HUNGARIAN JOURNAL
OF INDUSTRIAL CHEMISTRY
VESZPRÉM
Vol. 36(1-2) pp. 43-48 (2008)

SWEET SORGHUM JUICE AND BAGASSE AS A POSSIBLE FEEDSTOCK FOR BIOETHANOL PRODUCTION

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The aim of our study was to estimate the overall ethanol potential of a promising Hungarian sweet sorghum variety called 'Monori Édes' developed by Agroszemek Ltd. For ethanol production following parts of the plant can be utilized: the stem juice containing sucrose and the bagasse built up mainly from lignocellulose. As lignocellulosics have to be pretreated and hydrolyzed prior to fermentation, another purpose of our research was to apply weak alkaline pretreatment methods to enhance enzymatic digestibility of bagasse thus, to improve the ethanol yield. In our study the effect of two bases (NaOH and KOH) in two concentrations (1% and 2%) and at two temperatures (room temperature and 121 °C) was investigated on the efficiency of enzymatic hydrolysis. Every pretreatment type affected positively the hydrolysis efficiency but in different degrees. Best results were achieved with 2% NaOH at 121 °C. However highest ethanol conversion based on the glucan content of pretreated material was reached using 2% NaOH at room temperature. Summarizing the ethanol potentials of juice and bagasse an overall potential of about 8 300 L/ha was estimated.

Keywords: sweet sorghum, enzymatic hydrolysis, alkaline pretreatment, ethanol fermentation

Introduction

Sorghum is the forth most important forage crop in the world with a cultivation area over 40 million hectares [1]. In the dry zones of tropical and subtropical areas it is used for food purposes too while under the moderate climate the utilization as feed has priority. Two different sorts of sorghum are cultivated, the grain sorghum and the sweet sorghum which is a sugar cane-like plant with sucrose-rich juice in the stem. In contrast to sugar cane, sweet sorghum can be cultivated in nearly all temperate climatic areas in Europe, also in regions possessing weak arable land conditions.

Besides using sweet sorghum as animal feed there is anohter possibility to cultivate it as energy crop getting more and more attention. From the extracted juice fuel ethanol can be produced, in Asia there are already industrial scale factories based on this technology. In 2007 4.92 billion liters of ethanol were produced from sweet sorghum juice in China and India. The first factory of the USA utilizing sweet sorghum juice alone will be built in Florida by Renergie which has recently received a 1.5 million dollars grant to start the project [2].

In our study the ethanol potential of a Hungarian sweet sorghum variety called 'Monori Édes' developed by Agroszemek Ltd was estimated. Since this variety has been improved in Hungary, it has adapted to the climatic conditions and it can produce high green biomass yield, i.e. 80–100 t/ha. It is harvested in September–October when the sugar content of the stem is the highest. The sugar concentration of the juice can reach 14–20%. Nowadays, it is mostly used as cattle feed but there is a growing interest to utilize it as ethanol fermentation feedstock. In this case the harvested stems get pressed to extract the juice (40–50 t/ha); the by-product of the process is the bagasse, the leftover of the stem (40–50 t/ha, 50% dry weight) built up mainly from lignocellulose. Although bagasse could be used as raw material for second generation bioethanol production, regularly it is burnt to supply the energy demand in the juice-to-ethanol process.

While the technology based on juice is already available on industrial scale, the lignocellulose conversion is still in experimental phase. There are only a few papers available on this alternative utilization. Gnansounou et al. [3] studied the theoretical possibility for a sweet sorghum biorefinery under circumstances in North China. The purpose of our work was to analyse and utilize the bagasse of 'Monori Édes' as a possible raw material for ethanol production.

Before fermentation the lignocellulosic bagasse has to be pretreated and hydrolyzed to liberate glucose molecules. The aim of pretreatment is to break down the complex and resistant structure of lignocellulose and hereby increase the efficiency of subsequent enzymatic hydrolysis. Usually after grinding a chemical or physico-chemical method is applied. In case of chemical treatment acid, base or organic solvent is used. Based on previous

results achieved with other agricultural by-products alkaline pretreatment was used in our experiments [4]. Their effect on the efficiency of enzymatic hydrolysis was evaluated.

