# Improvements in Bioethanol Production Process from Straw

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**Abstract:** An efficient production of sustainable, carbon-neutral, renewable fuels like bioethanol and biogas from straw and other agricultural by-products has to be developed to guarantee mobility. The scientific focus is the improvement of the bioethanol production using straw as an alternative energy source.
The ethanol production process is already established on a laboratory scale. The process involves the following steps - the pretreatment of straw with steam explosion and enzymatic hydrolysis. Subsequently, yeast ferments the obtained glucose to ethanol. Unfortunately, inhibitors such as weak acids, furans and phenolic compounds are generated during the pretreatment and hydrolysis process, thereby reducing the glucose concentration and ethanol yield.
Glucose concentration was raised up to 140 g/l and ethanol content up to 7% by means of optimization of the process (washing steps and recirculation). Diverse substances inhibit the fermentation and reduce the ethanol content. One washing step prior to hydrolysis clearly reduced the inhibitory substances.
The ethanol and glucose yield was improved due to optimization of the bioethanol production. Now an efficient procedure to reduce the inhibitors has to be established to plan a pilot plant.

***Keywords:*** *bioethanol, lignocellulose, straw*

## 1. Introduction

Due to climate change, dramatic fluctuations of the oil price, decline of oil and increased prices for foodstuffs it becomes more and more important to find alternatives like bioethanol produced from renewable lignocellulosic residues without competition to foodstuff.

The bioethanol production process of the 2nd generation involves the following steps: the pretreatment to open the three-dimensional structure from lignocellulose, alternatively chemical hydrolysis or enzymatic hydrolysis to obtain sugars and subsequently, yeast ferments the obtained sugars to ethanol.

However, the essential requirement in bioethanol fermentation is a highly concentrated sugar solution which leads to an increased product concentration and furthermore reduced purification costs. Unfortunately the agricultural lignocellulosic by-products solubilize at relatively low concentrations. Increasing the dry matter and fed-batch process overcome this problem. The final solids content could be raised up to 21 % during the hydrolysis process [1-3]. Using fed-batch process, where fresh substrate is successively fed into the hydrolysis reaction, final solids loading can reach amounts of up to 17 % [2, 3].

Thirty percent solid loading and a final sugar concentration of 20 % were reached with corn stover based on combined pretreatments and fed-batch process [4]. Pretreatment and hydrolysis of straw cause the formation of compounds (organic acids, phenols, furanes) inhibiting the fermentation through yeast [reviewed in 5].

In this study the bioethanol production from staw was investigated and improved. Several improvements, particularly one washing step before hydrolysis and recirculation strategy, were made. These improvements increase both sugar concentration and bioethanol yield up to 7 %.

## 2. Methodology

### 2.1. Pretreatment, hydrolysis, recirculation

For pretreatment the milled wheat straw was heated in steam explosion process at various temperature and conditions (120-200 °C, 5-60 minutes). The pressure was suddenly released and made the material accessible to subsequent enzymatic hydrolysis. The removal of potential inhibitors was conducted by a washing step prior to enzymatic hydrolysis. After washing, wheat straw has been dried and milled. The enzyme mixture Accellerase TM1000 from Genencor® has been used with enzyme activities of 775 IU cellulase (CMC)/g solids and 138 IU beta-glucosidase/g solids.

Suspensions with various dry substances (10-20%) have been produced with the pretreated straw in citrate buffer (50 mM, pH 5.0). The suspensions have been enzymatically solubilised (aerobically) at a temperature of 50 °C for 96 hours in a shaking incubator. The hydrolysis of pretreated straw (10 to 20 % solid) has been repeated three times in a recirculation process. After the first hydrolysis step (96 hours), suspension has been centrifuged and the hydrolysate has been used for the next hydrolysis step with fresh substrate (10 to 20 % solid). Then, the hydrolysate has been recirculated in a third step.

### 2.2. Determination of sugars, ethanol, organic acids, furans

For precise sugar and ethanol analytics, as well as for determination of HMF, furfural and xylitol, HPLC from Jasco and BioRad AMINEX® HPX 87H with ultra-pure water as eluent and RI detection has been used. The bioethanol yield has additionally been determined using a Anton Paar Alcolyzer Beer from DMA. For precise organic acids analytic as well as for HMF-, furfural-concentrations, HPLC from Agilent Technologies, the ion exclusion column Varian Metacarb 87 H with $H_2SO_4$ (5 mM) as eluent, UV-detection at 210 nm and a RI detector (Jasco) has been used.

