

Bioethanol Production from Rice Straw

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Abstract: Problem statement: Rice straw has been treated with different chemical (acid, alkali) and physical (subcritical water, ultrasound) methods to convert lignocellulose material to sugar. In addition enzyme treatment of pretreated samples has been applied to improve the conversion of lignocellulose material in sugar. **Approach:** Sulfuric acid at concentration of 1-9% was applied for acid treatment. For alkali treatment was sodium hydroxide solution at concentration of 1-5% used. Subcritical water treatment carried out at 160°C (5 bar) and 200°C (15 bar) for 10 min. Ultrasound was applied as combination method after acid pretreatment. The condition during ultrasound treatment was 40 W at 50°C and 10 min. Finally the pretreated sample was fermented using *Saccharomyces cerevisiae* yeast and the amount of produced ethanol was measured. **Results:** Acid treatment at 121°C, 15 min is an effective pretreatment method for converting lignocellulose to sugar. Up to 21.45% sugar w/w could be measure after acid treatment. Combination of chemical pretreatment and subsequent enzyme treatment increased the sugar yield drastically. Up to 37 and 28% sugar w/w could be achieved for acid and alkali pretreated samples respectively. Subcritical Water (SCW) treatment method is an effective physical method. SCW treatment at 200°C and 10 min followed by enzyme treatment yielded up to 17% sugar w/w. Combination of acid pretreatment with ultrasonic before enzyme treatment increased the conversion of lignocelluloses to sugar. Sugar yield up to 44% w/w after combination of acid and ultrasonic pretreatment and subsequent enzyme treatment could be achieved. Fermentation of pretreated rice straw shown that after 3 days fermentation most of sugar (55-65%) will be converted to bioethanol. The remaining sugar could not be converted in ethanol even after 6 days fermentation. Under these conditions, the maximum ethanol of 1.69% (v/v) was obtained. **Conclusion:** The combination method of acid pretreatment combined with ultrasound and subsequent enzyme treatment result the highest conversion of lignocellulose in rice straw to sugar and consequently, highest ethanol concentration after 6 days fermentation with *S. cerevisiae* yeast.

Key words: Rice straw, bio ethanol, subcritical water, ultrasound, sugar, enzymes

INTRODUCTION

Ethanol from renewable resources has been of interest in recent decades as an alternative fuel to the current fossil fuels. Lignocelluloses biomass like wood and agricultural crops residue, e.g., straw and sugar-beet pulp are potential raw materials for producing several high-value products like fuel ethanol and biodiesel. Up to 80% of the lignocelluloses is polysaccharides (Kaparaju *et al.*, 2009). These renewable raw materials look promising for replacing environmentally unfriendly fossil hydrocarbon raw materials and hence, creating "green" products. In contrast to traditional fuels, fermentation ethanol does not contribute to the greenhouse effect, being a CO₂ neutral resource.

Rice straw is a by-product of rice production and great bio resource. It is one of the abundant lignocellulosic waste materials in the world. It is annually produced about 731 million tons, which is distributed in Africa 20.9 million tons, Asia 667.6 million tons and Europe 3.9 million. Rice straw can potentially produce 205 billion liters bioethanol per year, which is about 5% of total of consumption. It is the largest amount from a single biomass feedstock. Rice straw predominantly contains cellulose 32-47%, hemicelluloses 19-27% and lignin 5-24%, ashes 18.8%. The pentoses are dominant in hemicelluloses which xylose is the most important sugar followed by arabinose and hexoses. The carbohydrate of rice straw involves glucose 41-43.4%, xylose 14.8-20.2%, arabinose 2.7-4.5%, mannose 1.8%

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and galactose 0.4% (Maiorella, 1985; Roberto *et al.*, 2003).

Several pre-treatment processes have been developed for lignocelluloses, which function by an enlargement of the inner surface area. This is accomplished partly by solubilization of the hemicelluloses and partly by degradation of the lignin. The pre-treatments are: Milling and grinding, pyrolysis, high-energy radiation, high pressure steaming, alkaline or acid hydrolysis, gas treatment (chlorine dioxide, nitrogen dioxide, sulfur dioxide, ozone), hydrogen peroxide treatment, organic solvent treatment, hydrothermal treatment, steam explosion, wet oxidation and biological treatment (Fan *et al.*, 1982; Hormeyer *et al.*, 1988; McGinnis *et al.*, 1988).

