An Investigation of the Enhancement of Biogas Yields from Lignocellulosic Material using Two Pretreatment Methods: Microwave Irradiation and Steam Explosion

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Two pretreatment methods, microwave irradiation and steam explosion, were investigated in this work. The aim of the study was to investigate whether these methods would improve the biodegradability of wheat straw as a lignocellulosic feedstock. Microwave pretreatment was carried out on milled straw with an irradiation time of 15 minutes, at a temperature of either 200 or 300 °C in the oven. The steam explosion pretreatment was carried out on milled straw at 210 °C for 10 minutes. To determine the methane production potential, anaerobic digestion batch trials were run under mesophilic conditions for 60 days. The methane yields of the microwave-pretreated straw decreased by 65% for an attained temperature of 200 °C and by 92% for 300 °C. After steam explosion pretreatment, however, the methane yields of the straw increased by approximately 20% when compared to untreated straw samples. These results indicate that microwaving does not optimize methane production from wheat straw, while steam explosion yields positive results.

Keywords: Microwave; Steam explosion; Wheat straw; Batch trials; Methane yield

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INTRODUCTION

Straw is a major agricultural bio-product. It contains lignocellulosic materials, primarily cellulose, hemicellulose, and lignin. However, due to the recalcitrant nature of lignocelluloses, hydrolysis is the rate-limiting step in the production of biogas from lignocellulosic feedstock. Therefore, the lignocellulosic biomass must be pretreated to improve the efficiency of hydrolysis (Zhu *et al.* 2010). Currently, several different pretreatment methods (including biochemical, enzymatic, mechanical, and thermal treatments) are being applied to agricultural residues (Estevez *et al.* 2012; Horn *et al.* 2011; Jackowiak *et al.* 2011).

Microwave irradiation, which is a thermal pretreatment process, is a promising method to produce syngas, chars, and pyrolytic liquids, such as tar and pyroligneous acids (Robinson *et al.* 2010). However, few studies have been devoted to the application of microwave energy specifically (under atmospheric conditions) to pretreat biomass (Jackowiak *et al.* 2011; Shahriari *et al.* 2011). Therefore, a clarification of the ability of microwave irradiation to pretreat lignocellulosic feedstock may significantly contribute to this field.

Steam explosion, a current pretreatment method used on straw, involves heating the biomass to a high temperature, combined with a rapid pressure drop that physically disrupts the lignocellulosic structure of the fibers. Opening up the recalcitrant fiber structures has been shown to improve their biodegradability (Estevez *et al.* 2012).

The objective of this study was to compare the effects of microwave pretreatment and steam explosion pretreatment on the digestibility of wheat straw (WS) under mesophilic conditions by determining the biochemical methane potential in the pretreated straw. The biochemical methane potential was obtained by employing anaerobic batch experiments (Jackowiak *et al.* 2011; Zhu *et al.* 2010).

EXPERIMENTAL

Biomass Collection

Wheat straw (WS) was collected from the Norwegian University of Life Sciences (UMB) farm in Aas, Norway. During the harvesting process, which took place in the fall of 2010, the straw was baled while dry, and the bales were wrapped in plastic film in the field. The bales were not opened until the start of the experiment.

Pretreatment Processes

Drying and milling processes

To minimize any temperature differences and remove undesired moisture, the straw was stored at room temperature for two weeks before the start of the experiment. To obtain a small particle size, the air-dried WS was milled (2000 rpm, 3 min) with a Retsch Knife Mill (GRINDOMIX GM 300, Germany) and is referred to as untreated WS in this work.

After milling, the untreated WS was manually mixed in a large plastic container for approximately 10 min to obtain uniformity in the feeding material. The homogenized test sample was then stored at room temperature for future use. Table 1 indicates its proximate and physico-chemical properties.

