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EFFECTS OF ULTRASONIC DISINTEGRATION, HOT-COMPRESSED LIQUID WATER PRE-TREATMENT, AND STEAM EXPLOSION ON SOLVOLYSIS AND DIGESTIBILITY OF GRAIN SORGHUM STOVER

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One of the most promising renewable energy crops and biomass feedstock for biogas production in Europe is the C4 plant grain sorghum due to its high photosynthetic efficiency. The release of lignocellulosic material and therefore the acceleration of degradation processes of sorghum stalks and leaves can be achieved using mechanical and thermal pre-treatments, which assist to hydrolyse the cell walls and speed the solvolysis of biopolymers. This study is focused on hot-compressed liquid water, steam explosion and ultrasonic pre-treatments of grain sorghum stover. The effectiveness of pre-treatments was evaluated by means of soluble chemical oxygen demand, biochemical oxygen demand, and by the biogas and methane productivities. The results show that the pre-treatments disintegrated the lignocellulosic structure, increased and accelerated the biogas and methane production, and increased the mesophilic anaerobic digestion potential of grain sorghum stover. Our laboratory tests demonstrated that the steam exploded grain sorghum stover possess the highest biogas productivity.

Keywords: grain sorghum stover; hot-compressed liquid water pre-treatment; steam explosion; ultrasonic disintegration, biogas digestion.

Introduction

The grain sorghum is a possible carbohydrate resource for the simultaneous production of bioethanol and biogas due to its advantages over the traditional agricultural crops. The plants have remarkably good CO_2 absorption ability. Grain sorghum is the fifth most important cereal crop grown in the world. The drought tolerance of sorghum is greater than that of corn. Furthermore, it is able to regenerate after a period of drought. Its productivity is also higher than that of corn, even in dry periods or in lower quality soils. In Hungary, sorghum grows in arid areas as a substitution for fodder corn. Therefore sorghum could be used to cultivate alternative feedstock for bioethanol production [1] and could be a sustainable crop for energy production in Europe. The sorghum grain contains approximately the same amount of starch compared to corn kernels, which makes it attractive as raw material for the production of bioethanol. The agricultural by-product of sorghum-based bioethanol production, the sorghum stover is a valuable bioenergy substrate for biogas digesters, despite the complex lignocellulosic structures of sorghum stalks and leaves and its resistance to decomposition. The utility and the economic feasibility in biogas power plants can be highly increased by different pre-treatment methods, to assist the disruption of the cell wall structure and to speed-up the solubilisation of the biopolymers in the biogas feedstock [2–4]. The purpose of pre-treatments is to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase porosity [5,6] *(Fig.1)*.

Liquid hot-compressed water (LHCW) pre-treatment is a commonly used hydrothermal method. High temperature and high pressure are applied to maintain liquid phase and to avoid vaporization. During the pre-treatment, water is penetrated into the cell structure of the biomass, hydrating cellulose, solubilizing hemicellulose, and slightly removing lignin. LHCW pre-treatment is highly effective for enlarging the accessible and susceptible surface area of cellulose and improving cellulose degradability for microbes and their enzymes [7,8]. After the hydrothermal treatment, the biogas production was increased for pig manure, for municipal sewage sludge and for fruit/vegetable waste

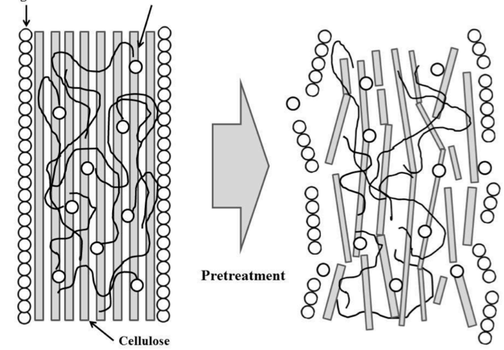

Figure 1: The disintegration of lignocellulosic material by pre-treatments

[9]. The hot water treatment increases the enzymatic hydrolysis and digestion of corn leaf, stalk and fibre [10,11] and the dissolution of solids and xylan of destarched corn fibre [12]. The liquid hot water pre-treatment of sorghum bagasse produced higher solubilisation of hemicellulose and cellulose content enrichment [13]. Combining hydrothermal treatment and enzymatic hydrolysis of sorghum bagasse resulted in improvement of cellulose and total polysaccharides hydrolysis [14].