The pretreated and hydrolized samples as well as the juice were fermented by baker's yeast to determine the ethanol potential of the whole plant.

Materials and methods

Raw materials

Both the frozen, with nitric acid acidified juice (pH = 3.0–3.5) and the bagasse were obtained from the site of Agroszemek Ltd. near to Hódmezővásárhely. Harvest and pressing were performed during the autumn of 2007. Before use the bagasse was chopped, dried and ground.

HPLC analysis

Water soluble sugar (cellobiose, glucose, xylose and arabinose) and ethanol content were determined by high-performance liquid chromatography (HPLC). Samples for HPLC analysis were prepared by filtration through a 0.45 µm pore size regenerated cellulose syringe filter (La-Pha-Pack, ProFill™, Langerwehe, Germany). An Aminex HPX-87H (BioRad, Hercules, CA, USA) column was used at 65 °C with 5 mM sulphuric acid mobile phase at 0.5 mL/min flow rate. Separated compounds were detected by a Shimadzu RID-10A refrective index detector (Shimadzu, Kyoto, Japan).

Raw material analysis

Initial sugar content of the juice was determined in triplicate by 'Sucrose, D-fructose and D-glucose' kit (Megazym).

Cellulose- and hemicellulose content of bagasse were determined in triplicate before and after pretreatments by a two-step sulfuric acid hydrolysis. The principle of this method was originally described by Hägglund [5]. Firstly 0.5 g of dry ground bagasse was hydrolysed in 2.5 mL of 72% sulfuric acid for 2 hours at room temperature. After that 77 mL of distilled water was added into it and further hydrolysed for 1 hour at 121 °C. After separation the reaction mixture on a G4 glass filter HPLC analysis was carried out from the liquid fraction. The solid fraction was washed with hot distilled water and dried. This residue was defined as the lignin content and was determined gravimetrically.

Pretreatments

Bagasse pretreatments were carried out on eight different ways. The effect of three parameters was investigated on two levels: type of base (NaOH versus KOH), concentration of base (1% versus 2%) and temperature – time combination (25 °C, 3 days versus 121 °C, 1 hour). Ground bagasse (0.3–1.4 mm) at 10% dry weight content (40 g DM in 400 g total mass) was suspended into NaOH or KOH solution in 1000 mL screw-capped bottles and left at room temperature for 3 days or autoclaved at 121 °C for 1 hour. After pretreatments the mixtures were separated and the solid phase was washed with hot distilled water to remove solubles. Filter cake was dried at 50 °C and used for raw material analysis and for enzymatic hydrolysis.

Hydrolysis

Pretreated bagasse samples were hydrolyzed at 50 °C at 2% dry weight content in 0.05 M acetate buffer (pH 4,8) by commercially available enzymes Celluclast 1.5L and Novozym 188 (Novozymes) at 20 and 40 IU/ g DM, respectively. As control untreated bagasse was also hydrolyzed. Process was carried out in 250 mL screw-capped bottles containing 4 g DM pretreated bagasse in 200 g total mass with stirring (250 rpm). 1.8 mL of samples were taken at start of hydrolysis and after 1, 3, 4, 6, 24 and 48 hours. Samples were centrifuged in Eppendorf tubes at 15 000 rpm for 5 minutes, subsequent reducing sugar determination was carried out from the supernatant. After 48 hours the hydrolisates were cooled down to 30 °C and moved to the fermentation step.

Reducing sugar determination

Hydrolysis was tracked by reducing sugar content determination according to Miller's colometric method [6]. A suitable volume (containing reducing sugar inside the applied calibration range) was pipetted from the samples into test tubes and completed to 1.5 mL by adding distilled water. 3 mL of DNS (3,5-dinitro-salicylic-acid) reagent was added and the mixture was boiled for 5 minutes. After cooling down to room temperature 16 mL of distilled water was added, mixed and the absorbance was measured at 550 nm against a blank sample. Results were obtained due to the calibration of the DNS reagent.

Fermentation

Batch fermentations were carried out at 30 °C in 250 mL stirred flasks (250 rpm) with measuring CO_2 production by an online fermentation module device, developed by Nonfood group (BME) and Stereo Vision Ltd. Baker's yeast suspension was added to 2 g dry weight per liter. At the end of fermentation when CO_2 production ceased, flasks were sampled. Samples were centrifuged in 50 mL centrifuge tubes at 9 000 rpm for 5 minutes. Supernatant was analyzed for sugar and ethanol concentration by HPLC.

