Sodium hydroxide pretreatment of ensiled sorghum forage and wheat straw to increase methane production
C. Sambusiti, E. Ficara, M. Rollini, M. Manzoni and F. Malpei

ABSTRACT

The aim of this study was to determine the effect of sodium hydroxide pretreatment on the chemical composition and the methane production of ensiled sorghum forage and wheat straw. NaOH pretreatment was conducted in closed bottles, at 40 °C for 24 h. Samples were soaked in a NaOH solution at different dosages (expressed in terms of total solids (TS) content) of 1 and 10% gNaOH/gTS, with a TS concentration of 160 gTS/L. At the highest NaOH dosage the reduction of cellulose, hemicelluloses and lignin was 31, 66 and 44%, and 13, 45 and 3% for sorghum and wheat straw, respectively. The concentration of soluble chemical oxygen demand (CODs) in the liquid phase after the pretreatment was also improved both for wheat straw and sorghum (up to 24 and 33%, respectively). Total sugars content increased up to five times at 10% gNaOH/gTS with respect to control samples, suggesting that NaOH pretreatment improves the hydrolysis of cellulose and hemicelluloses. The Biochemical Methane Potential (BMP) tests showed that the NaOH pretreatment favoured the anaerobic degradability of both substrates. At 1 and 10% NaOH dosages, the methane production increased from 14 to 31% for ensiled sorghum forage and from 17 to 47% for wheat straw. The first order kinetic constant increased up to 65% for sorghum and up to 163% for wheat straw.

Key words | alkaline pretreatment, anaerobic digestion, lignocellulosic, methane, sorghum forage, wheat straw

INTRODUCTION

Anaerobic digestion is considered to be a sustainable way to combine renewable energy generation with sustainable waste treatment. In Italy, sorghum and wheat straw represent two types of substrates for anaerobic digestion in agricultural biogas plants. Sorghum is an energy crop and therefore represents an interesting substrate for methane production. Wheat straw, as an agricultural by-product, could be an alternative to energy crops in anaerobic digestion plants. Nevertheless, as lignocellulosic substrates, the methane production of sorghum and wheat straw depends mainly on their complex structure, that limits their biodegradability. The structure of lignocellulosic materials is mainly composed of cellulose, hemicelluloses and lignin, strongly linked to each other. Cellulose and hemicelluloses (holocelluloses), which are the major components of most lignocellulosic materials, are easily degraded by anaerobic microorganisms and can be converted into biomethane. Nevertheless, lignin limits their accessibility to hydrolytic enzymes, preventing their degradation (Tong et al. 1990). However, the physical structure and composition of lignocellulosic materials can be altered through various methods of pretreatment, breaking down the linkage between polysaccharides and lignin to make cellulose and hemicelluloses more accessible to hydrolytic enzymes (Hendriks & Zeeman 2009). Pretreatments include mechanical, chemical, thermal, biological processes or a combination of them. Chemical pretreatments are classified into acidic, alkaline, oxidative, organosolv, and ionic liquids pretreatments. Among them, alkaline pretreatments (NaOH, KOH, lime, ammonia, and urea) are efficient in altering the structure of lignin, in solubilising hemicelluloses, and in increasing efficiently the accessibility of cellulose by swelling and a partial decrystallisation of cellulose (Cheng et al. 2010; McIntosh & Vancov 2010; Sills & Gossett 2011). Sodium hydroxide pretreatment has been studied for many years and it has been shown to disrupt the lignin structure of the biomass thus increasing the enzymatic accessibility to cellulose and hemicelluloses. Common substrates used for alkaline tests are straws, grasses, bagasses and corn...
stalks. Recently, Zhu et al. (2010) showed the effectiveness of sodium hydroxide pre-treatment to increase biogas production from corn stover by 37% compared with that of untreated substrate. Zhao et al. (2008) showed the effectiveness of sodium hydroxide pre-treatment for hardwoods, wheat straw, switch grass, and softwoods with less than 26% lignin content. Finally, Monlau et al. (2011) studied the effects of NaOH pre-treatments on sunflower stalks at a concentration of 4% gNaOH/gTS, a contact time of 24 h, and solid to liquid ratio of 50 gTS/L. The pretreatment temperatures tested were 30, 55, and 80 °C. The highest methane production (259 ± 6 mLCH4/gVS compared with 192 mLCH4/gVS for untreated substrate) was reached at 55 °C.