Steam explosion (SE) is one of the most effective methods for the pre-treatment of lignocellulosic biomass. In this process, the biomass is heated with high-pressure steam for a certain period, than the pressure is reduced to atmospheric pressure as quickly as possible. The biomass is undergone on an explosive decompression by this swift reduction of pressure [6,7]. The high efficiency of the steam explosion treatment is due to the thermo-mechano-chemical destruction applied in the method. Steam-explosion has been used to treat various kinds of lignocellulosic biomass (i.e. softwood, sugarcane bagasse, corn stalk) for enhanced bioethanol and biogas production [15–19]. SIPOS *et al.* [20] demonstrated the efficient effect of pre-treatment by steam on sorghum bagasse characterized by high degree of cellulose hydrolysis. ZHANG *et al.* [21] compared four pre-treatment processes including liquids, steam explosion, lime and dilute acid for enzymatic hydrolysis of sorghum bagasse and pointed out that steam-explosion showed the greatest improvement on enzymatic hydrolysis.

The biogas yields of anaerobic digestion can be also increased by ultrasonic treatments (UT) to support the solubilisation of raw materials. During the ultrasonic treatment, a cyclic sound pressure is used to disintegrate the cell walls by cavitation. The parameters of the sonication are the power, frequency and time of sonication [3,22]. ZHANG *et al.* [23] showed that ultrasonic pre-treatments caused indistinctive effects on bio-hydrogen production. The ultrasonic treatment accelerated the enzymatic hydrolysis of corn stover and sugar cane bagasse cellulose [24] and improved the liquefaction and saccharification of sorghum flour [25]. IMAM *et al.* [26] pointed out the positive effects of ultrasonic plus hot water pre-treatment on the conversion of sweet sorghum to hexose and pentose sugars and on lignin, cellulose and hemicellulose concentrations.

However, no systematic studies on pre-treatments of grain sorghum stover are available in literature. A correct comparison of the various pre-treatments and the selection of the suitable processes for the disintegration of grain sorghum stover as feedstock for enhanced biogas production are the goals and the novelty of this paper. However, the main message of this work is that a perfect testing is only possible, if there are no differences in the microbial communities in different biogas fermenters, in the inocula used, in the substrates cultivated and in the methods applied to assess pre-treatments: chemical analysis, batch tests or continuous AD.

Therefore this work is focused on liquid hot-compressed water, steam explosion and ultrasonic pre-treatments of grain sorghum stover using both fast analytical, biochemical and biomethane potential AD tests to study and comparison the effects of pre-treatments on anaerobic digestion without addition of chemicals. The influence of temperature, contact time of thermal treatments, and the effect of sonication energy on decomposition of lignocellulose on a chemical level were determined using laboratory scale experimental techniques. The effectiveness of thermal and ultrasonic treatments was evaluated by SCOD and BOD_5 concentrations and by the biogas and methane productivities during mesophilic fermentations.

Experimental

Materials

Sorghum stover investigated in this work consists of leaves and stalks of commercial grain sorghum (Milo) hybrid named *Mexican Sweet* (code: HF1) harvested from the B12 field at Kétegyháza, Hungary, and used for pre-treatments and biogas production experiments. This easily grown hybrid with mid-early maturation was developed by J. FECZÁK (Agroszemek Ltd., Szeged, Hungary). Post-harvest residue grain sorghum stover samples were chopped, homogenized, and analysed for dry substance (DS, 93.6%), organic dry substance (94.5%), and ash (5.5%). Deionized water was used in all experiments after cation and anion exchange.

Liquid Hot-Compressed Water Treatments

The LHCW pre-treatments were carried out in a 2 L capacity Parr 4843 type high-pressure reactor at the temperatures of 100, 150 and 175 °C (0.1, 0.5 and 0.9 MPa, respectively) and at various treating time from 10 to 120 minutes. 25 g of grain sorghum stover were used in each experiment and were added to 1000 g of water. The suspension was introduced in the thermal reactor at room temperature and heated to the set temperature. The pulp slurry was mixed by a built-in stirrer at 300 rpm during the entire treating process to avoid temperature gradients. After treatment, the reactor was cooled down to 30 °C by the built-in cooling system of the reactor using cold water-flow and the treated slurry was removed from the vessel.