Subcritical Water (SCW) treatment is an environmentally friendly “green” technique with wide range of application, such as extraction, hydrolysis and wet oxidation of organic compound (Holliday *et al.*, 1998; Kruse and Dinjus, 2007). Thus, SCW can be used for extraction of organic compound instead of using organic solvent which are environmentally unacceptable. On the other hand, SCW has been widely used for hydrolysis of organic compounds. Recently growing attention has led to extensive research activities using SCW for hydrolysis and conversion of biomass and carbohydrates to useful compound.

There is no information available in literature about the combination of ultrasound as pretreatment and enzymatic conversion of lignocellulose to sugar.

The aim of this study is to investigate the effect of combined physical and chemical methods as pretreatment on conversion of lignocellulose in rice straw to sugar and fermentation of sugar to bioethanol using *S. cerevisiae* yeast.

MATERIALS AND METHODS

Raw material: Rice Straw from Suphanburi province As enzyme mixture were 5 following technical enzyme cellulase/hemicellulase applied:

- Crystalzyme 200XL (valley Research, USA)
- Celluclast 1.5 LFG (Novozymes, Denmark)
- Alcalase 2.5 LDX (Novozymes, Denmark)
- Validase ANC-L (valley Research, USA)
- Xylanase (Dr. Luca and Partner Ingenieurkontor GmbH, Germany)
- The enzymes were mixed together in equal relation

Methods:

Alkali pre-treatment: About 50 g Chopped (≈ 2 cm length) dried rice straw was suspended in 1, 2, 3, 4, 5% NaOH in ratio of 1:10 (w/v) rice straw and NaOH. After that the samples were incubated in water bath 85°C for 1 h. Finally, hydrolysate was pressed through cheese cloth. The amount of reducing sugar in juice was measured by using Luff schoorl method (Matissek and Steiner, 2006).

Acid pretreatment: About 50 g Chopped dried rice straw was suspended in acid solution (1, 3, 5, 7 and 9% Sulfuric acid) in ratio of 1:10 (w/v) rice straw and Sulfuric acid. The mixtures were autoclaved at 121°C for 15 min. After that, hydrolysate was pressed through cheese cloth and the amount of reducing sugar in juice was measured as above.

Alkali/enzyme pre-treatment: The best alkali pretreatment condition was carried out for Alkali/Enzyme pre-treatment. The NaOH treated sample was pressed through cheese cloth. The juice was kept and the remaining pulp was mixed with distilled water (10:1 w/w on rice straw basis) containing enzyme mixture (0.8% v/w on of rice straw basis). The pH of sample was adjusted at pH = 4. Sample was incubated in a water bath at 55°C for 12 h (1st Enzyme). After that the sample was pressed through cheese cloth and the pulps of these pressed sample was applied for second enzymatic treatment as described above (2nd Enzyme). The juice of first press as well as “1st Enzyme” and “2nd Enzyme” were carried out for sugar content measurement.

Acid/enzyme pretreatment: The best acid pretreatment condition was chosen for acid/enzyme pretreatment. For enzymatic hydrolysis the sample were treated as described in alkali/enzyme pretreatment.

Subcritical water pre-treatment: For Subcritical Water (SCW) treatment a high pressure tube ($\varnothing = 15$ mm. Volume = 80 mL) equipped with pressure gauge and thermocouple was used. The heating of SCW tube was carried out in oil bath at constant temperature.

Chopped dried rice straw was mixed with distilled water in ratio 1:10 (w/v) rice straw and water. The mixture was poured in subcritical water tube and the experiment conducted at 160 and 200°C for 10 min. in oil bath. After subcritical water treatment, the tube was cooled in water bath and the content was poured in a beaker. Subsequently, the samples were treated with enzyme mixture (0.8% v/w on rice straw basis, pH = 4.0) for 12 h at 55°C. The samples were then pressed

through cheese cloth and the sugar content in pressed liquid was measured.

Ultrasonic pretreatment: Acid pre-treated (1% acid) sample was placed in a beaker and treated with ultrasonic at 40 W for 10 min. The temperature during ultrasonic treatment was <math><50^{\circ}\text{C}</math>. After ultrasonic treatment the sample was subjected to enzyme (4.0 % v/w on rice straw basis, pH = 4.0) treatment. The sample was pressed through cheese cloth and the amount of sample was measured.