Two different carbon sources were investigated:

- Enzymatic hydrolysates of sweet sorghum bagasse pretreated in different ways.
- Sweet sorghum juice (prior to fermentation the pH was adjusted to 5 by 10% NaOH solution).

Results and discussion

Sweet sorghum juice

Total sugar content of sweet sorghum juice was 163.8 g/L with a standard deviation of 7.3 g/L. Distribution of di- and monosaccharide molecules can be seen in *Table 1.* Sweet sorghum juice contains primary sucrose but due to acidification and storage sucrose hydrolyzed partially to fructose and glucose.

Table 1: Composition of sweet sorghum juice

Component	Initial g/l	After fermentation g/l
Fructose	14.2±0.3	7.6±0.1
Glucose	16.5±1.6	-
Sucrose	133.1±5.8	N/A
Ethanol	N/A	68.7±2.1

160 mL of sweet sorghum juice was inoculated with yeast suspension in a volume to correspond to 2 g DM/L. After approximately 20 minutes to inoculation the gas production started. It reached a constant velocity in 70 minutes for a 8 hour interval which was slowly declining and gas formation stopped in the 24th hour of fermentation, *Fig. 1.*

Tracking was stopped and broths were sampled for HPLC analysis; Table 1. HPLC detected also some remaining fructose which corresponds to the results of Phowchinda and Strehaiano [7], they have found that sugar remains in low concentration at the end of the fermentation. Based on the initial sugar content and final ethanol concentration conversion value of 78.9% was calculated. This high value corroborates that no complementary salt addition and pH regulation are necessary during fermentation to reach good conversion.

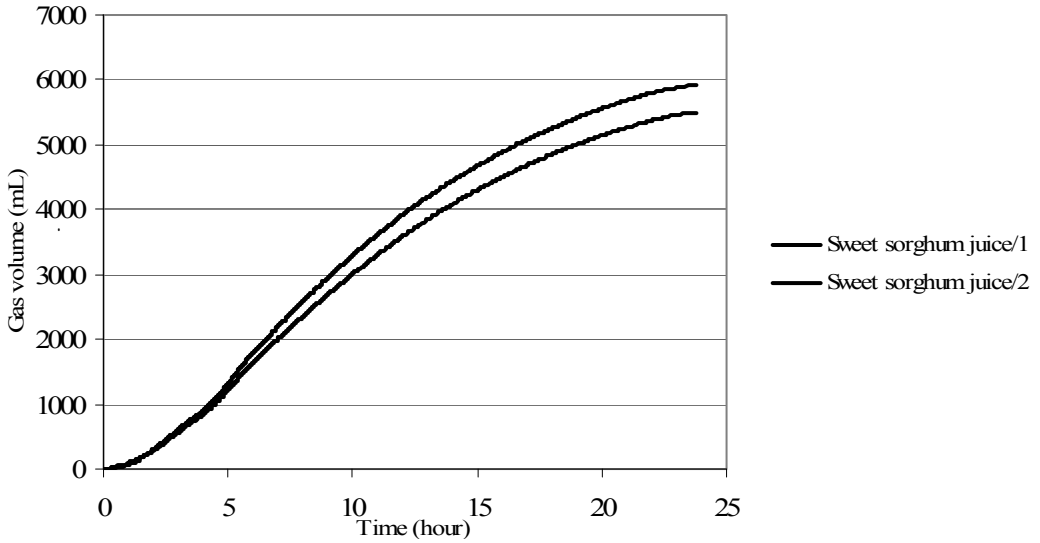

Figure 1: Ethanol fermentation of the juice tracked by CO_2 production in Online Fermentation Module device

Sweet sorghum bagasse

Prior to pretreatment ground bagasse contained 41.30±0.07% glucan which can be considered as cellulose content, 17.41±0.15% xylan and 17.62±0.11% lignin, on dry weight basis. Pretreatment resulted in an enhanced glucan and xylan content of bagasse. The sum of these was the basis for conversion calculations. Glucan content increased from 41% to 45–67% on dry weight basis depending on pretreatment. Xylan content reached a maximum of 29.37% using 2% KOH at elevated temperature. Detailed results are summarized in *Table 2.* Highest increase of glucan content was caused by bases in 2% concentration at elevated temperature. At room temperature 1% NaOH solution had the same effect on glucan content as the 2% KOH. Generally it can be said that NaOH is a more efficient pretreating agent than KOH in both concentrations and at both temperatures.

