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ORIGINAL PAPER

The Effect of Aqueous Ammonia Soaking Pretreatment on Methane Generation Using Different Lignocellulosic Biomasses

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Abstract In the present study aqueous ammonia soaking (AAS) has been tested as a pretreatment method for the anaerobic digestion of three lignocellulosic biomasses of different origin: one agricultural residue: sunflower straw, one perennial crop: grass and a hardwood: poplar sawdust. The methane production yield was evaluated in batch experiments at different organic loadings, in order to assess any inhibitory effects due to the pretreatment. The experiments showed that the increase of organic loading did not affect the final methane yield of either raw or AAS pretreated biomasses. Among the three biomasses tested, poplar sawdust exhibited the lowest methane yield, due to its high lignin content. AAS treatment led to an increase of the ultimate methane yields of all biomasses, with the increase in the case of poplar, sunflower straw and grass being 148.7, 37.7 and 26.2 %, respectively. AAS resulted in solubilization of hemicellulose and partial removal of cellulose for all biomasses. Higher cellulose degradation was observed in grass biomass, in which a different morphology than the other AAS treated samples, was shown in SEM images. No toxic compounds such as furaldehydes, were produced during AAS pretreatment.

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Keywords Aqueous ammonia soaking · Lignocellulosic biomass · Pretreatment · Methane potential · Sunflower straw · Grass · Poplar sawdust

Introduction

The anaerobic digestion process has been applied to a wide range of lignocellulosic biomass types such as agricultural and forestry solid residues, grasses or energy crops and has received increased attention during the past few years [1, 2]. Although abundant and almost zero-cost feedstocks, agricultural and forestry residues and grasses do not contain easily fermentable organic material such as free sugars and their biotransformation to methane is not an easy task. This is mainly due to the complex structure of lignocellulose (cellulose is embedded in an amorphous matrix of hemicellulose and lignin), which limits the access of microorganisms and enzymes, for efficient digestion [3, 4]. The most recalcitrant part of the biomass is the lignin, due to its hydrophobic nature [5]. In order to exploit lignocellulosic feedstocks to the highest possible degree, through accelerating the hydrolysis rate, a chemical, physical or biological pretreatment method has to be applied prior to anaerobic digestion [6]. Applying a proper pretreatment method, the structural and compositional barriers for the digestion of lignocellulosic biomass are decreased by breaking or partially removing the lignin seal, reducing cellulose crystallinity, and increasing the surface area [7]. This way, the subsequent liberation and uptake of simple fermentable sugars (hexoses and pentoses) that can be converted by the microorganisms to methane is facilitated, enhancing the efficiency of methane production [8].

Among the different pretreatment technologies that have been proposed, alkaline pretreatment is widely considered

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as essential for anaerobic digestion of lignocellulosic biomass [9–11]. Up to now, different alkaline treatment technologies have been proposed, for different kinds of lignocellulosic feedstocks. In general, alkaline pretreatment can be classified into "high concentration" and "low concentration" processes, depending on the concentration of the alkali used [12]. Low-concentration pretreatments are carried out at high temperatures and pressures, while high-concentration pretreatments are carried out at atmospheric pressure and relatively low temperatures. Using high temperatures for alkaline conditioning, problems such as formation of toxic compounds and loss of sugars have been reported [13]. Consequently, alkaline pretreatment at low temperatures is generally preferable [14].

Among alkaline pretreatments, ammonia recycle percolation (ARP) and ammonia fiber expansion (AFEX) have been reported to efficiently hydrolyze corn stover [15, 16], switchgrass [17] and sugarcane bagasse [18]. However, these methods require high temperatures and pressures and specialized equipment, leading to high capital costs and energy demands. The use of alkaline solutions such as NaOH, $Ca(OH)_2$ (lime) or ammonia, for lignin removal and partial hemicellulose solubilization, provides a low-cost alternative, enhancing enzyme accessibility to the cellulose. The effectiveness of an alkaline treatment depends on the lignin content of the biomass [19]. Monlau et al. [20] studied the effect of different thermo-chemical pretreatments, on the methane potential and the chemical composition of sunflower stalks and found that the most effective pretreatment for delignification, was the alkali pretreatment with 4 g of NaOH/100 g TS which also led to the highest biochemical methane potential (BMP). The application of optimized NaOH pretreatment (24 h, 55 °C, 4 % NaOH) to different sunflower stalks samples led to a BMP increase ranging from 29 to 44 % [21]. Xie et al. [11] showed that the solubility of grass silage increased by 45 %, with a 65.6 % lignin removal, when treated with 5 % NaOH at 100 °C. The highest methane yield was 452.5 mL/g VS added which was 38.9 % improved compared with the untreated grass silage. However, NaOH, can cause corrosion damage of the reactor and can also increase the risk of degradation and loss of carbohydrates, especially at high temperatures [13].

