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Impact of various thermo chemical pretreatments on solubilisation and methane production of sunflower stalks.

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Abstract

The conversion of sunflower stalks into biomethane by batch mesophilic anaerobic digestion was investigated in this work. Methane potential of sunflower stalks (192 ± 2 mLCH₄/g VS) was increased by several thermo-chemical pretreatments: NaOH, H₂O₂, Ca(OH)₂, HCl and FeCl₃. The highest production (259 ± 6 mLCH₄/g VS) corresponding to a 31.4% increase was reached for the treatment at 55°C with 4% NaOH during 24h. More than 90 % of hemicelluloses were removed by applying acidic pretreatment (10% FeCl₃ or 4% HCl) at 170 °C. Oxidative and alkaline pretreatment (4% NaOH, 4% H₂O₂, 4% Ca(OH)₂) at 55°C were found to be more efficient to dissolve lignin than acidic pretreatment with a lignin removal around 22%. A negative linear correlation ($R^2 = 0.895$) was found between the lignin content of raw and pretreated sunflower stalks and the methane potential.

Keywords

Anaerobic digestion, sunflower residues, acid, alkali and oxidative pretreatments, biochemical composition.

INTRODUCTION

Conversion of lignocellulosic materials through anaerobic digestion (AD) technology is one of the promising alternatives to fossil fuels (Nallathambi Gunaseelan, 2006). Among lignocellulosic biomass, sunflower residues with a world cultivated land of 24 million ha in 2009 (PROLEA) represent interesting feedstocks for methane production through anaerobic digestion. Few studies have investigated the methane potential from sunflower residues (Raposo *et al.*, 2008, Antonopoulou *et al.*, 2010, Torrijos *et al.*, 2008). Low methane potential of 268 mL CH₄ / kg_{DM} and 200 mL CH₄ / kg_{DM} were respectively determined from sunflower straws and sunflower oil cakes (Antonopoulou *et al.*, 2010, Torrijos *et al.*, 2008). Whereas sunflower oil cakes are generally used in animal feed, there is no profitable use of sunflower stalks, except the soil enrichment.

Their use for methane production is thus very interesting but they have a low conversion efficiency which may be explained by the low accessibility of biodegradable organic compounds (hemicellulose, cellulose) within the lignocellulosics network. Indeed, lignocellulosic biomass is mainly composed of cellulose, hemicellulose and lignin which are strongly linked to each other and form complex three dimensional structures less available for micro-organisms (Chaudhry *et al.*, 2000; Malherbe *et al.*, 2002).

For successful utilisation of lignocellulosic biomass, it is important to study pretreatment to increase its biodegradability and make holocellulose more accessible to enzymatic or bacterial attacks. An effective pretreatment prior to anaerobic digestion should break down the linkage between polysaccharides and lignin to make cellulose and hemicelluloses more accessible to bacteria (He *et al.*, 2008; Pavlostathis *et al.*, 1985). Various pretreatment methods, thermo-

chemical, mechanical, biological, have been applied for second generation ethanol production and, in a lower extend, to enhance the anaerobic digestion of lignocellulosic substrates (Taherzadeh *et al.*, 2008). Among them, thermo-chemical pretreatments seem to be effective as pretreatment of anaerobic digestion (Taherzadeh *et al.*, 2008). According to the literature, dilute-acid pretreatment (0.4-2%) are often used at high temperature (160°C – 220°C) during few minutes (Willfor *et al.*, 2005). They are very successful to hydrolyze hemicelluloses and increase the accessible surface of cellulose (Mosier *et al.*, 2005). Inorganic salts such as FeCl_3 are also efficient to dissolve hemicelluloses, high hemicelluloses removal of 90% were observed by applying 0.1M FeCl_3 for 20 min at 140 - 200°C (Liu *et al.*, 2009).