The aim of this study was to determine the effect of sodium hydroxide pretreatment on the chemical composition and the anaerobic biodegradability of an energy crop (ensiled sorghum forage) and an agricultural by-product (wheat straw).

METHODS

Substrates

Ensiled sorghum forage (Sorghum sudanense hybrid) and wheat straw (Aibusson), were collected from a farm near Cremona (Lombardy Region, Italy). After collection, samples were oven dried at 60 °C for two days to a moisture content of less than 10%, and ground into particles with a mean diameter of 1 mm by a kitchen blender, and finally stored at 4 °C in air-tight containers prior to use.

Analytical determinations

Total solids (TS) and volatile solids (VS) were determined according to the APHA Standard Methods (APHA 2005). The chemical oxygen demand (COD) of untreated substrates was determined according to the open reflux method (APHA 2005). Soluble chemical oxygen demand (CODs) in the liquid fraction after pretreatment, was determined after 45 μm filtration with a commercial photochemical test kit (Hach Lange GmbH, Dusseldorf, Germany).

The neutral detergent fibre (NDFom), the acid detergent fibre (ADFom), and the lignin (Lignin (sa)) were determined according to the Van Soest method (Van Soest & Wine 1967; Udén et al. 2005) with an ANKOM A220 system (ANKOM Technology). This is based on sequential extraction with neutral and acid detergent, followed by a strong acid extraction. Different fractions are: (a) soluble fraction in neutral detergent (1-NDFom); (b) hemicelluloses (NDFom-ADFom); (c) cellulose (ADFom-Lignin (sa)); and (d) Lignin (sa). Fats and proteins were determined with a NIR-system (5000 monochromator, Foss). Reducing sugars were determined by employing the Somogyi method (Somogyi 1952), while the total sugars content was determined through the phenol sulphate method (Dubois et al. 1956). All analytical determinations were performed in duplicate.

Alkaline pretreatment

The sodium hydroxide pretreatment was conducted in closed bottles. In each bottle, the sample was soaked in a solution at different NaOH dosages (1 and 10% gNaOH/gTS), with a solid to liquid ratio of 160 gTS/L. The soaked samples were kept in closed bottles at 4 °C for 24 h, without stirring. The pretreatment temperature (40 °C) and contact time (24 h) were chosen according to the best pretreatment results of our previous studies obtained on ensiled sorghum forage (data not shown), and according to literature suggestions on agricultural substrates (Zhu et al. 2010; Monlau et al. 2011). Control samples, soaked in tap water without NaOH addition, were included. After pretreatment, samples were filtered through a sieve of 0.20 mm of pore size. The sieve-separated solid and the liquid fractions were taken for compositional analyses.