Among the different alkaline pretreatment technologies, aqueous ammonia soaking (AAS) presents certain advantages, since ammonia is relatively safe to handle, noncorrosive when compared to NaOH, it can be easily recovered and presents a high selectivity towards the lignin reactions, while preserving the carbohydrates [14]. Ammonia can also penetrate the crystalline structure of cellulose and causes swelling [14]. Using ammonia at room temperature, the bioconversion and fermentation yields increase, while its interaction with hemicellulose is minimized, eliminating the possibility of toxic compounds formation [14]. AAS pretreatment is reported to be effective for low lignin feedstocks, such as agriculture residues.

ASS has been used for bioethanol production from corn stover [22], barley hull [23], switchgrass [24, 25] elephant grass [26] and oil palm empty fruit brunches [27] with satisfactory results. AAS at low temperature and pressure is a novel and promising pretreatment in the anaerobic digestion field. Switchgrass [28], corn stover [29], manure fibers [30], wheat straw, willow and miscanthus [31] are the lignocellulosic feedstocks, which were pretreated with AAS and used for methane production so far. AAS at six different concentrations of ammonia (5–32 %) and for 1, 3 and 5 days at 22 °C was applied on digested fibers, separated from the effluent of a manure-fed, full-scale anaerobic digester [32]. A methane yield increase from 76 to 104 % was achieved, while the different ammonia concentrations did not considerably affect the methane yield. It was shown that the optimal duration was 3 days for all ammonia reagent concentrations which were tested.

The objective of this study was to assess the effect of AAS pretreatment on the BMP of sunflower straw, grass and poplar sawdust. AAS pretreated and raw feedstocks were used at three different TS loadings, in order to assess any inhibitory effects caused by the pretreatment. A detailed characterization of all feedstocks, in terms of their lignocellulosic content, was carried out while analysis of the liquid fraction obtained after pretreatment was also performed. Scanning electron microscopy (SEM) was used to investigate the structural characteristics of the raw and pretreated feedstocks.

Materials and Methods

Feedstocks Used

Sunflower straw was collected after seed harvesting, in November 2012, in the region of Serres, in Macedonia, Northern Greece. Grass and poplar sawdust were collected in November and October 2012, respectively, in the region of Attica, Greece. The poplar sawdust used was the forestry residues generated during sawing poplar stems. All samples were initially air dried, chopped to a size of < 1 mm diameter with a house blender (izzy X3, E560T3, Titanium), milled with a lab grinder (IKA A11 basic) and the final product was collected as powder after passing through a sieved with a pore size of 0.71 mm. In the sequel, all the feedstocks were air-dried at ambient temperature and used for the experiments.

Aqueous Ammonia Soaking Pretreatment

AAS was applied for 3 days at 22 °C to maximize the feedstocks methane potential [30]. Specifically, all

feedstocks were soaked in ammonia reagent (32 % w/w aqueous ammonia) at a ratio of 10 mL reagent per g total solids (TS) and were kept in closed glass flasks to avoid evaporation. After 3 days, 10 mL water per g TS was added to facilitate the subsequent ammonia removal by a vacuum distillation step. Distillation was performed using a rotary evaporator (Buchi RII Rotavapor) with a vertical condenser under 0.32 atm and a gradually increased water bath temperature from 40 to 90 °C with a step of 20 from 40 to 80 °C. The retention time was 10 and 20 min at the first two and last two temperature levels, respectively. It should be mentioned here, that due to the vacuum, the distillation of the AAS mixture took place at temperatures lower than 60 °C and thus the effect of the distillation process on the methane potential of the AAS pretreated fibers was expected to be insignificant. Determination of the methane potential of fibers, which went through only distillation (with no previous ammonia treatment) confirmed the above, since the result was very similar with that of the raw fibers (data not shown).

Inoculum

The inoculum for the methane potential tests came from a 3L active volume mesophilic digester fed with a mixture of liquid swine manure (with a TS content of 2.6 %) and AAS treated raw manure fibers at a ratio 1:1 (TS based). The HRT was 25 days and the loading rate was 1.2 g TS/L/day or 0.7 g VS/L/day. The main characteristics of the inoculum were: pH: 8.3, TSS: 5.5 ± 0.5 g/L and VSS: 3.7 ± 0.3 g/L.