Table 1. Properties of Wheat Straw

	Constituent	
Proximate analysis (wt[a]%) ± MU[b]	Moisture	6.0 ± 10
	Volatile matter	74.1 ± 5
	Ash	3.3 ± 10
	Fixed Carbon	16.5 ± 7
Gross calorific value (MJ/TM[c] kg)		17.10
Ultimate analysis (TM%) ± MU	C	48.4 ± 5
	H	5.7 ± 10
	N	0.6 ± 10
	O	41.6
Physico-chemical analysis	pH	7.4 ± 0.0
	Conductivity (µS/cm)	982.5 ± 3.54
	Salinity	0.5 ± 0.0
	Total dissolved solid (mg/l) ± SD[d]	1110 ± 2.8

[a] weight presence of mass, [b] measurement uncertainty (%), [c] total matter, [d] standard deviation (n=3)

Microwave pretreatment process

Microwave treatments were performed in a laboratory-scale CEM Microwave Max asphalt oven (15 Amps, 50 Hz, and 220-240 V). The microwave's original thermocouple sensor was used to determine the temperature inside the microwave oven after a calibration check using a separate sensor (TENMA 72-7712 CE dual input digital thermometer with type K thermocouple probes). Therefore, the temperatures given in this work indicate the nearby temperatures of the microwave pretreatment reactor, as has been previously performed by Huang *et al.* (2008). Microwave pretreatment of WS (100 g) was performed at 200 °C and 300 °C. The average heating rate was 5 °C min^{-1}. The pretreated samples were stored in 1 L plastic zip bags at room temperature before the experiment.

Steam explosion process

The steam explosion of the straw was conducted at 210 °C for 10 min, as described in detail by Horn *et al.* (2011). The process was performed at the UMB Department of Chemistry, Biotechnology and Food Science. The pretreated samples were stored in 5 L plastic vacuum bags at 4 °C before the start of the treatment.

Anaerobic Digestion Process

Inocula

These microwave and steam explosion experiments were carried out as two separate experiments at different times. Therefore, two types of inocula were used. To experiment with microwave-pretreated WS, an inoculum from the biogas plant at Tomb's Agricultural College in Norway was used. To experiment with steam-exploded WS, the inoculum used was from the Nordre Follo Wastewater Treatment Plant in Aas, Norway. The pH value and the volatile solids of the inoculum from the biogas plant were 7.4 ± 0.1, and 3.9% (w/w), while those from the wastewater plant were 7.5 ± 0.1, and 1.5% (w/w), respectively.

Both inocula were incubated at 37 ± 0.5 °C under anaerobic conditions for two weeks prior to their inoculation to acclimate the microbes and reduce their endogenous biogas production.

Batch experiments

Thirty-six identical 1125 mL laboratory-scale anaerobic batch reactors were run in triplicate to investigate the methane yield of pretreated straw using the same organic load, retention time, and incubation conditions.

Following pre-incubation, vials were filled with diluted inoculum (700 mL, approximately 6 g volatile solid (VS)/L) and the pretreated straw substrate (approximately 4 g VS/L). While filling the vials, the inoculum tanks were continuously stirred using a magnetic rod to prevent sedimentation. After the addition of the substrates, the vials were closed with rubber stoppers and aluminum crimps. In addition, diluted inocula without any substrate were run as blanks. The diluted inocula were combined with pure cellulose ($(C_6H_{10}O_5)n$, < 20 µm, Merck Chemical Corp.) as a control, checking and comparing the performance of both inocula. Finally, the vials were centrifuged and incubated (90 rpm, 37 ± 0.5 °C), and biogas production was observed by measuring the developing pressure. Excessive pressure was released by inserting a needle into the rubber stoppers.

Analytical Methods

To determine the particulate size of the milled WS, the sample was screened using Endecott's test sieves mesh series (England).

The pH values of the untreated and pretreated WS were determined using a WTW Multi 350i multimeter after the samples were diluted with ultra-pure water (1:5), using the protocol for solid analyses described by Blakemore *et al.* (1987).

The contents of both dry matter and VS were analyzed according to standard methods (APHA 1995).

