AgroLife Scientific Journal - Volume 5, Number 2, 2016
ISSN 2285-5718; ISSN CD-ROM 2285-5726; ISSN ONLINE 2286-0126; ISSN-L 2285-5718

MILD ALKALINE PRETREATMENT APPLIED IN THE BIOREFINERY OF SORGHUM BIOMASS FOR ETHANOL AND BIOGAS PRODUCTION

Adrian TRULEA, Teodor VINTILĂ, Nicolae POPA, Georgeta POP

University of Agricultural Sciences and Veterinary Medicine of Banat "Regele Mihai I al României", 119 Calea Aradului, Timişoara, Romania

Corresponding author email: e-mail: tvintila@animalsci-tm.ro

Abstract

Production of bioenergy from lignocellulosic biomass has gained more and more interest over the past years. Due to its high content in carbohydrates, Sorghum bicolor is one of the best suited candidate for bioenergy production. However, the lignocellulosic nature of the sorghum biomass raises difficulties to the access of the microbial enzymes to cellulose and hemicellulose and inhibits processes such as hydrolysis, fermentation and anaerobic digestion of biomass to produce biofuels. In this study, the effects of thermo - chemical pretreatment (mild alkaline pretreatment), applied to improve biodegradability of three different hybrids of sorghum biomass were investigated. Alkaline pretreatment have positive effects on the production of lignocellulosic ethanol, increasing both cellulose and hemicellulose content while breaking the lignocellulosic bonds and reducing lignin content. The achieved methane yields ranged from 320 to 345 l_n CH_4/kg VS for the pretreated biomass, approximately 22% higher than the yields obtained from the untreated biomass. Important increase of methane production has been noticed as well in the case of anaerobic digestion of spent bagasse resulted after ethanol fermentation. Mild alkaline pretreatment is a suitable pretreatment method for conversion of sorghum biomass to ethanol and biogas.

***Key words**: sorghum, lignocellulose, pretreatment, ethanol, methane.*

INTRODUCTION

The dwindling supply and high prices of fossil fuels, energy independence, together with the adverse effects they have on the environment, greenhouse gas emissions and climate change, have pushed the global community towards the development on new, renewable energy sources (Cesaro, 2015; Guo, 2015).

Biomass, as a renewable energy source, is an important substitute for fossil fuels. One of its great advantages is its versatility. Biomass can be used to produce liquid (bioethanol), solid (wood pellets) and gaseous (biogas) biofuels.

Current technologies employed to produce biofuels impede their penetration on the large scale market. To guarantee the economic competitiveness of biofuels, besides constant improvement in conversion technologies, a biorefinery system seems to be an attractive solution (Leitner, 2016).

Different types of raw material are currently being used in the biofuel industry and investigated for their energy potential. Sweet sorghum (*Sorghum bicolor* (L.) Moench) is considered to be one of the most important plants for energy production. Sweet sorghum has a high content of soluble and fermentable sugars, high yields, is drought and flood tolerant and has the ability to grow in a wide range of environmental conditions (Larnaudie, 2016; Maw, 2016).

The aim of this study was to determine the influence and effects of mild alkaline pretreatment on sorghum biomass, when used as feedstock for bioethanol and biogas production. In order to maximize the energy output of the raw material processed, three different conversion pathways were examined:

1. Untreated biomass to biogas via anaerobic digestion.
2. Pretreated biomass to biogas via anaerobic digestion.
3. Pretreated biomass to ethanol via alcoholic fermentation followed by anaerobic digestion of the spent fermentation mass resulted after the alcoholic fermentation.

MATERIALS AND METHODS

Biomass from three different sorghum hybrids, namely *Sorghum bicolor x sudaneze* cv. Jumbo, originally from Australia, *Sorghum bicolor x sudaneze* Sugargraze II, originally from the United States of America and *Sorghum bicolor* var. *saccharatum* cv. F135ST, originally from

Romania, was used as biological raw material. The biomass was air - dried after harvest and then milled to 2 cm theoretical length using a Retsch SM100 mill with 2 cm mesh.