Steam Explosion Pre-treatments

SE treatments were performed in a steam explosion laboratory unit *(Fig.2)* consisted of a steam generator (2), a digester vessel (6) and a separator cyclone (8). A 2 L capacity PARR 4843 reactor was used for the production of steam with temperatures of 185, 200, and 215 °C and working pressures of 1.1 MPa, 1.6 MPa, and 2.1 MPa, respectively. The SE unit operated in batches and equipped with a digester vessel (6) of 200 cm^3 volume. The digester vessel was filled with 10 g

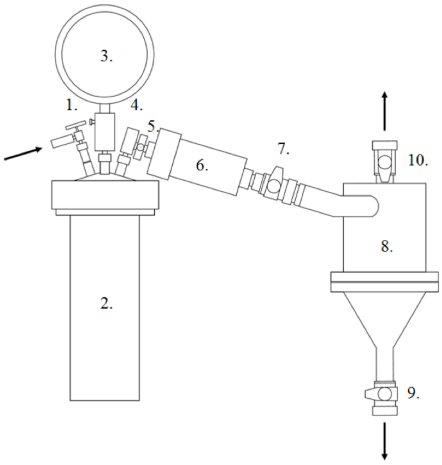

Figure 2: Scheme of the steam explosion apparatus (1. input valve, 2. steam generator, Parr 4843 reactor, 3. pressure gauge, 4. evacuation valve, 5. steam inlet valve, 6. digester vessel, 7. expansion valve, 8. cyclone, 9. sampling valve, 10. steam outlet valve)

feedstock per batch and was heated to the desired temperature, directly with saturated steam led to the vessel, by opening the steam inlet valve (5). No catalysts (additional chemicals) were applied in the process. After the steaming time (1–10 minutes), the expansion valve (7) was opened to rapidly reduce the vessel pressure to the atmospheric one. The steamed sample slurry explosively released into the separator cyclone (8).

Ultrasonic treatments

The ultrasonication was performed in a Vibracell VCX 750 (Sonics & Materials, USA) ultrasonic processor operated at constant sonication frequency of 20 kHz with maximum power input of 750 W. The radiation was carried out by a 25.4 mm diameter titan alloy (Ti-6Al-4V) probe with removable tip and maximum amplitude of 35 μm. Samples of the mixture of grain sorghum stover (1 g) and water (99 g) placed in a temperature-controlled vessel. They were subjected to ultrasonic pre-treatment without additional agitation at different power inputs by adjustment of the amplitude (50, 70 and 100%) and at various sonication durations of from 1 to 10 minutes for each power level. The temperature of the vessel was held constant at 25 °C during the treatment by a high-precision thermo-regulator (Huber Kältemaschinenbau GmbH, Germany).

Aliquot parts of the resulting sorghum stover pulp slurries of samples obtained from pre-treatments were used as substrate in AD test. The rests were filtered to separate the solid fraction from the liquid fraction for analytical measurements (SCOD, BOD_5, pH). A set of untreated, blank samples was also tested for chemical analysis and methane potential by AD as the control points of reference for the treated samples.

Mesophilic Anaerobic Digestions

The anaerobic degradability of untreated, LHCW, SE, and UT pre-treated grain sorghum stover slurries was determined in laboratory scale, using a fermenter system contained 12 Pyrex batch reactors of 1 L capacities. Inoculum for the AD tests was taken from the effluent line of an anaerobic pilot fermenter [27] treating a mixture of swine manure and corn stillage (spent mash remaining after bioethanol distillation) at biogas test facility of DENK Ltd (Kövegyűrpuszta, Hungary). The batch reactors were inoculated by 700 g inocula. Afterwards a batch of 100 g of grain sorghum stover pulp slurry samples was added under nitrogen atmosphere. Furthermore, the digester materials were bubbled with nitrogen gas for 10 minutes to get rid of the air from the liquid phase before each experiment. The batch reactors were kept in a temperature-controlled water bath at 37 °C until they stopped producing biogas. The mesophilic AD tests performed for grain sorghum stover substrates including controls were all done in duplicate. During the 36–40 days mesophilic AD tests the flows of the produced biogas were on-line measured by digital flow meters and the daily and cumulated biogas volumes were stored digitally. The methane content was determined by gas chromatography.