Contrary to acidic pretreatments that require high temperature and low time, alkaline or oxidative pretreatments are often realised at low temperature during longer time (6-24h) (Taherzadeh *et al.*, 2008; Mosier *et al.*, 2005). Such pretreatments are more efficient than acidic pretreatments in the solubilisation of lignin. By applying 2% H_2O_2 at 30°C for 8h on sugarcane bagasse, 50 % of lignin were removed (Azzam, 1989). Alkaline pretreatments (NaOH , $\text{Ca}(\text{OH})_2$...) are efficient to remove lignin and part of hemicelluloses and efficiently increase the accessibility of cellulose (Taherzadeh *et al.*, 2008). Pretreatment of miscanthus with 12% NaOH at 70°C for 4 h led to 77% of delignification and 44% of hemicelluloses hydrolysis (Vrije *et al.*, 2002).

The objectives of this study were: (1) to make a screening of several thermo-chemical pretreatments (NaOH , H_2O_2 , $\text{Ca}(\text{OH})_2$, HCl and FeCl_3) and to assess their impact on the methane potential of sunflower straw (2) to study the impact of temperature on each of these pretreatments at a fixed concentration and fixed time (3) to study the impact of thermo-chemical pretreatments on sunflower straw solubilization (4) to establish correlations between chemical composition and methane production.

MATERIAL AND METHODS

Raw material:

The substrate used in this study was sunflower stalks “NK Kondi” cultivated in the South of France. Sunflower stalks were air-dried and milled into a size of 2-3mm using a cutting milling SM 100. The main characteristics and composition of the sample used in the experiments were: Total Solids (TS): $0.94 \pm 0.01 \text{ g/gStalks}$, Volatile Solids (VS): $0.87 \pm 0.02 \text{ g/gStalks}$, Van Soest soluble fraction: $3.64 \pm 0.02\%$, cellulose: $54.79 \pm 0.51\%$, hemicellulose: $19.59 \pm 0.32\%$, lignin: $21.99 \pm 0.53\%$ (average values of three determinations with standard deviations).

Pretreatment:

For all pretreatment methods tested the mass/volume ratio of solid (g) to liquid (mL) was 3:100. Different pretreatments: basic (NaOH and $\text{Ca}(\text{OH})_2$), oxidative (H_2O_2 at pH=11.5, with 3% NaOH addition) and acidic (HCl and FeCl_3) were investigated at fixed concentration and fixed time in the range of conditions usually used in the literature (table 1). Alkaline and oxidative pretreatments were realised in flasks of 500 mL in a water bath whereas acidic pretreatments were performed in a 1 L autoclave Zipperclave series 02-0378-1 (Autoclave France). This autoclave can reach a temperature of 250 °C and a pressure of 79 bars and the stainless steel body has a capacity of 1 L.

Anaerobic digestion:

The untreated and the treated sunflower straws were digested in batch anaerobic flasks. The volume of each flask was 0.5 L, with a working volume of 0.4 L and the remaining 0.1 L volume served as head space. Each flask was composed of: macroelements solution (sources of N, P, Mg, Ca, and K....), microelements solution, buffer bicarbonate solution, anaerobic inoculum (5 g VS /L) and

raw or treated sunflower straw (0.5 g/g VS_{inoculum}). Inoculum was granular sludge from a mesophilic anaerobic digester of a sugar factory. The sludge contained 64.27 g/L total solids (TS), 46 g/L volatile solids (VS). Once flasks were prepared, a degasification with nitrogen was carried out to obtain anaerobic conditions and the bottles were closed with butyl rubber septum-type stoppers, which were air impermeable. Duplicate bottles were incubated at 35°C for 35 days and two endogenic controls were realized and subtracted from the results.

Table 1. Conditions of pretreatments

Chemical	Temperature (°C)	Concentration (% w /w)*	Time (h)
Thermal alone	30, 55, 80 170	-	24 1
NaOH	30, 55, 80	4	24
Ca(OH) ₂	30, 55, 80	4	24
H ₂ O ₂ (pH =11.5)	30,55, 80	4	24
HCl	80, 125, 170	4	1
FeCl ₃	80, 125, 170	10	1

* g chemical/100 g stalks

Analytical methods:

Biogas analyses.