After milling, the biomass was subjected to mild alkaline & steam pretreatment process. The biomass was moistened with 2% NaOH solution (1:6 w/v) and autoclaved for 30 minutes at 121°C (Silverstein, 2007; Zhao, 2008; Chen, 2009). After pretreatment, the biomass was washed with tap water and neutralized with 2% H_2SO_4 solution until pH 5-5.5 ± 0.2. The next step of the process consisted of enzymatic hydrolysis of pretreated biomass catalyzed by the enzymatic complex NS22192, provided by Novozymes®. Pretreated biomass was immersed in citrate buffer pH 5 in concentration of 5% w/v dry matter and autoclaved for 30 minutes at 121°C in order to assure aseptic conditions. Before autoclaving, 2% w/v peptone, 1% w/v yeast extract and 0.5% Tween 80 (Chen 2009), were introduced in the hydrolysis media. All these components are necessary in the fermentation step of the process. The enzymatic complex NS22192 was added in the hydrolysis media in concentration of 15 IU per 1 gram of cellulose, according to the manufacturer's indication. The flasks where incubated at 50°C for 24 hours under constant shaking (150 rpm).

After 24 hours of hydrolysis, the temperature of the incubator was lowered to 30°C and fresh inoculums of *Saccharomyces cerevisiae* CIMT2.21 was aseptically added. The inoculums was obtained from the Collection of Industrial Microorganisms of the USAMVB, Timişoara. The incubation of the flasks continued at 35°C for 24 hours. To each flask two NIR sensors where attached (BlueSens, Germany): one detecting the concentration of ethanol and one sensor detecting the concentration of CO_2. Anaerobic digestion of the biomass was carried out according to the VDI 4630 guidelines (VDI, 2006) in a batch test. The inoculum used was a combination of four digestates collected from different agriculture biogas plants. Methane concentration in the produced biogas was analysed using a Dräger X-AM 7000 gas analyser. During the experiments dry matter, organic dry mater, cellulose, hemicellulose and acid insoluble lignin content where monitored. Analysis were performed according to NREL standard laboratory procedures (L.A.P., Sluiter, 2005; Sluiter, 2008).

RESULTS AND DISCUSSIONS

The structural characteristics of the untreated and pretreated sorghum biomass are summarized in Table 1. Our data show that in all three cases, mild alkaline/steam pretreatment has a positive effect on the biomass, breaking the lignocellulosic complex, thus liberating cellulose and hemicellulose. Higher concentration in both cellulose and hemicellulose content has been recorded in pretreated biomass. The concentration of cellulose in the pretreated biomass increased from 16.3% to 22.4% and the concentration of hemicellulose increased from 4.3% to 6.2%, depending of the sorghum variety.

The next step in the conversion pathway of sorghum to biofuels is the hydrolysis of lignocelluloses and alcoholic fermentation of the obtained sugars. As shown in figure 1, the fermentation process followed the classic path. In the first hours of incubation the active yeast immediately started the fermentation, after a very short lag phase, producing in three hours the main quantity of ethanol. After six hours the ethanol concentration reached the maximal values, varying only in small margins.

Calculating the ethanol yields, the following yields where obtained in the three sorghum hybrids: 0.33 g/g Jumbo biomass, 0.34 g/g F135ST biomass and 0.38 g/g Sugargraze II biomass, (all reported to dry matter).

Table 1. Structural characterization of sorghum biomass (%)

		Dry matter	Ash	Organic dry matter	Cellulose	Hemicellulose			Lignin (AIL)
						Total	Xylose, galactose and mannose	Arabinose	
Jumbo	untreated	90.67	9.08	90.92	28.76	21.49	19.58	1.91	17.11
	pretreated	18.73	2.09	97.91	51.23	27.72	26.04	1.68	10.33
Sugargraze II	untreated	91.5	8.37	91.63	32.58	22.31	20.54	1.77	18.58
	pretreated	21.42	2.64	97.73	54.38	27.92	25.99	1.93	8.54
F135ST	untreated	90.86	9.69	90.31	32.46	22.92	21.21	1.71	16.95
	pretreated	18.58	5.1	94.9	48.78	27.24	24.63	2.61	10.24