Analyses of the ultimate, proximate, and calorific values, as well as a determination of the carbohydrate content of the straw samples, were performed at a commercial laboratory (Eurofins Norsk Miljøanalyse AS, Norway). The proximate and calorific values and the quantity of neutral detergent fibers (NDF) were determined using the standard methods of the European Committee for Standardization (CEN), and the results are shown in Table 1. The amount of acid detergent lignins (ADL) and acid detergent fibers (ADF) were determined using the standard method of the Association of Official Analytical Chemists (AOAC 2006). The amount of hemicelluloses in each sample was calculated as NDF minus ADF. An elemental analysis was performed using both the CEN standards and the American Society for Testing and Materials standards (ASTM 2008).

The volume of the produced biogas in each anaerobic batch reactor was determined by measuring the amount of pressure in the vials with a barometer (GMH 3161 Greisinger Electronic, Germany), as previously reported by Eskicioglu *et al.* (2009).

The methane concentration in the headspace was determined using an SRI gas chromatograph (Model 8610 C) equipped with a thermal conductivity detector (TCD) and a 3' × 1/8" SS molecular sieve 13X stainless steel column. The operational temperatures of the injector, detector, and column were kept at 41, 153, and 81 °C, respectively. Helium was used as a carrier gas at a flow rate of 20 mL/min. A gas mixture of CH_4/CO_2 (65/35% volume) was used as the standard for calibration. The gas chromatograms were analyzed using the PeakSimple 3.67 program.

After determining the methane concentration in the headspace, the methane yield was calculated using the method reported by Estevez *et al.* (2012), based on the European Chemical Industry Ecology and Toxicology Center (ECETOC) procedure (Stringer 1998). The differences in methane production between the substrate vials and the blank vials were considered the methane yields of the investigated samples.

Statistical Analyses

A one-way ANOVA was performed using the Minitab®16 statistical software package. An alpha (α) level of 0.05 was used to determine the statistical significance of all analyses. The mean results and the 95% confidence intervals of the means were assessed to determine the statistical significance between the groups.

The biogas volume and biogas quality studies in the anaerobic batch systems were performed in triplicate. All of the standard deviations reported in this study were calculated using the statistical functions in Microsoft Excel® 2007 spreadsheets.

Biomass samples with larger particle sizes have been shown to display higher levels of cellulose, hemicelluloses, and lignin and a lower ash level than those with smaller particle sizes (Ross and Mazza 2011). In this study, the effect of particle size was controlled so that it would not influence the compositional analyses.

RESULTS AND DISCUSSION

Particle Sizes

After the milling process, percentages of straw in the particle size distributions of >5.66 mm, 5.66 - 1.190 mm, 1.190 - 0.590 mm, 0.590 - 0.297 mm, 0.97 - 0.25 mm, and <0.25 mm were found to be 1%, 33.3%, 30.5%, 16.2%, 3.1%, and 15.9%, respectively.

Effects of Two Pretreatment Processes on Wheat Straw Characteristics

The physico-chemical parameters of untreated WS and pretreated WS were measured, and the results were compared to understand the effects of treatment on pH, VS, moisture, C/N, and the straw's lignocellulosic contents (Table 2).

The contents of celluloses and hemicelluloses in the untreated WS were similar to those reported in the literature (Ross and Mazza 2011; Deniz *et al.* 2004). Although the lignin content of the untreated WS was lower than that in most other reports (Ross and Mazza 2011; Deniz *et al.* 2004), Jackowiak *et al.* (2011) and Antongiovanni and Sargentini (1991) found similar lignin levels.

It was observed by Menardo *et al.* (2012) that when the temperature in either the microwave oven or the steam explosion unit was increased to approximately 200 °C, the pH of the pretreated straw decreased. A low pH in the pretreated WS is most likely due to the solubilization of the acidic hemicellulose components as well as the oxidation of some hemicellulose and lignin fragments into carboxylic acids (Ahring *et al.* 1996). However, when the temperature in the microwave oven was increased from 200 °C to 300 °C, the pH of the pretreated straw increased to a level similar to that of the untreated WS.