Biogas volume was monitored every day by water displacement method. Acidified water (pH = 2) was used to minimize the dissolution of carbon dioxide in water. Biogas composition was determined using a gas chromatograph (Varian Micro-GC-CP4900) equipped with two columns operated with He carrier gas. The first one (CP91 4509 molecular sieve, 1 0 m) was used at 11 0°C to analyse O₂, N₂, CH₄. The second one (CP914510 PQQ, 10 m) was used at 70°C to analyse CO₂. The detection of gaseous compounds was done using a thermal conductivity detector. The calibration was carried out with a standard gas composed of 25 % CO₂, 2 % O₂, 10 % N₂ and 63 % CH₄.

Substrate analyses.

The untreated and treated sunflower stalks were analysed for TS and VS according to the APHA standard method (APHA, 1992). The content of cellulose, hemicellulose and lignin was determined according to the procedure of Van Soest (Van Soest *et al.*, 1967) with the FIBERBAG system (Gerhardt). It is based on sequential extraction under neutral and acid detergent, followed by strong acid extraction. Different fractions are: i) soluble in neutral detergent fraction (soluble van Soest), ii) hemicellulose which is extracted by acid detergent, iii) cellulose which is extracted by 76 % sulfuric acid, iv) lignin. All the chemical composition analyses were realised in duplicate.

Statistical analysis:

The analysis of variance (ANOVA) method was first used to analyse the whole series of BMP results. It was also used to analyse the impact of temperature in each kind of pretreatment. Finally, different pretreatments (best conditions) were compared two by two by Student test and were classified into groups. Confidence interval was fixed at 95%, thus the probability to reject the null hypothesis was 0.05.

RESULTS AND DISCUSSION

Effect of thermo-chemical pretreatments on the methane production

Impact of temperature

The methane production (mL CH₄ / g VS_{added}) for pretreated sunflower straw is presented in Figure 1. Anova test was applied to each kind of pretreatment to assess the impact of temperature.