Detoxification: The pretreated samples from acid/enzyme pretreatment and ultrasound pretreatment was mixed with wood activated charcoal (20:1 w/w sample: Charcoal) and then agitate for 2 days on magnetic stirrer at room temperature. After charcoal treatment the sample was filtered using filter No. 5 (Whatman, Germany) to remove the charcoal. The filtrate was subjected for sugar measurement.

Fermentation: The pretreated samples from acid/enzyme pretreatment and ultrasound pretreatment with or without detoxification were carried out for fermentation experiments.

The yeast *S. cerevisiae* was used for fermentation. The initial yeast count in fermentation sample was $2-8 \times 10^8$ cfu mL⁻¹. After 3 and 6 fermentation days the ethanol content was measured by gas chromatography. In addition the remaining sugar during fermentation was measured.

For calculation of ethanol yield the following equation was applied:

$$\text{Ethanol.Yield} = \frac{\text{Measured.Ethanol.in.Sample (g)}}{\text{Theoretical.Ethanol (g)}}$$

Theoretical ethanol (g) = Amount of initial sugar content (g) in fermentation solution $\times 0.5$

All the measurements were duplicated and the data reported are average of two replications.

RESULTS

Effect of acid pre-treatment methods on sugar content: In the Fig. 1 the relation between sugar content and acid concentration is illustrated. Increasing the acid concentration showed reverse effect on sugar concentration in sample. This is maybe because of degradation of monomeric sugars (xylose, glucose) in furfural and hydroxymethyl furfural. The highest sugar

up to 21.45% sugar (on the rice straw basis) could be obtained using 1% sulfuric acid.

Effect of alkali pre-treatment methods on sugar content: Higher concentration of alkali leads to slight increasing the sugar in sample (Fig. 2). Generally, very low sugar content of $\leq 0.55\%$ (on the rice straw basis) could be observed.

Effect of acid and alkali pre-treatment and subsequent enzyme treatment on sugar content: Application of technical enzymes have positive effect on conversion of lignocellulose material to sugar. This was observed for acid pretreated as well as alkali pretreated samples (Fig. 3).

The sugar yield was in acid pretreated and subsequent enzyme treated sample distinct higher (36.95%) compare to only acid pretreated sample (21.45%). Whereas, nearly no effect of alkali pretreatment on conversion of cellulose and hemicelluloses to glucose could be observed, a drastically increase of sugar concentration (24.6% on rice straw basis) in alkali pretreated and subsequent enzyme treated sample could be achieved.