Biochemical Methane Potential (BMP) Assays

Biochemical methane potential (BMP) experiments were carried out in duplicate at 35 °C, in batch experiments, where raw and AAS pretreated feedstocks (the whole pretreated biomass (whole slurry: liquid and solid fractions obtained after pretreatment)), were used, as substrate. Based on Jurado et al. [30], no organic or inorganic material was lost during AAS treatment and ammonia removal, as confirmed by TS mass balances. Thus, for BMP experiments, where the whole pretreated biomass, was used, the percentage of whole material recovery (gTS pretreated biomass per g TS initial biomass) of all feedstocks, was considered as 100 %. The methane production rate and yield were evaluated at different organic loadings, in order to assess any inhibitory effect of the AAS pretreatment on the digestion process. AAS pretreated as well as raw feedstocks were used at three different TS loadings: 0.1, 0.2 and 0.6 g TS per 10 mL of inoculum. Thus, appropriate amounts of treated and raw samples were added in serum bottles of 160 mL and seeded with 20 mL mixed anaerobic inoculum and deionised water to a final volume of 100 mL. The microbial culture was supplemented with 10 mL/L of a $(NH_4)_2HPO_4$ (7.21 g/L) solution, 10 mL/L of a $FeSO_4.7H_2O$ (0.7 g/L) solution and 10 mL/L of a trace metals solution [33]. Control experiments for checking the methanogenic biomass activity were carried out using glucose. Blank experiments were also carried out in order to determine the background gas productivity of the inoculum. The content of the vials was gassed with a mixture of N_2/CO_2 (80/20) in order to secure anaerobic conditions. The vials were sealed with butyl rubber stoppers and aluminum crimps and methane production was monitored as a function of time according to Owen and Chynoweth [34].

Analytical Methods

Raw samples were air-dried and then used for compositional analyses. Carbohydrate and lignin content were determined according to the National Renewable Energy Laboratory (NREL)'s [35] standard laboratory analytical procedure (LAP) for determination of structural carbohydrates in biomass [36]. A two-step extraction (with water and ethanol) was conducted for grass, while one step (only ethanol extraction) was performed for sunflower straw and poplar biomass. In each case, the extractive free biomass (0.3 g sample) was used to determine the structural carbohydrates with a two-step acid hydrolysis method. After initial hydrolysis at 37 °C with 3 mL of 72 % (w/w) sulfuric acid, the samples were diluted with distilled water to a total volume of 84 mL and autoclaved for 1 h in pressure tubes. Detection and quantification of sugar monomers (glucose, xylose and arabinose) were performed with HPLC-RI with an Aminex HPX-87H column (Biorad) at 60 °C and a Cation H micro-guard cartridge (biorad Laboratories) using H_2SO_4 0.006 N as an eluent at a flow rate of 0.6 mL/min. Acid soluble and insoluble (Klason) lignin contents were calculated according to NREL's standard laboratory analytical procedure [36], respectively. For the characterization of the AAS pretreated samples, a separation of liquid and solid fractions was made, through filtering with 0.7 μm filters. The solid fractions were washed, air-dried and characterized as described above for the raw samples, but without performing an extraction process, prior to the characterization. Since for the purpose of chemical compositional analysis, only the solid fraction obtained after AAS pretreatment was used, the solid material recovery due to the loss of weight, has been taken into account. Thus, in order to calculate the lignocellulosic content of the pretreated biomass per 100 g of initial TS, the loss of solid material (g TS/100 g $TS_{initial}$) (Table 1) was multiplied by the values of lignocellulosic content expressed per kg of pretreated TS. The liquid fractions were used for soluble charbohydrates' content determination, according to Joseffson [37]. The liquid fractions

Table 1 The main characteristics of the raw feedstocks used in this study

Characteristic	Sunflower straw	Grass	Poplar
TS (%)	90.9 ± 0.1	92.2 ± 0.1	93.2 ± 0.1
VS (g/100 g TS)	79.5 ± 0.1	83.4 ± 0.1	92.0 ± 0.2
Cellulose (g/100 g TS)	32.0 ± 0.2	20.4 ± 0.1	32.7 ± 1.1
Hemicellulose (g/100 g TS)	18.7 ± 2.4	24.0 ± 2.0	16.8 ± 0.7
Lignin (g/100 g TS)	22.3 ± 0.3	12.3 ± 1.2	34.2 ± 0.1
Acid insoluble lignin	19.3 ± 0.1	4.7 ± 1.1	27.0 ± 0.1
Soluble lignin	3.0 ± 0.1	7.6 ± 0.4	6.2 ± 0.3
Extractives (g/100 g TS)	8.1 ± 0.1	25.6 ± 3.1	5.0 ± 0.6
Proteins(g/100 g TS)	1.7 ± 0.2	10.5 ± 0.5	1.0 ± 0.1