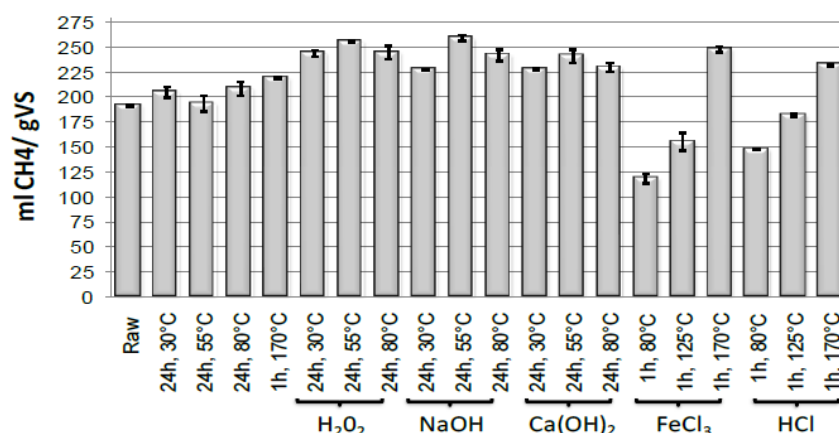


Figure 1. Methane potential of thermo-chemical pretreated sunflower straw

Results showed that the four samples after thermal treatment alone belong to the same group ($p=0.335$) indicating no significant impact of treatment temperature. Maybe a higher temperature should be applied to see a more significant increase of methane potential. Liquid Hot Water (LHW) treatment is generally applied at higher temperatures (200-230°C) for few minutes (Mosier *et al.*, 2005). In the same way, temperature had no significant impact on hydrogen peroxyde and lime pretreatments with Anova p-values of 1.16 and 0.39, respectively and no clear conclusion could be drawn about the impact of temperature of NaOH pretreatments ($p=0.051$). Nevertheless, for NaOH, H₂O₂ and Ca(OH)₂ pretreatments, the highest BMP values were obtained at 55°C. In contrast, temperature seemed to be an important parameter for HCl ($p=7.10^{-5}$) and FeCl₃ ($p=0.004$) treatments. Indeed, quite low methane potentials were observed at 80°C and 125°C, which may be due to a problem of manipulation because of heterogeneity of sampling for acidic pretreatment realized in autoclave at these temperatures. Indeed, treated samples were more homogeneous after 170°C pretreatments. This problem was not observed with other treatments at 30, 55 or 80°C because sampling was not necessary as pretreatments were carried out directly in the flasks for further anaerobic digestion. However, the very low standard deviations observed do not support this assumption. These unexpected results may also be due to an inhibition problem or to the formation of refractory compounds; these results have to be more explored in further studies.

For the following, only one condition of each kind of treatment was kept: 55°C for NaOH, Ca(OH)₂ and H₂O₂ and 170°C for thermal treatment alone and HCl and FeCl₃.

Impact of treatment on sunflower stalks methane potential

Table 2 shows the values of methane potentials of sunflower stalks before and after pretreatments in selected conditions and the groups resulting from pair wise comparisons of BMP values.

Table 2. Different statistical groups based on methane potential values.

Pretreatments	BMP (mL CH ₄ / gVS)	Groups
Raw	192 (± 1)	A
170°C	219.0 (± 0.7)	B
55°C 4 % Ca (OH) ₂	241 (± 6)	B/C/D
170°C 4 % HCl	233 (± 1)	C
170°C 10 % FeCl ₃	248 (± 3)	C/D
55°C 4 % H ₂ O ₂	256 (± 1)	D
55°C 4 % NaOH	259 (± 3)	D

For all thermo-chemically pretreated samples considered, an increase in the methane production as compared to the raw sunflower stalks (192 mL CH₄ / g VS) was observed. The highest increase in methane potential was observed for the conditions: 4% NaOH, 55°C, 24h (+ 35 %) and 4% H₂O₂, pH=1.5, 55°C, 24h (+ 33%). The treatment at 4% Ca (OH)₂, 55°C, 24h led also to interesting results (+26%) but this sample belonged to several groups in reason of a quite high standard deviation on the BMP values.

Among acidic pretreatment at 170°C during 1 h, 10 % FeCl₃ seems to be better than 4 % HCl with a methane potential increase of 29.5% and 21.4% respectively.

The effect of thermo-chemical (H₂SO₄ and NaOH) pretreatment on sunflower straws was investigated by Antonopoulou et al. (2010) and contrary to our results they did not observe any increase of methane potential. However, whereas they worked with alkali and acid concentrations (0.1%-1.5% w/v) of the same order as this study (0.12% w/v), the tested temperature (121°C) was higher than the temperature we used for alkali pretreatments and lower than the one used for acid pretreatments.

Effect of pretreatment on biochemical composition

The composition (soluble part from pretreatment, soluble Van Soest, cellulose, hemicelluloses and lignin in % VS) of raw and pretreated sunflower straws were determined and are shown in Figure 2).