The C/N ratio of the samples was investigated because of its importance to the biogas potential (Estevez *et al.* 2012). To calculate the ratio, elemental analyzes were conducted on the pretreated WS. These results demonstrated that when the temperature in the microwave oven rose from 200 °C to 300 °C, the percentage of C in the microwave-pretreated WS increased, as was reported by Wang *et al.* (2009). Similarly, the N content for microwave-pretreated WS was higher than that for untreated WS. These contents in the pretreated WS were probably increased due to loss of water. In fact, the calculated C/N ratio for microwave-pretreated WS at 200 °C was higher than that observed at 300 °C. A likely reason for this result is that the WS had more volatile C than volatile N. Although the C/N ratio in both microwave-pretreated WS samples was determined to be more than 80 (Table 2), the mixture ratio of microwave-pretreated WS with inocula in the reactors were found around 35. Estevez *et al.* (2012) reported that the optimum C/N ratio of pretreated lignocellulosic feedstocks with co-digestion for a biogas reactor is between 35 and 40.

In other assessments of how pretreatment affects WS properties, levels of both moisture and VS in the pretreated-WS were determined and compared to untreated WS (Table 2). While the moisture content in the microwave-pretreated WS was lower than that in untreated WS, the moisture content in the steam-exploded WS was higher because the water vapor was added during the steam explosion process. The VS content in the microwave-pretreated WS at 200 °C increased due to hemicellulose degradation; the untreated WS had 7.6 g more of hemicellulose per 100 g (Table 2). The VS content in the steam-exploded samples increased as well, most likely due to the addition of water vapor and/or the degradation of hemicelluloses (Horn *et al.* 2011; Ramos 2003). However, the

VS content in the microwave-pretreated WS at 300 °C was the same as in the untreated WS.

Table 2. Characteristics of the Pretreated Biomasses Used in Biogas Batch Experiments

	Cellulose (g/100 g)	Hemicelluloses (g/100 g)	Lignin (g/100 g)	C/N ratio	pH	Moisture (%w)	Volatile solids (%TS)
Untreated WS	45	24.2	9.0	80.6	8.4	6.0	90
Microwave-pretreated WS at 200 °C for 15 min	46.9	16.6	19.1	91.2	6	1.4	92
Microwave-pretreated WS at 300 °C for 15 min	19.4	0.6	65.6	83.1	8	1.2	90
Steam-exploded WS at 210 °C for 10 min	n.a.	n.a.	n.a.	n.a.	5	61	91

n.a.: not available, TS: total solid

The microwave-pretreated WS and the steam-exploded WS were darker in color than the untreated WS samples. Others have observed a similar phenomenon in ligno-cellulosic biomasses (Ramos 2003; Lanzetta and Di Blasi 1998).

Effects of Two Pretreatment Processes on Methane Production from Wheat Straw

Figure 1 shows the accumulated production of methane after approximately 60 days. As explained above, two different inocula were used in the experiments. The first inoculum originated from a biogas plant and was composed of cattle manure and food waste; it was used for the anaerobic digestion of microwave-pretreated WS (Fig. 1A). The second inoculum originated from a wastewater treatment plant and was used for the digestion of steam-exploded WS (Fig. 1B). It was found that the two inocula yielded similar amounts of methane from celluloses (Fig. 1A and 1B) ($p > 0.05$). However, the amount of methane produced from the untreated WS differed because the inoculum from the biogas plant was more adapted to the substrate.