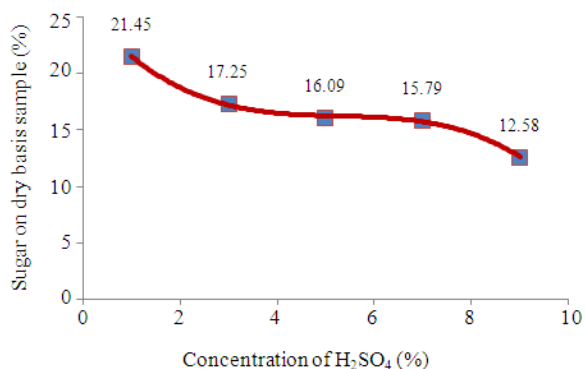


Fig. 1: Sugar content in acid pre-treated rice straw

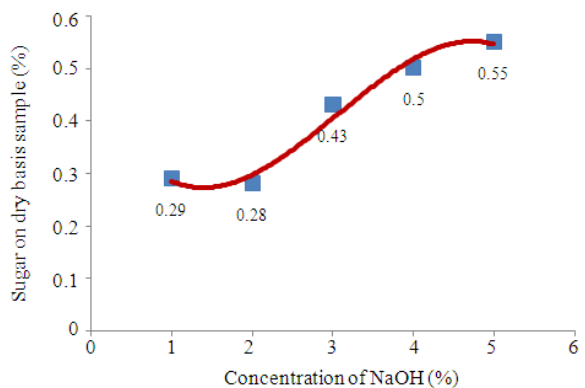


Fig. 2: Sugar content in alkali pretreated rice straw

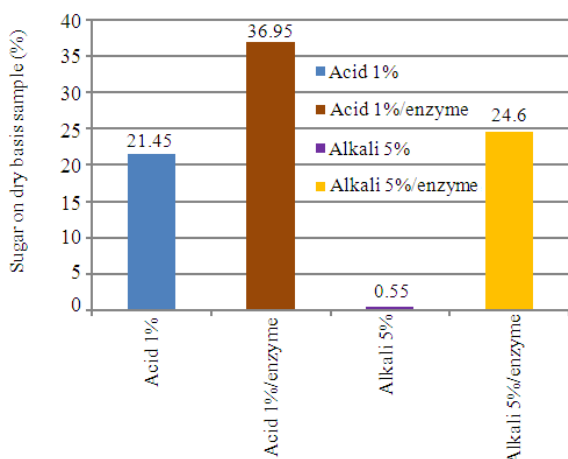


Fig. 3: Effect of acid and alkali pretreatment with or without subsequent enzyme treatment to sugar content of rice straw

Table 1: Sugar content of Subcritical Water (SCW) pretreatment and subsequent enzyme treated rice straw

Condition	Temperature (°C)	Percentage of sugar*
SCW/enzyme	160	7.4
	200	16.9
Only enzyme	30	3.4

*: On rice straw basis

Table 2: Lignocellulose conversion to sugar and sugar yield of different treated rice straw

Pretreatment	Sugar (%)*
Acid 1%	21.45
Acid 1%/enzyme (0.8% v/w)	36.95
Acid 1%/enzyme (4% v/w)	39.10
Alkali 5%	0.55
Alkali 5%/enzyme (0.8% v/w)	24.60
SCW/enzyme, (0.8% v/w)	16.90
Enzyme, (0.8% v/w)	3.40
Acid 1% + ultrasonic + enzyme (4% v/w)	43.93
Acid 1% + ultrasonic + enzyme (4% v/w/and detoxified)	32.29

*: On rice straw basis

In contrast enzyme treatment of sample without pretreatment have nearly no effect (3.4% sugar on rice straw basis) on sugar content of rice straw.

Effect of subcritical water treatment: Table 1 shown the effect of subcritical water and subsequent enzyme treatment on conversion of lignocelluloses to sugar. Increasing the temperature during SCW from 160-200°C increased the sugar concentration more than 2 times (from 7.4-17% sugar on rice straw basis).

Effect of enzyme concentration: Increasing the enzyme concentration from 0.8 v/w (on rice straw basis) to 4% v/w lead to increasing of sugar in acid pretreated sample from 36.96 to 39.10% respectively (Table 2).

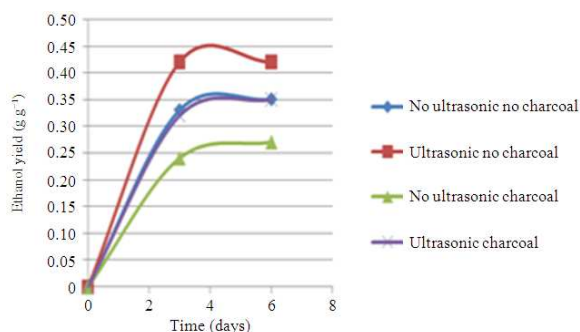


Fig. 4: Ethanol yield during fermentation

Table 3: Sugar concentration (g/100 g solution) during fermentation

Fermentation time (day)	A	B	C	D
0	4.35	5.13	4.02	4.49
3	2.00	2.13	1.67	1.98
6	1.60	1.77	1.51	1.75

Note: A = No ultrasonic/No detoxification; B = With ultrasonic/No detoxification; C = No ultrasonic/with detoxification; D = With ultrasonic/with detoxification

Effect of combined Ultrasonic (US) and acid pretreatment on sugar content: The sugar content of combined acid and ultrasonic pretreated and subsequent enzyme (4% v/w on rice straw basis) treated sample was higher (43.93% sugar on rice straw basis) compare to sample without ultrasonic treatment (39.1%) (Table 2). This results show the positive effect of ultrasonic treatment during pretreatment of rice straw on polysaccharide conversion into sugar.

Effect of Detoxification on sugar content: During detoxification of samples part of sugar could be adsorbed on activated charcoal leading reduction of total sugar in sample. In Table 2 is the amount of sugar before and after charcoal treatment illustrated.