### 2.3. Determination of phenols

Phenols have been determined as gallic acid equivalent, using folin-ciocalteu reagent [6].

### 2.4. Fermentation

Diverse salts were added to the wheat straw hydrolysate for the fermentation (di-ammonium hydrogen phosphat, 4.4 g/l, calciumchlorid 3 g/l, potassiumhydrogenphosphat 2.86 g/l, magnesiumsulfat 1.5 g/l. The pH-value has been adjusted to 4.6. Exclusively, a wild-type strain of *Saccharomyces cerevisiae* has been used. Fermentation process has been conducted at a temperature of 30 °C in a shaking incubator for one week.

## 3. Results

### 3.1. Straw pretreatment

Pretreatment of lignocellulose offer a rapid and efficient hydrolysis of cellulose [7]. Steam explosion is one possibility for removing lignin and hemicellulose as well as making cellulose accessible to subsequent conversion into monomers with enzyme [reviewed in 8-13].

### 3.2. Enzymatic batch and fed-batch hydrolysis

The glucose and xylose concentrations reached amounts of 64 g/L and 16 g/L after enzymatic hydrolysis with 20 % solids using unwashed straw, respectively. Due to a washing step prior to hydrolysis, glucose concentration could have been increased to 80 g/L. Xylose concentration has reduced from 16 g/L to 5 g/L after washing. Final glucose and xylose amounts after hydrolysis using unwashed and washed straw are shown in table 1 and 2.

*Table 1: Sugar yield after the first hydrolysis with unwashed straw (10, 15 and 20 % solids).*

| | 10 % solid | 15 % solid | 20 % solid |
|---|---|---|---|
| Glucose [g/L] | 20 | 39 | 64 |
| Xylose [g/L] | 5 | 10 | 16 |

*Table 2: Sugar concentrations after the first hydrolysis of washed straw (10, 15 and 20 % solids).*

| | 10 % solid | 15 % solid | 20 % solid |
|---|---|---|---|
| Glucose [g/L] | 31 | 52 | 80 |
| Xylose [g/L] | 2 | 3 | 5 |

### *3.3. Recirculation strategies and fermentation*

Recirculation strategies have been developed, where the sugar solution of a first hydrolysis reaction is recycled to fresh straw and subsequent hydrolysis reaction.
For this approach, wheat straw has been pretreated with steam explosion. After the first recirculation step to fresh unwashed solids and subsequent hydrolysis (20 % solids, 2$^{nd}$ hydrolysis), glucose and xylose concentrations have reached 108 g/L and 28 g/L, respectively. Due to a washing step prior to hydrolysis, a glucose concentration of more than 116 g/L could have been reached. Xylose was removed to some extent during this washing step.
However, glucose concentration has been further increased by a second recirculation step to fresh washed solids and subsequent hydrolysis (20 % solids, third hydrolysis) to an amount of 143 g/L. After fermentation with *Saccharomyces cerevisiae*, an ethanol yield of 7.5 %vol. could have been produced.

In figure 1 a, b the final glucose concentrations after hydrolysis (10, 15 % and 20 %) and recirculation processes with unwashed and washed straw, as well as produced bioethanol yields after fermentation were demonstrated.

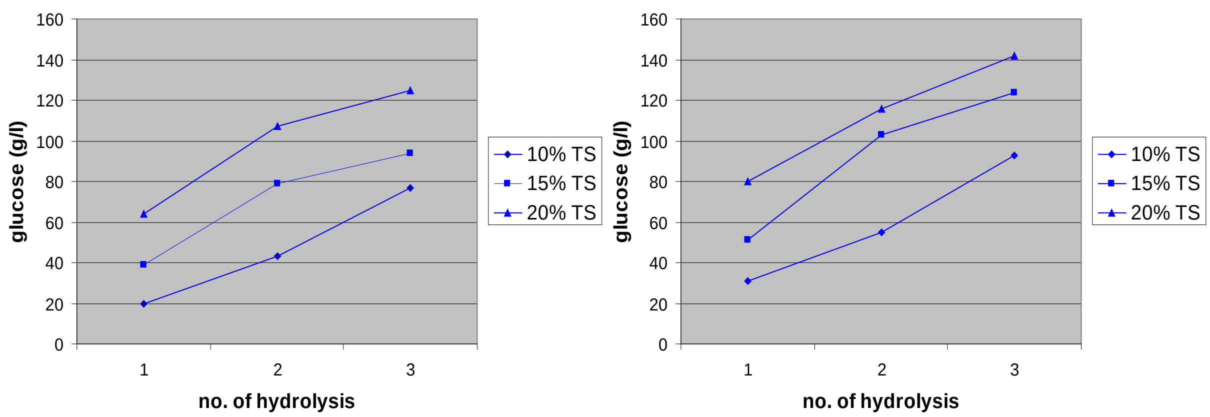


*Fig. 1a: Final glucose concentrations after recirculation process (1$^{st}$, 2$^{nd}$, 3$^{rd}$ hydrolysis) with 10, 15 and 20 % solids loading of unwashed (left) and washed straw (right).*