Table 2: Composition of pretreated bagasse samples

Pretreatment		Glucan		Xylan	
		average	std dev	average	std dev
25 °C	1% NaOH	48.24	0.57	25.16	0.35
	2% NaOH	50.02	0.52	23.34	0.13
	1% KOH	44.59	1.19	24.25	0.29
	2% KOH	48.63	0.68	24.44	0.30
121 °C	1% NaOH	49.57	0.67	25.75	0.46
	2% NaOH	66.86	0.39	28.76	0.35
	1% KOH	45.78	0.59	24.56	0.74
	2% KOH	64.85	0.36	29.37	0.16

Decrease of the lignin content was observed, which is in line with what had been expected. In case of alkaline pretreatment it is supposed that the lignin fraction get partly solved. Lignin content of sweet sorghum bagasse showed 2–7% decrease depending on pretreatment conditions.

During hydrolysis of pretreated material in every case the reducing sugar contentration started to increase rapidly and then the velocity decreased depending on substrate availibility. Hydrolysis curves of the room temperature pretreated bagasse can be seen on *Fig. 2* while the hydrolysis data of the pretreatment experiment performed at 121 °C are summarized on *Fig. 3.*

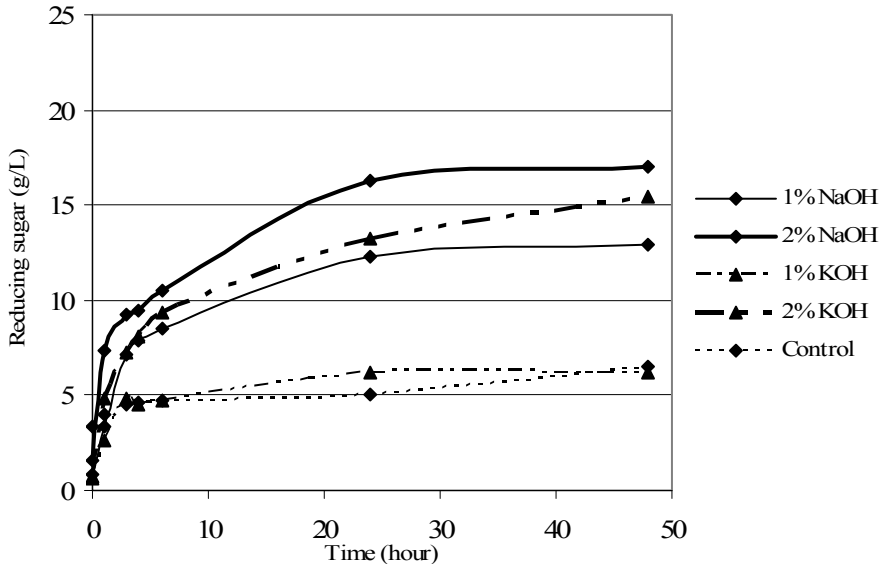

Figure 2: Reducing sugar concentrations during enzymatic hydrolysis on 25 °C pretreated materials