The methane yield from untreated WS was higher than that of the microwave-pretreated WS samples. Meanwhile, the yield of the low temperature-pretreated WS (200 °C) was significantly higher ($p < 0.05$) than that of the high temperature-pretreated WS (300 °C). The VS content in the microwave-pretreated WS at 200 °C increased due to hemicellulose degradation. The degradation in the microwave-pretreated WS at 300 °C was more extensive than the microwave-pretreated WS at 200° C (Table 2). However, the VS content in the microwave-pretreated WS at 300 °C was lower than the microwave-pretreated WS at 200 °C. This VS loss in the microwave-pretreated WS at 300 °C could be due to the high temperature. Additionally, the loss between 200 and 300 °C may cause a low methane yield. Jackowiak *et al.* (2011) reported similar results on the yield of methane from pretreated WS, based on microwave heating at 150 and 180 °C. They reported that hemicelluloses and some lignins are solubilized at ≥ 160 °C during thermal pretreatment. The resulting compounds are usually similar to phenolic compounds (Hendriks and Zeeman 2009), which could have an inhibitory or toxic effect on bacteria, yeast, and methanogens/archaea (Jackowiak *et al.* 2011). It has been shown that these compounds inhibit biodegradation enzymes such as cellulase, xylanase, and glucosidase (Taherzadeh and Karimi 2008). Therefore, the low methane yield from the microwave pretreatment processes at 200 and 300 °C could be due to the presence of inhibiting compounds. In addition, microwave-pretreated WS could be resistant to microbial degradation, as has been reported for other torrefied biomasses (Medic *et al.* 2012).