The hydrolyses from unwashed straw operated definitely superior to the hydrolyses from washed straw. Obviously the washing step reduced inhibitory compounds released during pretreatment and hydrolysis. However, even the sugar concentration from 2nd and 3rd hydrolysis with washed straw didn´t increase linear possible due end product inhibition.

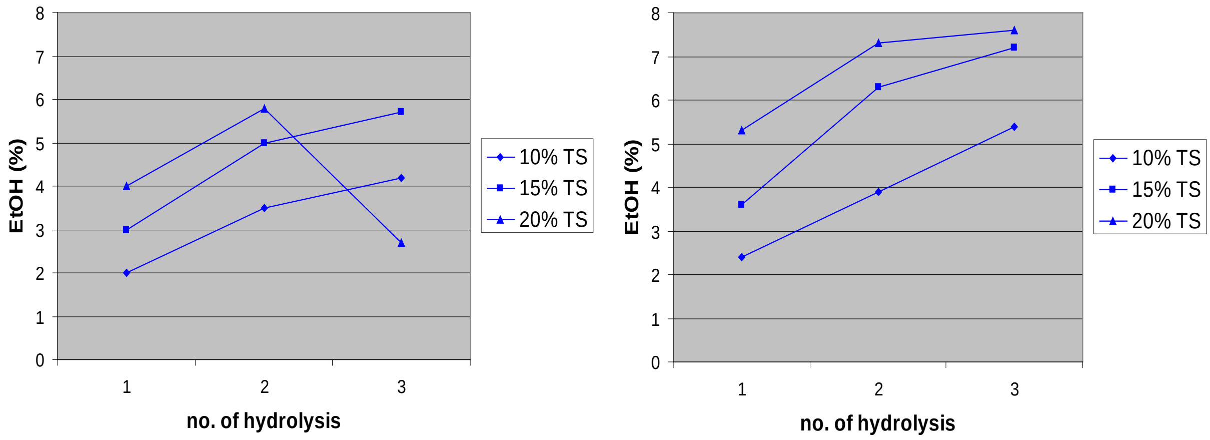


*Fig. 1b: Produced bioethanol yields after fermentation of unwashed (left) and washed straw (right).*

However, during pretreatment and hydrolysis of straw, groups of inhibitory compounds (phenols, organic acids, furanes) were generated, which had a negative influence on the ethanol-producing yeast. During recirculation procedures, the inhibitory compounds accumulate, resulting in a dramatic decrease of fermentative efficiency. The washing step before hydrolysis removed presumably the inhibitory compounds.

The concentration of diverse potential inhibitors was determined with HPLC analysis and phenol using folin-ciocalteu reagent (figure 2 and 3).

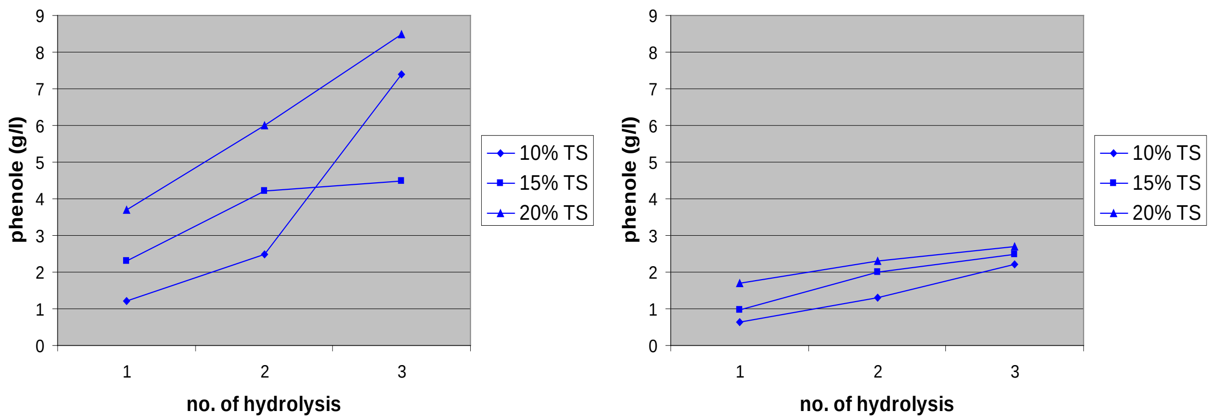


*Fig. 2: Phenole concentrations after recirculation process (1st, 2nd, 3rd hydrolysis) with 10, 15 and 20 % solids loading of unwashed straw (left) and washed straw (right).*