Analytical and Biochemical Measurements

The SCOD, pH, and BOD_5 measurements were carried out on the liquid fractions after 10 min centrifugation at 14,000 rpm and 20 °C with a refrigerated universal high-speed, filtration (0.45 μm) centrifuge (UniCen MR, Herolab, Germany). Colorimetric SCOD concentration was measured by the Standard Methods procedure [28] using a Nanocolor Vario Compact heating block (MACHEREY-NAGEL, Germany) and a MultiDirect photometer (Tintometer/Lovibond, Germany) according to DIN ISO 15705. Biochemical oxygen demand (BOD_5) was determined according to EN 1899-1 and EN 1899-2 by using OxiTop Control 6 OC 100 (WTW, Germany) system. The pH values were measured by a C831 type pH meter (Consort, Belgium) with an RA-0903P sensor (Radelkis, Hungary) after two-point pH calibration (pH = 4.01 ± 0.01 and pH = 7.00 ± 0.01) using DuraCal pH buffers (Hamilton, Switzerland). The surfaces of the grain sorghum stalk were investigated by a PHILIPS XL30ESEM environmental scanning electron microscope (ESEM) with 20 kV accelerating voltage and resolution of 3.5 nm, in secondary electron image mode (SEI), and backscattered electron image mode (BEI).

Results and Discussion

Liquid Hot-Compressed Water Treatments

The influence of LHCW pre-treatments at various temperatures and treating times on SCOD concentration of grain sorghum stover slurry is visualized by 3D mesh segmentation on *Fig.3*.

The results of LHCW pre-treatments show that hot-compressed water has a positive effect in grain sorghum stover disintegration, leading to significant increments in SCOD concentration: +262% at 100 °C, +419% at

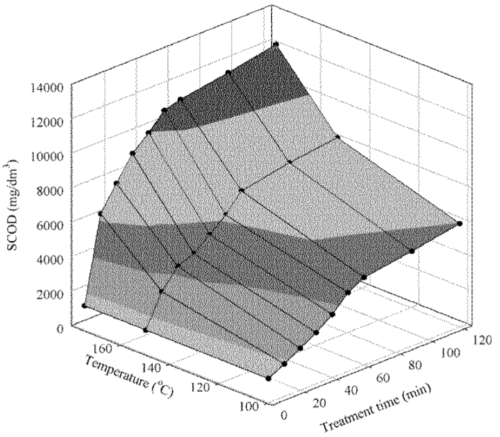

Figure 3: Effects of temperatures and contact times of LHCW pre-treatments on SCOD concentration of grain sorghum stover suspension (●: experimental data points)

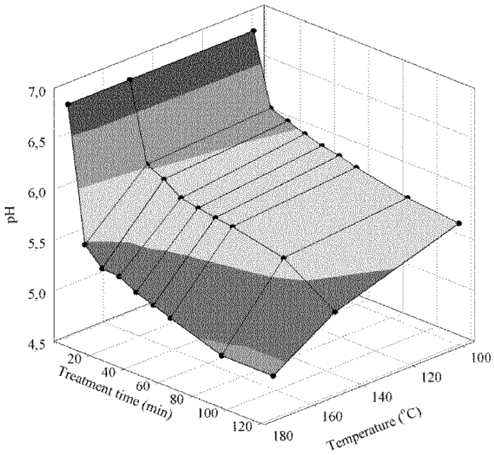

Figure 4: Acidity of grain sorghum stover slurries as the function of LHCW contact time and temperature (●: experimental data point)

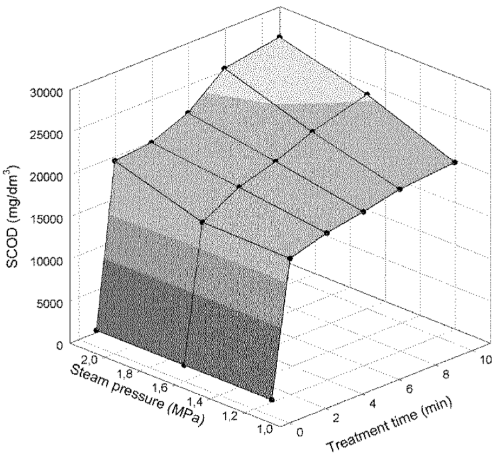

Figure 5: Effects of steam pressures on SCOD concentration levels of grain sorghum stover slurries as the function of the contact time during steam explosion (●: experimental data points)

