested in NSSF.
The wet oxidised material was hydrolysed for 24 h at 50°C with 5-20 FPU/g DM enzyme loading.

The substrate was added to the reaction in 3 portions, resulting in final substrate concentration between 10 and 17% DM. After pre-hydrolysis cellulase enzyme (5-20 FPU/g DM) was added again to the hydrolysate simultaneously with dried baker's yeast.
The fermentation was carried out at 30°C for 120h.

Conclusion

Alkaline wet oxidation at 195°C, 15 min, 12 bar O_2 and 2 g/L Na_2CO_3 was an efficient pre-treatment of corn stover, producing a solid fraction with high enzymatic convertibility and fermentability, resulting 41% conversion of the cellulose to ethanol.
50 g/l ethanol concentration was achieved, which matches the technical and economical limit of the industrial-scale alcohol distillation.

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