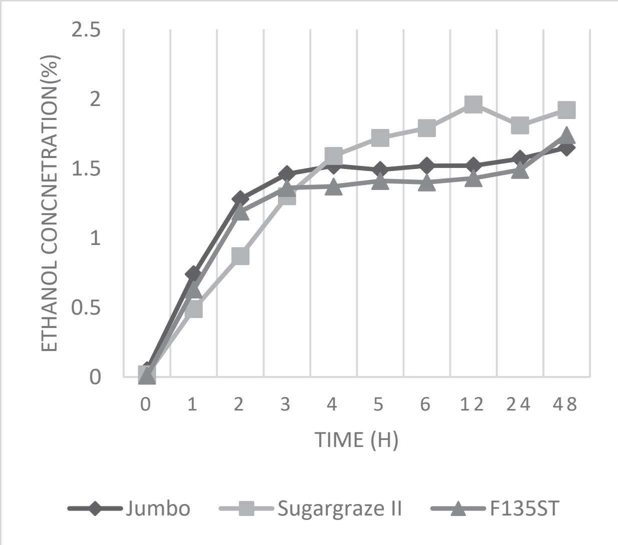

Figure 1. Evolution of ethanol concentration during fermentation

The final step of the process and the cornerstone of this study was the anaerobic digestion of sorghum biomass in order to observe the effects of mild alkaline/steam pretreatment on the production of biogas.

In figure 2 the quantities of biogas and methane produced during the anaerobic digestion for each sorghum variety as well as the differences between the three conversion pathways are displayed.

The obtained results show important differences in biogas and methane yields obtained in the case of anaerobic digestion of untreated and pretreated biomass. Regarding the methane yield, the pretreatment increases productions with an average of 61 l_N/kg organic dry matter, which is translated as 21% increase of methane yields.

In the batches performing anaerobic digestion of the spent fermentation mass resulted after alcoholic fermentation of the pretreated sorghum biomass higher yields were obtained. In all three sorghum varieties, the methane yields were on an average 49% higher than in the case of the untreated samples and 22% higher than in the case of the of the pretreated samples.

Although parts of the carbohydrates present in the biomass were degraded and converted into ethanol during the fermentation process, the highest yields of biogas and methane were recorded in the samples containing spent fermented mass. This atypical result can have two reasons at its foundation. First of all, during the alcoholic fermentation of the biomass, yeasts convert the soluble sugar faction into ethanol and other by-products, such as proteins and other substances, that are used as a substrate by the microorganisms employed in the anaerobic digestion process and converted into biogas.

Secondly, the alcoholic fermentation process can be considered as an additional biological pretreatment step that loosens the bonds in the lignocellulosic structure and thus liberating an extra amount of carbohydrates that can be converted in the anaerobic digestion step.