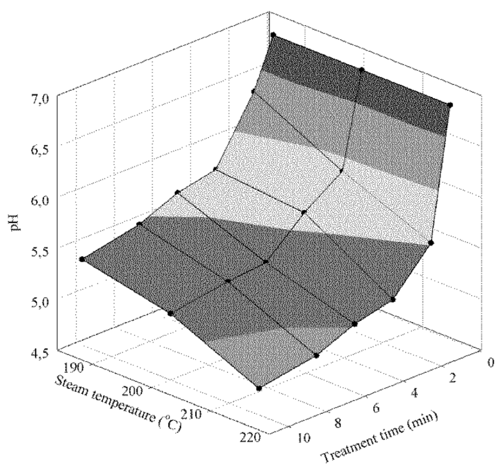

Figure 6: The effect of steam explosion on pH values of treated grain sorghum stover slurry at various steam pressures as the function of the treatment time (●: experimental data points)

150 °C and + 698% at 175 °C with an operating time of 60 min, respectively. The control experiments were performed using untreated grain sorghum stover suspension. Both temperature and time have positive, but not linear effects on SCOD and not to the same degree. The temperature raising has more effect on the solubilisation of grain sorghum stover after 60 minutes than the treating time in agreement with the results of VALO *et al.* [29] who found that treating time had less influence than temperature during thermal treatment of sludge. It is notable that higher solvolysis temperatures resulted in more acidic products as reflected by the low pH level of the grain sorghum stover slurry *(Fig.4)*.

Steam Explosion Pre-Treatments

The pressures and temperatures of SE pre-treatments had significant effect on digestibility of grain sorghum stover. Elevated pressures and temperatures resulted in enhanced solubilisation *(Fig.5)*. The positive pressure effect may be explained by the increased disintegration caused by higher-pressure drop at the end of treatment. Compounds that are more soluble were released from the grain sorghum stover during the steam explosion and made the resulting slurry available to subsequent bacterial degradation.

The pressure effect is quasi-linear and the effect of contact time can be modelled by an exponential-to-maximum function. Compared to the untreated grain sorghum stover, the SCOD concentrations of the slurries increased by a factor of 8.9, 9.6, 10 at 185, 200, and 215 °C, respectively, with an operating time of 3 min, respectively. Additional, the pH value decreased with pre-treatment pressure (steam temperature) and with the contact time *(Fig.6)*. The formation of acidic degradation products during disintegration of grain sorghum stover slurry by steam explosion agree well with the observation of GUO *et al.* [30] at corn stalk steam explosion.

Ultrasonic Treatments

The efficiency of ultrasonic irradiation of grain sorghum stover was quantified by SCOD concentration of the slurry as a function of contact time and power input in terms of ultrasonic amplitude level (%) and specific sonication energy. Results of ultrasonic treatments are shown in *Fig.7*, where the values of the SCOD

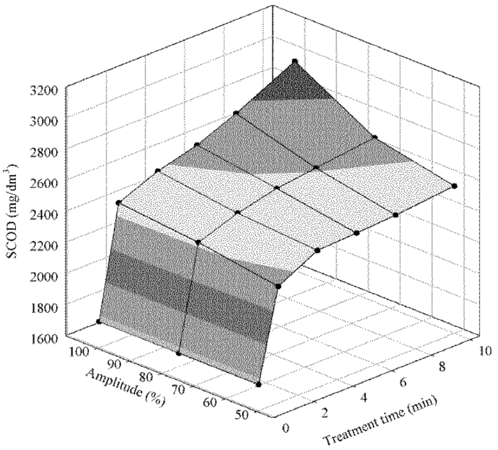

Figure 7: The effect of sonication energy on SCOD concentration level of grain sorghum stover slurry as the function of sonication time and relative ultrasound amplitude (●: experimental data points)