Biochemical methane potential tests

Biochemical Methane Production (BMP) tests were performed in duplicate, by using a commercial laboratory instrument (AMTPS, Bioprocess control, Sweden). This is a volumetric device consisting of 15 gas-tight glass bottles (500 mL of working volume) placed in a water bath at 35 ± 0.5 °C. Each bottle was continuously mixed with a rotary stirrer. The biogas produced passes through a NaOH solution (3M), for CO2 absorption. Methane flows through a liquid-displacement automated measuring unit with a resolution of 11–13 mL. A data acquisition system allows flow-rate data to be recorded continuously. The inoculum used for BMP tests was obtained by mixing two digested sludge samples: (1) collected from a digester fed on waste activated sludge, with a solid content of 20 ± 4 gTS/L, 12 ± 2 gVS/L; (2) collected from a digester fed on agro-wastes (cattle manure and corn silage), with a solid content of 55 ± 3 gTS/L, 37 ± 2 gVS/L. The mixture was made of 50% each on a VS basis, with a final VS content of 15 gVS/L. Digested sludge samples were also characterised in terms
of maximum specific methane activity (SMA). The SMA of each sludge was determined in duplicate using the AMTPS system, as well. The sludge (diluted to 5 gVS/L in each bottle) was supplemented with 1 gCOD/L of sodium acetate. The SMA, expressed as mLCH4/gVS/d, was calculated as the ratio between the average methane production rate (mLCH4/d) and the amount of the VS of the sludge in each bottle (gVS). The corresponding SMA was 55 mLCH4/gVS/d and 19 mLCH4/gVS/d for the two sludges, respectively.

Before the BMP test, the inoculum was kept under endogenous anaerobic conditions at 35 °C for about 7 days to reduce non-specific biogas generation. An amount of 2.5 gVS of samples (raw and pretreated before sieve-separation) were then mixed with 2.5 gVS of inoculum (corresponding to a volume of 166 mL), obtaining a substrate/inoculum ratio around 1 gVS/gTS, as suggested by Raposo et al. (2011) and Chandra et al. (2012). Finally, to reach 500 mL of working volume, 50 mL of mineral medium of macronutrients (as suggested by OECD 311 2006) and deionised water were also added to each bottle. A blank sample was performed by mixing the inoculum, the mineral medium, and the deionised water, without the addition of substrate. The samples pretreated with 10% gNaOH/gTS had a final pH of 12 and 13 for pretreated sorghum and wheat straw, respectively. So these samples were neutralised to pH 7 with a concentrated HCl solution prior to BMP tests. Sorghum and wheat straw, pretreated with 1% gNaOH/gTS, had an initial pH ranging between 9 and 11. At the end of 24 h of pretreatment at 40 °C, the pH fell to 7. Therefore, in this case, no pH adjustment was necessary. The BMP test duration was 31 days.

The BMP production was calculated as follows:

\[
\text{BMP}(\text{LCH}_4/\text{gVS}) = (V_{\text{CH}_4,s} - V_{\text{CH}_4,\text{blank}})/\text{VS_s}
\]

where: \(V_{\text{CH}_4,s} - V_{\text{CH}_4,\text{blank}}\) (LCH4) is the net volume (at standard temperature and pressure, STP) of methane production measured at the end of the test; VSs (gVS) is the mass of VS of the added substrate. All gaseous volumes hereafter reported are referred to at STP conditions.

A kinetic study was also performed on BMP test results. The anaerobic degradation process was assumed to follow a first order kinetic as it is the case of slowly degradable lignocellulosic substrates for which the disintegration and hydrolysis are the limiting steps (Angelidaki et al. 2009). To quantify the kinetic advantage of the pretreatment on the anaerobic methane production, the first order kinetic constant was calculated by using the least-squares fit of methane production data during time \(t\) in the following equation:

\[
\text{BMP}_t = \text{BMP}_{t \to \infty} \cdot (1 - \exp(-k_h \cdot t))
\]

where: \(\text{BMP}_t(mLCH}_4/gVS)\) is the cumulative methane yield produced during at the time \(t(d)\), \(\text{BMP}_{t \to \infty}(mLCH}_4/gVS)\) is the ultimate methane yield of the substrate, \(k_h\) (d\(^{-1}\)) is the first order kinetic constant and \(t(d)\) is the digestion time.

### RESULTS AND DISCUSSION

#### Chemical composition of substrates

The chemical compositions of ensiled sorghum forage and wheat straw are summarised in Table 1. Both samples have an average COD/VS value of almost 1.2, which is close to the typical value for carbohydrates; this is in agreement with their chemical composition. A similar content of cellulose and hemicelluloses was observed both for ensiled sorghum forage and wheat straw. Sorghum had a higher protein and fat content and a lower lignin content than wheat straw.