were also used for the identification of furaldehydes (5-hydroxymethylfurfural (HMF) and furfural) and aliphatic acids (formic and acetic acid), as well as ethanol, which were probably released during pretreatment. For the analysis, an HPLC-RI with an Aminex HPX-87H column (Biorad) at 60 °C using H_2SO_4 0.006 N, as an eluent, at a flow rate of 0.7 mL/min, was used. The measurements of total solids (TS) and volatile solids (VS), total suspended solids (TSS) and volatile suspended solids (VSS) were carried out according to Standard Methods [38]. Raw and extractive-free samples were also used to determine Total Kjeldahl Nitrogen (TKN) according to Standard Methods [38]. Crude protein content was determined by multiplying TKN by a factor of 6.25.

The methane content of the produced gas was quantified with a gas chromatograph (SRI 8610c MG#1) (two columns in series: molecular sieve column, 6 ft., O.D. 1/8 in., I.D. 2.1 mm and silica gel column, 6 ft., O.D. 1/8 in) equipped with a TCD (thermal conductivity detector). The column oven temperature was 80 °C, the injector valve 90 °C and the TCD oven 100 °C. Helium was used as carrier gas at 20 mL/min. SEM images were captured using a Zeiss SUPRA 35VP, after coating the samples with a homogeneous Au layer by ion sputtering.

Statistical Analysis

Two-sample t test with a threshold p value of 0.05 was applied to analyze the effect of AAS pretreatment on BMP yields, using excel software.

Results and Discussion

Feedstocks Composition

The composition of sunflower straw, grass and poplar sawdust, used for the experiments, is presented in Table 1. It should be mentioned that the table values are referred to the air-dried raw feedstocks. Thus, the TS content of air dried grass, which was used in this study, was 92.2 ± 0.1, while the respective value for fresh grass before air drying, was 25.9 ± 0.6 %. As anticipated, poplar sawdust, being a hardwood biomass had the highest lignin content, while its hemicellulose fraction was quite low. Grass and sunflower straw were characterized by lower lignin and higher hemicellulose fractions, compared to the poplar sawdust. Especially for grass, the hemicellulose fraction was 24.0 ± 2.0 %, representing the higher biopolymer fraction of the plant. The compositional analysis of all feedstocks is comparable to other reports [21, 39]. However, direct comparison of compositional data is not feasible, since the chemical composition of a lignocellulosic material depends on several factors, such as the variety, location, agricultural practices used to grow the crop and the analytical method applied for cell wall composition analysis [40].

Table 2 summarizes the effect of AAS pretreatment on biomass fractionation in terms of lignin, cellulose and hemicellulose. The values in Table 2 are expressed per kg of initial TS, taking into account that the loss of biomass (gTS solid pretreated biomass/g TS initial biomass) after AAS was 75.8 % for sunflower straw, 63.0 % for grass and 87.8 % for poplar sawdust, respectively. The same values expressed per kg of pretreated TS could be calculated by dividing the lignocellulosic content relative to the initial biomass (g/100 g $TS_{initial}$), by the loss of solid material (g TS/100 g $TS_{initial}$). The percentage of the solids remaining after reaction with ammonia depends on the feedstock used, as well as on the severity of the pretreatment process. For example, Kim and Lee [41], who applied AAS pretreatment on corn stover at different temperatures and different ammonia concentrations, found that the percentage of the solids remaining after pretreatment (with 15 % NH_3), decreased from 76.0 ± 1.6 % at 40 °C to 71.4 ± 2.1 % at 60 °C and 67.3 ± 1.0 % at 90 °C.

From Table 2, it is obvious that, the lignocellulosic content of all pretreated solids, was lower than the respective of the untreated materials, due to the loss of the overall mass. However, AAS pretreatment affected the chemical composition of the three different lignocellulosic

Table 2 The lignocellulosic content (g/100 g $TS_{initial}$) and the loss of solid material (g TS/100 g$TS_{initial}$) of all feedstocks, after AAS pretreatment

Characteristic	Sunflower straw	Grass	Poplar
Loss of solid material (gTS/100 g $TS_{initial}$)	24.1	37.0	12.2
Cellulose (g/100 g$TS_{initial}$)	28.9 ± 0.7	16.0 ± 0.1	29.1 ± 1.5
Hemicellulose (g/100 g $TS_{initial}$)	13.9 ± 1.3	16.7 ± 0.4	12.0 ± 0.9
Lignin (g/100g $TS_{initial}$)	19.9 ± 0.7	11.4 ± 2.1	34.4 ± 1.0

biomasses in a different way. Specifically, the reduction of lignin for all substrates was low, since for grass it was 7.3 %, for sunflower straw 10.8 % and for poplar it was not affected. Cellulose solubilization or degradation during AAS treatment was insignificant for sunflower straw and poplar sawdust (9.7 and 11.0 %). However, for grass biomass, a reduction of cellulose content by 21.6 % was observed. A hemicellulose reduction by almost 28.6 % occurred for poplar sawdust and 25.7 % for sunflower straw, due to hemicellulose solubilization. The solubilization was higher for grass (30 %) due to the higher loss of mass which was indicated by the low percentage of the solids remaining after pretreatment.