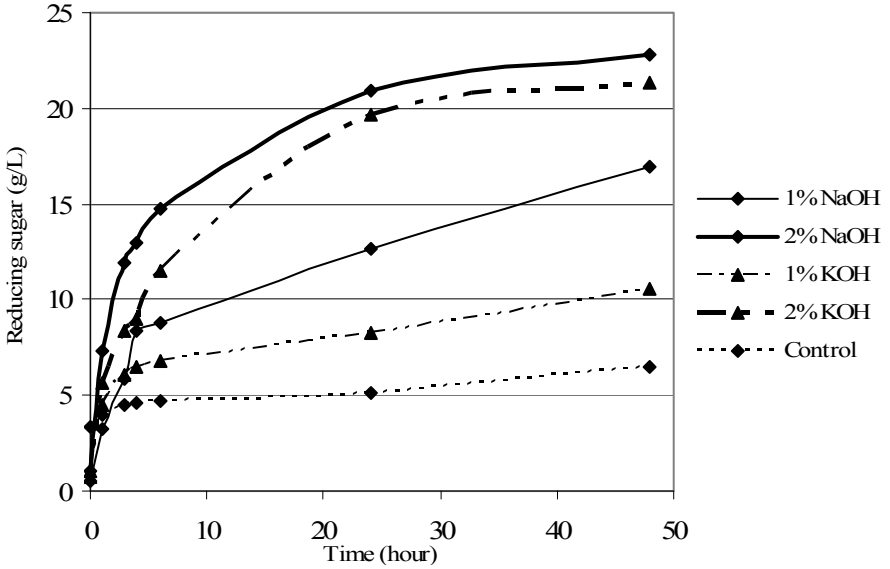

Figure 3: Reducing sugar concentrations during enzymatic hydrolysis on 121 °C pretreated materials

In pretreatment at room temperature KOH was not effective in 1% concentration the reached reducing sugar content was nearly the same as the one for control sample (6.5 g/L). Although the pretreatment with 2% NaOH gave the highest reducing sugar concentration (17.0 g/L), the pretreatments with 1% NaOH (RC = 12.9 g/L) and 2% KOH (RC = 15.4 g/L) can be considered also efficient.

Hydrolysis of the bagasse pretreated at 121 °C resulted in a more diverse pattern where hydrolysis on bagasse pretreated by 2% NaOH and 2% KOH gave the

highest reducing sugar concentrations, 22.9 and 21.3 g/L respectively.

For the calculation of conversion the actual reducing sugar content was divided by the monomer equivalent of the xylan and glucan content of pretreated material. Since there were differences in the composition of the pretreated bagasse depending on the applied pretreatment, the order of conversion values is not always the same as that of the reducing sugar contents. The final (at 48 hours) conversion values can be seen in *Table 3*.

Based on conversion results the bagasse pretreated with 2% bases (both KOH and NaOH) gave the highest values under room temperature pretreated materials. Among on elevated temperature pretreated materials the bagasse treated with NaOH showed better results than the ones with KOH in both concentrations. The highest conversion value (94.6%) was achieved in case of 2% NaOH pretreatment performed at elevated temperature.

In ethanol fermentation of various hydrolyzates a short lag phase could be observed, the constant gas formation rate was reached in less than an hour. It took only 3–5 hours for the yeast cells to consume the liberated glucose which after no more CO_2 gas formation could be detected, *Fig. 4.*

Table 3: Conversion values at the end of hydrolysis (48 h) – based on reducing sugar measurements

Pretreatment		Conversion %
25 °C	1% NaOH	68.4
	2% NaOH	86.8
	1% KOH	33.1
	2% KOH	83.2
121 °C	1% NaOH	90.5
	2% NaOH	94.6
	1% KOH	57.6
	2% KOH	89.2
Control		20.4