#### Effect of pre-treatment on fibrous composition

The compositions of untreated and pretreated substrates were determined in terms of %VS (referring to the untreated substrate) and are shown in Figure 1. SOLU is the soluble fraction (1-NDFom), CEL is the cellulose fraction and H-CEL is the hemicelluloses fraction. The sum of CEL, H-CEL and Lignin (sa) represents 100% of the NDFom fraction.

Hemicelluloses removal of 45 and 66% was observed after a NaOH pretreatment at 10% NaOH dosage for...

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ensiled sorghum forage</th>
<th>Wheat straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (% wet weight)</td>
<td>95 ± 4</td>
<td>94 ± 4</td>
</tr>
<tr>
<td>VS (%TS)</td>
<td>86.6 ± 0.4</td>
<td>92.7 ± 0.4</td>
</tr>
<tr>
<td>COD/VS</td>
<td>1.21</td>
<td>1.15</td>
</tr>
<tr>
<td>Protein (%VS)</td>
<td>9 ± 3</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>Fat (%VS)</td>
<td>1.8 ± 0.3</td>
<td>0.9 ± 0.8</td>
</tr>
<tr>
<td>Cellulose (%VS)</td>
<td>49 ± 1</td>
<td>49 ± 1</td>
</tr>
<tr>
<td>Hemicelluloses (%VS)</td>
<td>35 ± 2</td>
<td>34 ± 2</td>
</tr>
<tr>
<td>Lignin (%VS)</td>
<td>4.1 ± 0.0</td>
<td>6.5 ± 0.0</td>
</tr>
</tbody>
</table>

Table 1 | Composition of ensiled sorghum forage and wheat straw
ensiled sorghum forage and wheat straw, respectively. Cellulose removal was observed for both substrates after the pretreatment at 1 and 10% NaOH dosages. Nevertheless, at the highest alkaline dosage, the cellulose removal was higher for sorghum than for wheat straw (up to 31 and 13%, respectively). An effect on lignin removal was observed after an alkaline pretreatment at 10% NaOH dosage (up to 3 and 44% for wheat straw and sorghum, respectively).

**Total and reducing sugars released**

The amount of total and reducing sugars released from ensiled sorghum forage and wheat straw are shown in Figure 2. The sugars release is presumably due to the swelling and hydrolysis of hemicelluloses and cellulose, as reported by Carrillo et al. (2005). Comparing the total sugars content of the control (0% gNaOH/gTS) and pretreated substrates, alkaline pretreatment at 10% NaOH dosage was found to increase the total sugar level up to five times for both substrates. The reducing sugars content, that represents the fraction promptly available for microbial fermentation, did not significantly increase with sodium hydroxide dosage, due to the different monomeric sugars, reducing or not, present in hemicelluloses and cellulose structures. Moreover, the amount of total sugars released, comprising also polymeric carbohydrates needing hydrolysis before fermentation, was found to be slightly higher for sorghum than for wheat straw.

**Effect of pre-treatment on COD solubilisation**

The 24 h soaking resulted in the release of CODs (Table 2). According to these results, very limited differences were observed in the COD released under neutral (0% gNaOH/gTS) and mildly alkaline conditions (1% gNaOH/gTS) and mildly alkaline conditions (1% gNaOH/gTS). In contrast, a significantly higher solubilisation was observed for samples soaked at 10% gNaOH/gTS.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>NaOH dosage (g/100 gTS)</th>
<th>0</th>
<th>1</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorghum</td>
<td></td>
<td>15</td>
<td>14</td>
<td>58</td>
</tr>
<tr>
<td>Wheat straw</td>
<td></td>
<td>12</td>
<td>11</td>
<td>42</td>
</tr>
</tbody>
</table>
Effect of pretreatment on methane production

In Figure 3 the methane yield trend (LCH4/gVS) of raw and pretreated substrates as a function of the digestion time is represented.