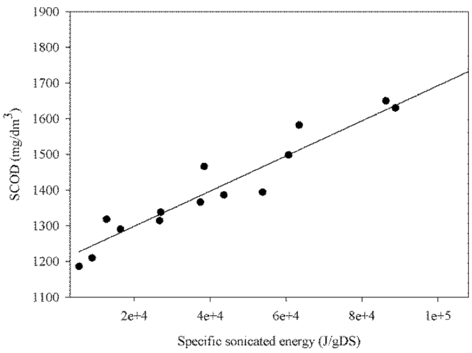

Figure 8: SCOD change in grain sorghum stover slurry as a function of specific sonication energy (●: experimental data points)

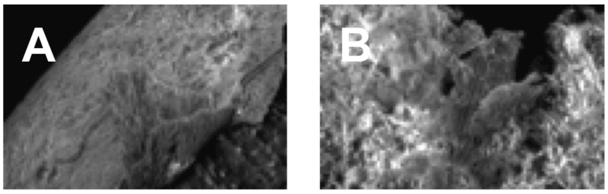

Figure 10: ESEM pictures of the surface on untreated (A) and by ultrasonic treated (B) grain sorghum stalks at 100× magnification

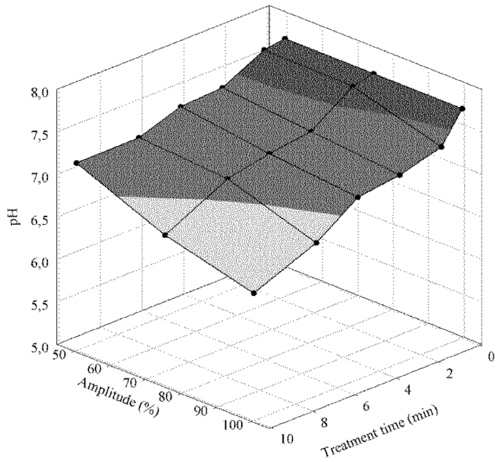

Figure 9: The effect of sonication power on the pH values of grain sorghum stover slurries as the function of the sonication time (●: experimental data points)

concentrations are presented for each value of the ultrasonic amplitude level (%), and sonication time. The SCOD of the pulps increased with the ultrasonic power demonstrating the effectiveness of the cavitation energy to disrupt the cell walls of the plant parts. The SCOD concentration excess reached +105%, +109%, and +133% at 50%, 70%, and 100% amplitude level, respectively, with a contact time of 3 min.

The ultrasonic power and the pre-treatment time affect the SCOD to a different degree. The raising of SCOD values by treating time is stabilized after 3 minutes. These can be described by an exponential-to-maximum function. The effect of energy-input on SCOD is quasi-linear (R^2 = 0.942) as demonstrated by the data shown in *Fig.8*, where SCOD values are plotted against the specific sonication energy. The SCOD concentrations were increased by the sonication energy; however, no power-saturation phenomenon was observed. The sonication energy over the minimal energy of 1kJ g^{-1} DS is the most effective for breaking up cells [31].

The pH values of the sonicated grain sorghum stover slurries decreased monotonously with pre-treatment time and sonication power on a small scale *(Fig.9)* suggesting that local temperature effects caused by cavitation bubbles were not enough to accelerate the hydrolytic processes resulted in acidic degradation product.

Environmental Scanning Electron Microscopy

The ESEM pictures of the surfaces on untreated and by ultrasonic treated grain sorghum stalks are shown in *Fig.10* at 100× magnification. The surface of the treated sorghum stalk was altered *(Fig.10B)* from the appearance of defective plant tissues. *Fig.10B* shows a heterogeneous appearance compared to the homogeneous surface of the untreated stalk *(Fig.10A)*.