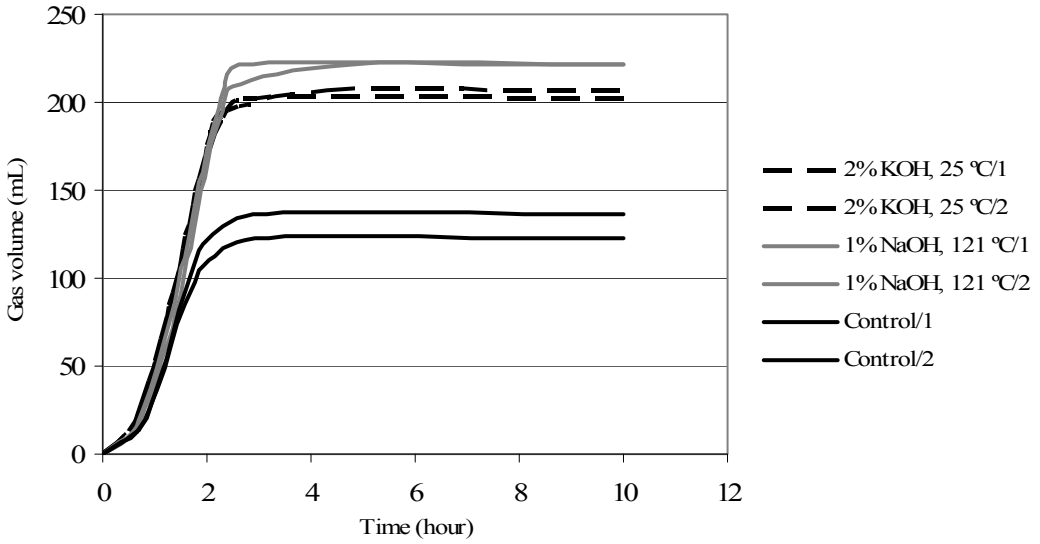

Figure 4: Ethanol fermentation tracked by CO_2 production in Online Fermentation Module device

The curves recorded by the Online Fermentation Module device show a good parity between the duplicates, indicating that measuring the gas volume is an appropriate method to track ethanol fermentation. Largest difference between paralels was about 40 mL (data not shown) which corresponds to less than 0.1 g ethanol (calculated according to the ideal gas law and stochiometry of hexose fermentation).

According to the final ethanol concentrations measured by HPLC combined hydrolysis and fermentation conversion values were calculated based on the glucan content of the pretreated materials, *Table 4* as *Saccharomyces cerevisiae* used in the experiments was unable to utilize xylose. These conversion values vary in a wide range, but no correspondence could be observed between them and the hydrolysis efficiency (Table 4 versus Table 3). Some acetic acid formation was detected in nearly every sample suggesting by-product formation.

Table 4: Results of ethanol fermentation and combined hydrolysis and fermentation conversion values

Pretreatment		Ethanol conc. g/L	Conversion %
25 °C	1% NaOH	2.777 ± 0.370	48.2
	2% NaOH	4.158 ± 0.337	69.6
	1% KOH	1.160	21.8
	2% KOH	2.413 ± 0.047	41.6
121 °C	1% NaOH	3.107 ± 0.556	52.5
	2% NaOH	3.829 ± 0.199	48.0
	1% KOH	1.910 ± 0.004	34.9
	2% KOH	4.461 ± 0.355	57.6

Ethanol potential

Sweet sorghum juice has high and directly fermentable carbohydrate content which can be fermented to ethanol without any salt addition effectively. According to the ethanol concentration reached in our fermentation (68.7 g/L) an ethanol yield (L/ha) was calculated equal to 3 729 L/ha assuming 45 t/ha juice yield.

Based on the results 4 560 L/ha ethanol yield was calculated for bagasse which corresponds to the highest ethanol conversion rate (69.9% – pretreatment: 2% NaOH at room temperature) in case of 45 t/ha bagasse yield with 50% dry weight.

Adding up the ethanol yields of juice and bagasse an overall ethanol potential of 8 289 L/ha can be calculated.

Summary

Sweet sorghum juice fermented during our research had an initial sugar concentration of 163.8 g/L and which a conversion value of 78.9% it yielded 68.7 g/L ethanol.

In order to improve the degradability of sweet sorghum bagasse eight different alkaline pretreatments were performed. Best hydrolysis conversion based on reducing sugar measurement was reached in case of pretreatment at 121 °C in 2% NaOH solution, namely 94.6%.

Enzymatic hydrolyzates were fermented to ethanol using *Saccharomyces cerevisiae*. Highest ethanol concentration, 4.461 g/L was reached when bagasse pretreated with 2% KOH at 121 °C was used. Concerning conversion, based on the glucan content of pretreated material, the highest combined hydrolysis and fermentation conversion was 69.9% by the pretreatment 2% NaOH at room temperature.

The overall ethanol yield (L/ha) of the whole plant is about 8 300 L/ha.

Our research demonstrated that the Hungarian sweet sorghum variety ‘Monori Édes’ is promising feedstock for bioethanol production with a high overall ethanol yield.

ACKNOWLEDGEMENT

The authors gratefully acknowledge to OTKA TS 049849 and NKFP3-00006/2005 for their financial support. Novozymes (Denmark) is kindly thanked for providing enzymes.

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