The methane yield of untreated ensiled sorghum forage (0.269 ± 0.022 LCH4/gVS) was higher than that of wheat straw (0.204 ± 0.017 LCH4/gVS). These experimental data are well in agreement with literature values. Previous studies have indicated a methane yield of untreated straw in the range of 0.162–0.241 LCH4/gVS (Sharma et al. 1988; Møller et al. 2004; KTBL 2005). Jerger & Tsao (1987) and Chynoweth et al. (1993) have indicated a specific methane yield of untreated sorghum (0.8 mm size) in the range of 0.260–0.390 LCH4/gVS. The alkaline pre-treatment resulted in an increase of methane yield for both substrates (Figure 3). The increase of methane yield appeared to be higher for wheat straw than for ensiled sorghum forage both for the lowest (up to 14 and 17%, respectively) and highest (up to 31 and 47%, respectively) tested dosages. Moreover, experimental results (Table 3) make it evident the positive effect of the alkaline pre-treatment on the anaerobic degradation of samples in terms of ultimate methane yield (BMPt–∞) and anaerobic digestion kinetics. Table 3 summarises the k_h and BMPt–∞ estimated values with 95% confidence limits. The first order kinetic model was successful in interpreting the experimental production trend, as demonstrated by the high R^2 values, suggesting that such a simple methanisation model can be used in practice to describe the complex anaerobic degradation processes for those substrates, such as lignocellulosic ones, for which hydrolysis is the rate-limiting step, as reported by Angelidaki et al. (2009). An increase in the first order kinetic constant was observed by increasing the NaOH dosage (up to 65 and 163% for sorghum and wheat straw, respectively). The increase in the first order kinetic constant would result in an increase in biogas production in full-scale anaerobic digesters, although the actual increase would depend on the reactor fluid-dynamics and on its average retention time.

Table 3 | BMPt–∞ and k_h values and their relative increase with respect to the untreated sample, with 95% confidence limits for each condition tested (data are mean of duplicates, standard deviations are in the range of 0.000–0.001 LCH4/gVS)

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>BMPt–∞ (LCH4/gVS)</th>
<th>k_h (d^-1)</th>
<th>R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated sorghum</td>
<td>0.271</td>
<td>0.171</td>
<td>0.9708</td>
</tr>
<tr>
<td>Sorghum, 1% gNaOH/gTS</td>
<td>0.295</td>
<td>0.229</td>
<td>0.9954</td>
</tr>
<tr>
<td>Sorghum, 10% gNaOH/gTS</td>
<td>0.345</td>
<td>0.283</td>
<td>0.9949</td>
</tr>
<tr>
<td>Untreated wheat straw</td>
<td>0.208</td>
<td>0.104</td>
<td>0.9723</td>
</tr>
<tr>
<td>Wheat straw, 1% gNaOH/gTS</td>
<td>0.230</td>
<td>0.159</td>
<td>0.9932</td>
</tr>
<tr>
<td>Wheat straw, 10% gNaOH/gTS</td>
<td>0.289</td>
<td>0.272</td>
<td>0.9911</td>
</tr>
</tbody>
</table>

CONCLUSIONS

This study confirms that NaOH pretreatment (at 1 and 10% gNaOH/gTS, at 40 °C for 24 h) positively affects lignin and hemicelluloses reduction, COD solubilisation, and thereafter the anaerobic biodegradability of ensiled sorghum forage and wheat straw. Anaerobic digestion of ensiled sorghum forage and wheat straw is possible without any pretreatment, as confirmed from methane yields previously reported. However, alkaline pretreatment was efficient in enhancing the methane production of these substrates, especially at the highest dosage (10% gNaOH/gTS). Moreover, experimental results make evident the positive effect of the alkaline pre-treatment on anaerobic digestion kinetics. However, to date insufficient experimental data, especially at pilot scale, are available in the literature to draw any definite conclusion on the efficacy and therefore the potential application of such pretreatment at full-scale.
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