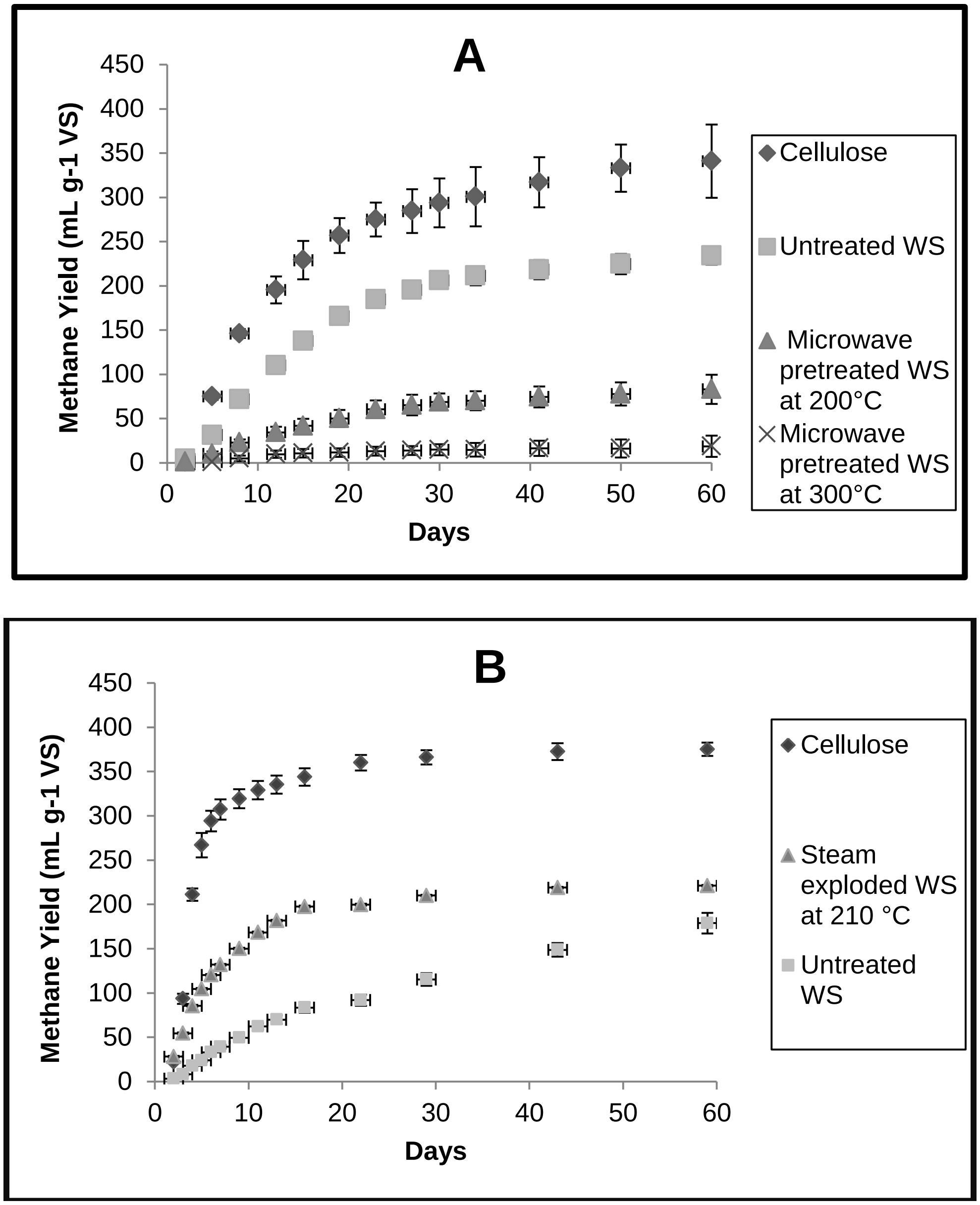

Fig. 1. The profiles of the accumulated production of methane from untreated WS and WS pretreated with microwave irradiation at 200 and 300 °C for 15 min (A) and from untreated WS and WS that was steam exploded at 210 °C for 10 min (B).

These results showed that the methane production of steam-exploded WS was higher (approximately 20%) than that of the untreated straw samples. Similarly, it has been reported that the methane yield from steam-exploded *Salix* at 210 °C for 10 min increased up to 50% compared to untreated *Salix* (Estevez *et al.* 2012). Cui *et al.* (2012) investigated changes in carbohydrate content of steam-exploded WS. While percentages of cellulose, hemicellulose, and lignin in dry weight of untreated WS were determined to be 36.14%, 23.16%, and 17.74%, respectively, steam-exploded WS at 220 °C for 6 min was found to give the following corresponding values: 55.23%, 9.65%, and 21.67%. These results indicated that the cellulose content of the WS increased 19.09%, and hemicellulose content of the WS decreased 13.51% after the steam explosion pretreatment process. In addition, a cellulose fraction of steam-exploded biomass has been shown to be more susceptible to the enzymatic attack of anaerobic bacteria (Ramos 2003). Steam explosion is an important pretreatment method for the biodegradation of lignin in WS (Zhang *et al.* 2008). In this study, steam explosion as pretreatment provided striking benefits for the production of methane from WS, though the application of a thermal microwave process did not.

CONCLUSIONS

1. An analysis of the elemental content of WS indicated that the composition changed during the microwave process. The C/N ratio was higher in microwave-pretreated straw at 200 °C than at 300 °C.
2. When the temperature was increased to approximately 200 °C, the pH values of the WS pretreated with both the microwave and steam explosion processes decreased. However, when the temperature following microwave treatment was increased to 300 °C, the pH increased to nearly the pH value of the untreated WS.
3. It was observed that the two different inocula (taken from either the biogas plant at an agricultural collage or from a municipal wastewater treatment plant) had quite similar methane yields from the hydrolysis of cellulose (358 ± 17 ml gVS^{-1}), but had yields that were quite different when applied to untreated WS (234 ± 10 ml gVS^{-1} and 179 ± 12 ml gVS^{-1}, respectively).
4. The methane yield of WS after 60 days of microwave pretreatment decreased at both 200 °C and 300 °C. The subsequent performance of anaerobic batch digestion from WS was not improved by increasing the temperature of the microwave pretreatment. However, after steam-explosion pretreatment, the methane yield of WS increased by approximately 20% compared to untreated WS samples. Therefore, in this study, it can be concluded that steam explosion is the more efficient pretreatment process to improve methane production from wheat straw (compared with microwave pretreatment) at both 200 and 300 °C.

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