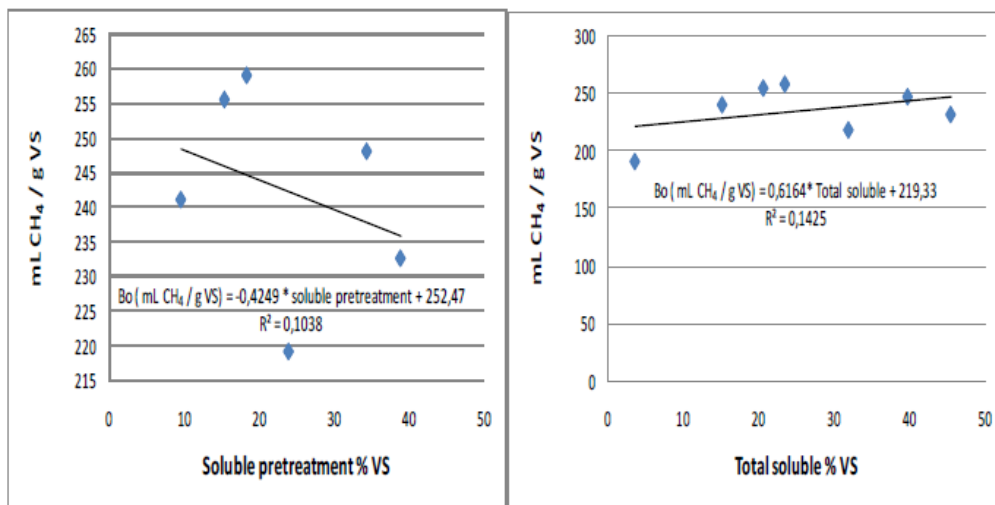
Figure 2. Lignocellulosic fractions for raw and pretreated sunflower stalks (SS)

Hemicelluloses removal of 71% was observed with thermal pretreatment (170 °C) and higher removals were noticed for acidic pretreatment with 93.7% and 94.4% hemicelluloses removal for 170°C - 10% FeCl₃ and 170°C - 4% HCl respectively. These hemicelluloses removals explain the high increase in the soluble pretreatment part for high temperature with acidic conditions. Such results were observed during FeCl₃ and H₂SO₄ pretreatment of corn stover (Liu *et al.*, 2009; Kumar *et al.*, 2009). With FeCl₃ pretreatment of corn stover in aqueous solutions heated between 140°C and 200°C, a significant increase of hemicelluloses removal was observed with 90% of xylose solubilisation (Liu *et al.*, 2009). By applying dilute acid pretreatment (0.06% H₂SO₄) at 190°C on corn stover, more than 72.5% of hemicelluloses were removed (Kumar *et al.*, 2009). Lower cellulose removals were observed even with high temperature (170°C) and acidic conditions (HCl or FeCl₃) as only 21.8% and 34.4 % cellulose were removed by 10% FeCl₃ and 4% HCl respectively. Moreover, low lignin removal of 13.6% and 15.4% were respectively observed for 170°C - 4% HCl and 170°C - 10% FeCl₃. These results confirm Knappert *et al* conclusion: acidic pretreatments are efficient to remove hemicelluloses by breaking ether bonds in lignin/phenoliccarbohydrates complexes without dissolving lignin (Knappert *et al.*, 1981).

In term of lignin removal, alkaline and oxidative pretreatments are better than acidic pretreatments as a lignin removal around 22 % was observed for basic and oxidative pretreatments. However these results are quite low in comparison to other studies. By applying 12% NaOH at 70°C for 4h , 77% of delignification were observed on miscanthus (Vrije *et al.*, 2002). Furthermore, 66% of delignification were observed by pretreating corn stover with 1% H₂O₂ (pH= 11.5) at 65°C for 3h (Selig *et al.*, 2009).

Correlation between biochemical composition and methane potential

In this part, we have attempted to correlate: the methane potentials (mL CH₄ / g VS) obtained after the pretreatment conditions selected in Figure 2 and the changes in their composition induced by the various pretreatments. Indeed, Van Soest method leads to fractions which are classified according their accessibility to chemical attack. It can be assumed that the accessibility to chemical attack may be correlated to the accessibility to microorganisms. Thus, methane potential was plotted against: 1) the percentage of organic matter solubilised by pretreatment, 2) the total soluble organic matter which represents the sum of soluble parts from Van Soest and pretreatment, 3) the sum of total soluble organic matter and hemicelluloses and 4) the sum of total soluble organic matter, hemicelluloses and cellulose (which corresponds to the remaining lignin content) (Figure 3).