The comparison of the pre-treatment results of the chemical analysis, the SCOD concentration, and pH changes of grain sorghum stover slurries suggested that the most effective disintegration method is the steam explosion to disrupt the lignocellulosic structure of grain sorghum stover and partial hydrolysis of it molecular components. However, results of these fast methods can only indicate how much structures of lignocellulosic materials broke down on a chemical level. Nevertheless, greater decomposition of the lignocellulose structures does not necessarily prove enhanced biogas production, because inhibitors for methanogenesis reactions can also be produced through hydrolytic processes during steam explosion. Furthermore, one has to take into account the high energy needs of treatments at elevated temperatures. According to the results of fast chemical analyses (SCOD and pH), the optimal conditions for the LHCW, steam explosion and ultrasonic treatment of grain sorghum stover were established as 150 °C / 30 min, 200 °C / 3 min and 100% ultrasound amplitude / 3 min (at 25 °C), respectively. However, to study the real effect of optimal conditions of the investigated pre-

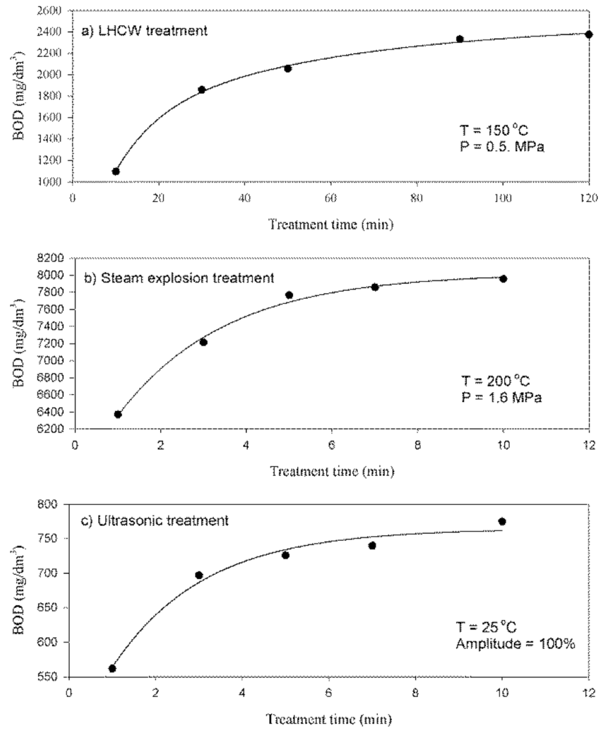

Figure 11: Comparison of the BOD_5 test of filtrates of grain sorghum stover slurries after various pre-treatment methods

treatments additional biochemical tests like BOD and biomethane potential determinations are necessary.

Biochemical Oxygen Demand

A comparison of the biochemical oxygen demands of filtrates of grain sorghum stover slurries after pre-treatments at the optimal conditions is given in *Fig11*. The results of the BOD_5 tests support the observations from chemical analysis. Actually, all three methods investigated are appropriate for pre-treatment of grain sorghum stover, because the BOD_5 values systematically increased in filtrates of grain sorghum stover slurries with the treatment time under the conditions investigated. However, the highest BOD_5 values were obtained in filtrates after steam explosion, therefore the steam explosion seems to be the most effective disintegration method to disrupt the lignocellulosic structure of grain sorghum stover and to partially hydrolyse its molecular species, resulting in the formation of bacterially digestible substrate.

Biogas Production by Mesophilic Anaerobic Digestions

Biogas and biomethane production tests were carried out using grain sorghum stover slurries pre-treated under stated optimal conditions (LHCW: 150 °C / 30 min, steam explosion: 200 °C / 3 min, and ultrasonic treatment: 100% ultrasound amplitude / 3 min at 25 °C). Daily as well as the final biogas and methane production (normalized volumes at 0 °C and 101.15 kPa) were determined. The results are shown as a function of the digestion time in *Figs.12* and *13*. The specific gas productions were calculated by dividing the

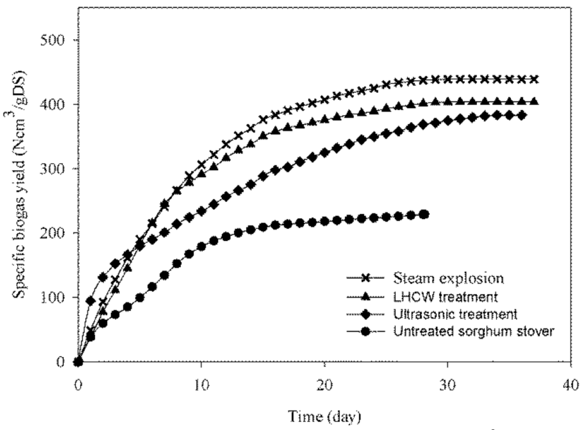

Figure 12: Specific cumulative biogas yield (Ncm^3/g DS) of grain sorghum stover substrates after pre-treatments