Fermentation: Figure 4 show the effect of fermentation time on conversion of sugar to ethanol in acid pretreated, with or without ultrasonic and subsequent enzyme treated rice straw. During 3 days fermentation time nearly all for *S. cerevisiae* fermentable sugar (glucose) will be converted to bioethanol. Longer fermentation time up to 6 days have only slight effect on bioethanol production. The sugar concentration in sample decreased from 4-5% w/v to nearly 1.5% w/v after 6 days fermentation. This is equal to 55-65% bioconversion of sugar to bioethanol (Table 3).

The ethanol yield in this study was about 0.42 g g⁻¹ (Fig. 4).

DISCUSSION

The decrease of sugar content in acid treated samples with increasing of acid concentration is may be because of degradation of monomeric sugars (xylose, glucose) in furfural and hydroxymethyl furfural. These substances are toxic substances for yeast and can inhibit the yeast growth.

The positive effect of acid pre-treatment and subsequent enzyme treatment could be explained as better access of enzyme to lignocellulose materials in rice straw. Vlasenko *et al.* (1997) have found that after acid pretreatment (10% solid mater, 0.8% acid, 160°C) followed by enzyme treatment (cellulose 100 L + cellulbiase) the yield of glucose was 43 g L⁻¹ or 43% conversion of cellulose to glucose. The results in present study is slightly less than Valsenko *et al.* (1997) reported. This is may be due to lowe temperature (121°C compare to 160°C) during acid treatment or because of different conditions during enzyme treatment.

There was clear relationship between temperature during SCW and effectiveness of this “green technology”. Increasing the temperature and pressure during SCW treatment indicated higher conversion of lignocellulose to sugar after subsequent enzyme treatment. Karimi *et al.* (2006) reported the conversion of rice straw to sugar by SCW. SCW treatment at 15 bar and 200°C without adding acid resulted 9.9% sugar (glucose and xylose) after 2 times SCW treatment. Bobleter and Bonn (1983) found out that hemicellulose sould be completely separated from the lignocellulose and enzymatic digestability of cellulose can be significantly increased by treating the lignocellulose material with SCW in flow through system.

Power ultrasound produce cavitations. Cavitations can break down cell structure and cell polymers (such as cellulose, hemicellulose) structures. Combined acid pre-treatment and ultrasonic followed by enzyme treatment resulted highest sugar yield up to 44% (on rice straw basis). This results show the effectiveness of combination of physical and chemical pre-treatment prior to enzymatic hydrolysis of lignocellulose material to sugar.

Detoxification of samples prior to fermentation showed no positive effect on ethanol yield. It could be postulated that the concentration of phenolic substances after acid treatment was less and had nearly no effect on *S. cerevisiae* growth.

Abedinfar *et al.* (2009) have investigated the fermentation of rice straw (pretreated with diluted acid and subsequent enzyme treatment) rice straw using *Mucor indicus* and *Rhizopus oryzae*. They have found

an ethanol yield of 0.36-0.43 g g⁻¹ using *Mucor indicus* which was comparable with the corresponding yield by *S. cerevisiae* (0.37-0.45). *Rhizopus oryzae* produce 0.33-0.41 g g⁻¹ ethanol.

The ethanol yield in this study was about 0.42 g g⁻¹. That is in accordance with ethanol yield reported from literature (Abedinfar *et al.*, 2009).

The remaining sugar of 35-45% w/v is may be xylose. Xylose is pentose sugar that can not be digested by *S. cerevisiae*.

CONCLUSION

The comparison between different chemical pretreatment methods has shown that the acid treatment combined with subsequent enzyme treatment is a suitable method for conversion of lignocellulose to sugar. Up to 36% sugar could be observed after this method. Combination of acid pretreatment and ultrasonic before enzyme treatment lead to much higher sugar up to 44%. Detoxification of sample before fermentation showed decrease of sugar concentration on sample upon absorption of sugar on activated charcoal.

It should be noted, that the ethanol concentration achieved in this study was not higher than 15 g L⁻¹ as a consequence of low rice straw loading. Therefore, in order to achieve higher concentration of ethanol attractive for industrial application, higher rice straw loading is necessary.

ACKNOWLEDGEMENT

We would like to thank Mahidol University for financial support of this project.

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