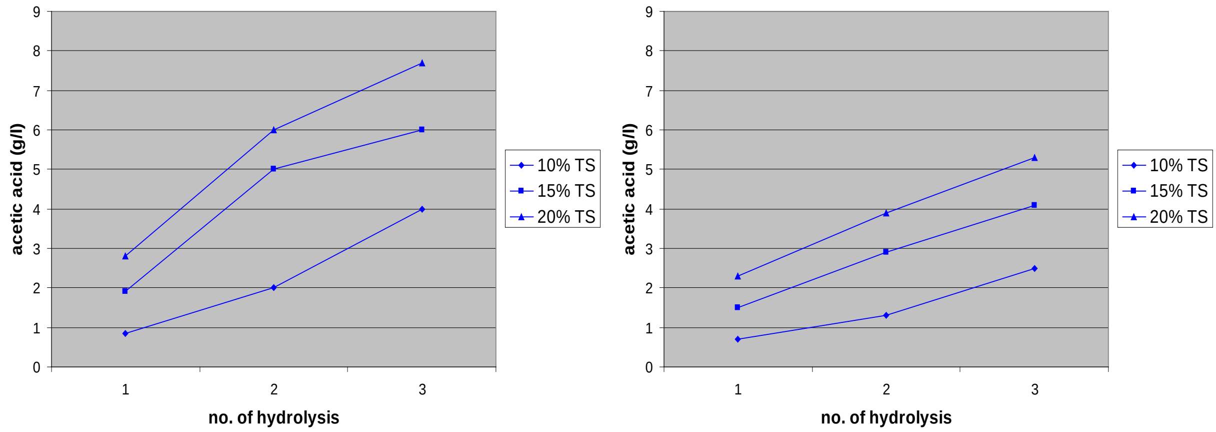


*Fig. 3: Acetic acid concentrations after recirculation process (1$^{st}$, 2$^{nd}$, 3$^{rd}$ hydrolysis) with 10, 15 and 20 % solids loading of unwashed straw (left) and washed straw (right).*

A clearly reduction of potential fermentation inhibitors like phenol (figure 2), acetic formic acid (figure 3), propionic acid, HMF and furfural were detected between unwashed and washed straw (data not shown).

## 4. Discussion and Conclusions

An efficient bioethanol production requires an effective pretreatment, hydrolysis and fermentation resulting high sugar concentration and subsequent high ethanol yield without inhibitory compounds.

In this study one new strategy – recirculation – was chosen to raise the sugar and ethanol yield. Therefore steam exploded straw was washed, hydrolyzed and fermented reaching sugar concentration of 140 g/l and EtOH yield of about 7.5 %. A comparison with unwashed straw revealed that obviously the inhibitory compounds formed during pretreatment were removed through the washing step.

This washing step must be considered critically - a high water consumption and pollution – could be a tender point in the bioethanol production from straw. In the future the washing water can maybe purified through membrane technology and reused and/or fed in a biogas plant.

Unfortunately also xylose was removed through this washing step. At present xylose can not efficient ferment to bioethanol but maybe xylose could be used in another way for example as biopolymer. Therefore the recovery of xylose has to established to get more products from a bioethanol plant.

Furthermore the recirculation strategy must be improved – the actual sugar and bioethanol yield is two times lower than the theoretical value and is at the moment to inefficient for an industrial application. The cellulase activity during the hydrolyses was obviously reduced through end production inhibition and/or inhibitors. New enzyme and/or enzyme mixture can enhance the sugar yield.

In the future diverse improvements have to be done:

- particularly the inhibitory compound(s) or the combination of the inhibitory compounds have to be determined and
- an efficient reduction method of the relevant inhibitors has to be established
- and/or adapted yeast to the inhibitors has to be developed
- creation of product(s) from xylose.

In addition the cycle of materials must made to enable a cost-effective bioethanol production from straw. The energy for steam explosion step should be provided from a biogas plant and the fermentation remains fed in a biogas plant. The biogas remains should be manure the agricultural area.

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