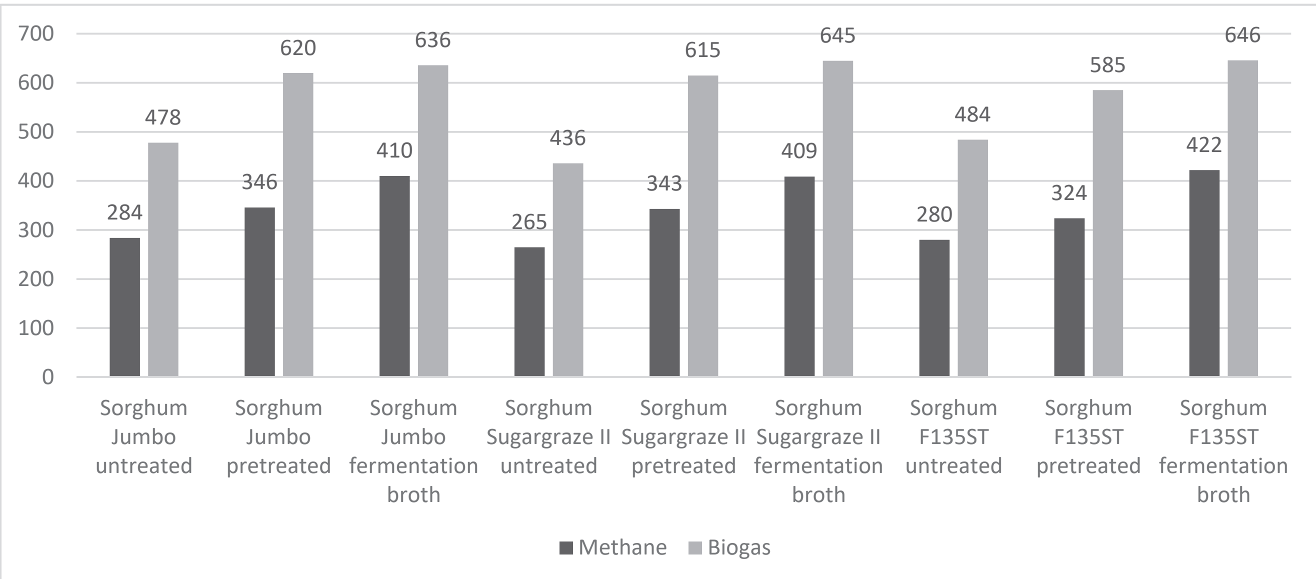

Figure 2. Biogas and methane production during anaerobic digestion

CONCLUSIONS

Mild alkaline/steam pretreatment is a suitable pretreatment method for converting sorghum biomass to ethanol and biogas. The combination of sodium hydroxide solution and steam pretreatment opens up the lignocellulosic bonds, liberating a substantial quantity of cellulose and hemicellulose.

Between the three different conversion pathways studied in this work: (1) Anaerobic digestion of untreated biomass; (2) Anaerobic digestion of pretreated biomass; (3) Anaerobic digestion of bagasse resulted after alcoholic fermentation of pretreated biomass; the highest yields were obtained in the third conversion pathway, by connecting ethanol fermentation to anaerobic digestion. This means that an integrated, cascading, conversion pathway is the most suitable in terms of energy yields from sorghum biomass.

REFERENCES

Chen M., Zhao J., Xia L., 2009. Comparison of four different chemical pretreatments of corn stover for enhancing enzymatic digestibility. Biomass and Bioenergy, 33(10), p. 1381-1385.

Cesaro A., Belgiorno V., 2015. Combined biogas and bioethanol production opportunities for industrial application. Energies, 8, p. 8128-8144.

Guo M., Song W., Buhain J., 2015. Bioenergy and biofuels: History, status and perspective. Renewable and Sustainable Energy Reviews, 42, p. 712-725.

Lanaudie V., Rochon E., Ferrari M.D., Lareo C., 2016. Energy evaluation of fuel bioethanol production from sweet sorghum using very high gravity (VHG) conditions. Renewable Energy, 88, p. 280-287.

Leitner V., Lindorfer J., 2016. Evaluation of technology structure based on energy yield from wheat straw for combined bioethanol and biomethane facility. Renewable Energy, 87, p. 193-202.

Maw M.J.W., Houx J.H., Fritschi F.B., 2016. Sweet sorghum ethanol yield component response to nitrogen fertilization. Industrial Crops and Products, 84, p. 43-49.

Silverstein R.A., Chen Y., Sharma-Shivappa R.R., Boyette M.D., Osborne J.A., 2007. Comparison of chemical pretreatment methods for improving saccharification of cotton stalks. Bioresource Technology, 98(16), p. 3000-3011.

Sluiter A., Hames B., Hyman D., Payne C., Ruiz R., Scarlata C. et al., 2008. Determination of Total Solids in Biomass and Total Dissolved Solids in Liquid Process Samples - Laboratory Analytical Procedure (LAP). National Renewable Energy Laboratory.

Sluiter A., Ruiz R., Scarlata C., Sluiter J., Templeton D., 2005. Determination of Ash in Biomass - Laboratory Analytical Procedure (LAP). National Renewable Energy Laboratory.

Sluiter A., Hames B., Ruiz R., Scarlata C., Sluiter J., Templeton D. et al., 2008. Determination of Structural Carbohydrates and Lignin in Biomass - Laboratory Analytical Procedures (LAP). National Renewable Energy Laboratory.

Zhao X., Zhang L., Liu D., 2008. Comparative study on chemical pretreatment methods for improving enzymatic digestibility of crofton weed stem. Bioresource Technology, 99(9), p. 3729-3736.

***VDI, 2006. Fermentation of organic materials. Characterization of the substrate, sampling, collection of material data, fermentation tests. VDI - Gesselschaft Energie und Umwelt.