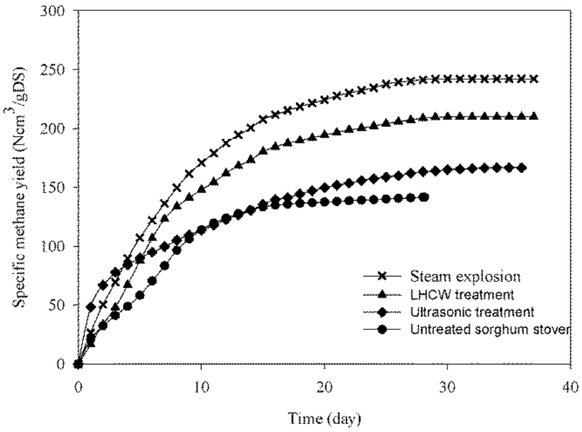

Figure 13: Specific cumulative methane yield (Ncm^3 g^{-1} DS) of grain sorghum stover substrates after pre-treatments

cumulated gas amount with the total DS of the substrates. As reference, the untreated material was used in the same amount. In agreement with the results of chemical analytical and biochemical tests after pre-treatments, the specific total biogas and methane yields obtained for treated grain sorghum stover substrates were significant higher than that of the untreated control.

As seen in *Fig.12*, biogas productions began immediately, without a lag period after starting the degradation of grain sorghum stover. The fastest biogas production at the beginning was detected at the ultrasonicated substrate. However, the highest biogas production was observed during the fermentation of steam-exploded substrate. The biogas production proceeded in constant and high daily biogas production for treated substrates until the 30^{th} day of hydraulic retention time. This observation is notable, because the application of pre-treatments can decrease the usual cycle time for biogas fermentation and increase the production of biogas with higher methane content. The comparison of the biomethane yields is given in *Fig.13*. Methane production, as the major result of anaerobic digestion of pre-treated grain sorghum stover, was markedly increased by the three pre-treatments. The highest biomethane production rate was observed at the substrates after ultrasonic and steam explosion pre-treatments. The biomethane production rate of LHCW treated substrate was lower, probably due to the low pH of the pre-treated grain sorghum stover slurry.

Our laboratory tests have shown that the steam exploded substrate possess has the highest biogas production potential (439 Ncm^3 g^{-1} DS) during hydraulic retention times of 36 days with 55% methane content. The steam explosion pre-treatment increased the biomethane yield by +72% to 242 Ncm^3 g^{-1} DS. The LHCW pre-treated substrate has the second highest biogas production potential (403 Ncm^3 g^{-1} DS) during hydraulic retention times of 36 days with 52% methane content, the LHCW pre-treatment increased the biomethane yield by 49% to 210 Ncm^3 g^{-1} DS. This yield increment is higher than that was obtained by QIAO *et al.* [9] for various biomass wastes after hydrothermal treatment. The substrate after ultrasonic pre-treatment has a biogas production potential of 383 Ncm^3 g^{-1} DS during hydraulic retention times of 36 days with 44% methane content, the ultrasonic pre-treatment increased the biomethane yield by 18% to 167 Ncm^3 g^{-1} DS. The low biogas and methane yields of ultrasonic treated grain sorghum stover can be explained by the superficial effect of ultrasonic cavitation.

Conclusions

The results of chemical, biochemical analyses and biogas/biomethane potential test obtained by mesophilic anaerobic digestion were presented. It was found that LHCW treatment and steam explosion are the most effective biomass pre-treatment for increasing the solubilisation of the organic matters of grain sorghum stover. Laboratory testing of anaerobic digestibility of pre-treated slurries have shown that the grain sorghum stover has high biogas potential. It can serve as biogas feedstock and could reduce the energy consumption of sorghum-based bioethanol production in the future. The LHCW and steam explosion biomass pre-treatment techniques can reduce the cycle time and improve the biogas production via disintegration of the complex and compact plant structures of grain sorghum stover.

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