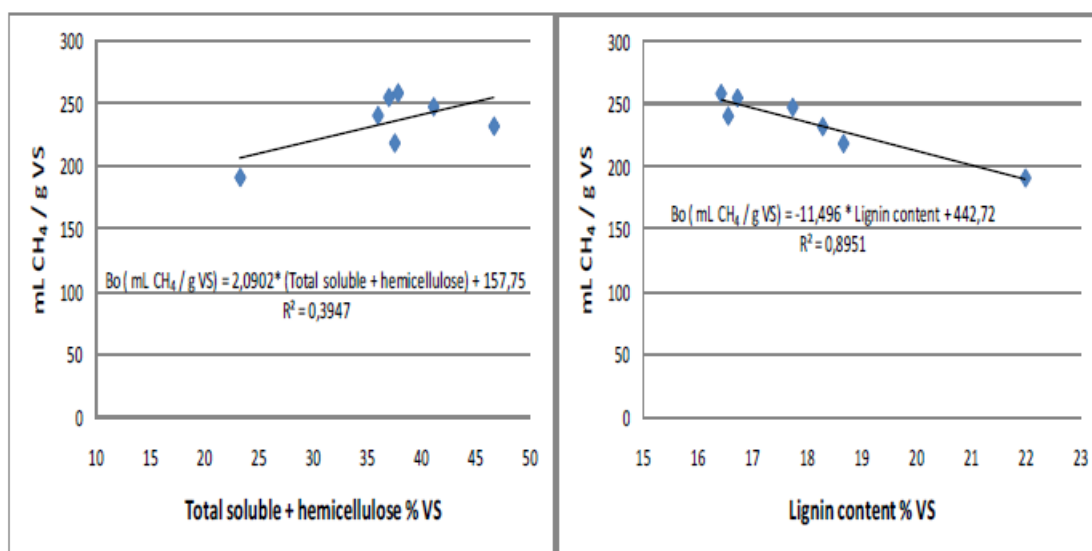


Figure 3. Correlation between chemical compositions with methane potential

In Figure 3 we can see that the methane potential is not correlated to the organic matter solubilised by pretreatment ($R^2 = 0.104$) nor to the total soluble fraction ($R^2 = 0.143$) which represent the most available and accessible organic matter. If hemicellulose fraction is added to the previous fractions, the correlation is slightly better ($R^2 = 0.395$) but is absolutely not satisfying. Finally, a good correlation ($R^2 = 0.895$) was observed between methane potential and the lignin content. It was observed that the methane potential decreases when the lignin content is higher. These results were also observed by Kobayashi *et al.* on steam exploded bamboo, a linear regression showed a strong negative correlation ($R^2 = -0.946$) between the amount of methane produced and the amount of lignin (Kobayashi *et al.*, 2004). The lignin content plays a major role in methane production by limiting the access of holocelluloses. The holocelluloses which are anaerobically biodegradable compounds in their pure form appear to be less biodegradable or even completely refractory when combined with lignin (Tong *et al.*, 1989).

CONCLUSIONS Anaerobic digestion of sunflower stalks is possible without any pretreatment with a methane potential of 192 mL CH₄ / g VS. However, thermo-chemical pretreatments were efficient to enhance the methane production from sunflower stalks. The highest production (259 ± 6 mLCH₄/ g VS) was reached for the pretreatment at 55°C with 4% NaOH during 24 h. Acidic pretreatments (HCl or FeCl₃) removed more than 90% of hemicelluloses and basic and oxidative pretreatments were more efficient to dissolve lignin, with a lignin removal around 22%. Moreover, it was shown that lignin content plays a major role on the methane potential of chemically pretreated lignocellulosic biomass: the lignin content must be reduced to increase the methane potential. Thus, alkaline and oxidative pretreatments can be recommended to optimise anaerobic digestion of lignocellulosic residues.

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