In general, loss of hemicellulose and removal of lignin during ammonia pretreatment depend upon the reaction conditions and the lignocellulosic material used [42]. The delignification efficiency of poplar sawdust obtained in this study was much lower than in other studies. Thus, when hybrid high lignin poplar was soaked in 15 % ammonia for 24 h at a temperature of 150 °C, a 30 % delignification, accompanied by an 8 % hemicellulose reduction, was observed [43]. The difference could be attributed to the different conditions applied, since it is well known that aqueous ammonia-mediated pretreatment at low temperatures, leads to a considerable loss of hemicellulose and a lower lignin removal [23, 44]. However, a complete delignification from lignocellulosic biomass is extremely difficult to occur, even at the most severe conditions, due to the location of lignin within the lignin- carbohydrate complex, the strength of the poly-ring bonds of C–O–C, C–C and its hydrophobicity [23, 45].

For example, Chandel et al. [42], who optimized AAS pretreatment of sugarcane bagasse, through testing different ammonia concentrations, temperatures and residence times, found that among the conditions studied, the pretreatment at 20 % (v/v) NH_4OH at 70 °C for 24 h, led to a maximum lignin removal (41.5 %) and a hemicellulose loss of 68.7 %. Aqueous ammonium hydroxide pretreatment of barley hull (15 % w/w hydrated ammonia, 24–72 h, 75 °C) led to a 50–66 % lignin removal while a 65–76 % of xylan was maintained in the solid matrix accompanied by a negligible loss of glucan [23]. When rice straw was soaked in an aqueous-ammonia solution (70 °C, 12 h and 20 % w/w hydrated ammonia), a 60.6 ± 0.3 % lignin removal and retaining of 86.9 ± 1.1 % glucan, were observed [44]. Isci et al. [24] applied an aqueous ammonium treatment (ammonium hydroxide 30 %) in switchgrass, using different liquid–solid ratios for either 5 or 10 days, at atmospheric conditions without agitation. A delignification of 40–50 % (Klason lignin basis) was achieved, whereas the cellulose content did not change and the hemicellulose content decreased by approximately 50 %. Finally, when corn stover was soaked in aqueous ammonia over an extended period (10–60 days), at room temperature, without agitation, a lignin removal of 55–74 % occurred, while nearly 100 % of the glucan and 85 % of the xylan were retained [15]. However, the required reaction time of 10 days, could be considered a barrier for this process, and an optimization of AAS was performed by using different temperatures (40–90 °C) and aqueous ammonia concentrations (15–30 wt%) in order to reduce the reaction time to 6–24 h. The optimum treatment conditions were found to be 15 wt% of NH_3, 60 °C and 12 h of treatment time, resulting in a 62 % of lignin removal, while glucan was retained at 100 % and xylan at 85 % [41].

Analysis of the Liquid Fractions Obtained After AAS Pretreatment

Before and after AAS treatment of all feedstocks, the soluble sugars were measured and are presented in Fig. 1. For AAS treated feedstocks, the measurement of soluble sugars was performed in the liquid fraction obtained, after AAS treatment and expressed per g of initial TS. From the figure, it is obvious that the soluble sugar content (measured as glucose equivalent) of all feedstocks increased during AAS-treatment, confirming that some solid material was solubilized. The concentration of soluble sugars in the liquid fraction of AAS treated sunflower straw, grass and poplar sawdust was found to be 3.6 ± 0.1, 5.6 ± 0.8 and 3.4 ± 0.1 g/L corresponding to a 7.9 ± 0.1, 12.2 ± 1.5 and 6.8 ± 0.1 g/100 g TS.

In Table 3, the analysis of sugars contained in the AAS pretreated samples, in terms of glucose, xylose, arabinose and cellobiose, is presented. In the same table, the concentrations of ethanol, aliphatic acids such as formic and acetic acid, as well as of furaldehydes such as 5-hydroxymethylfurfural (HMF) and furfural, which could be released during pretreatment as a result of lignin and carbohydrates degradation

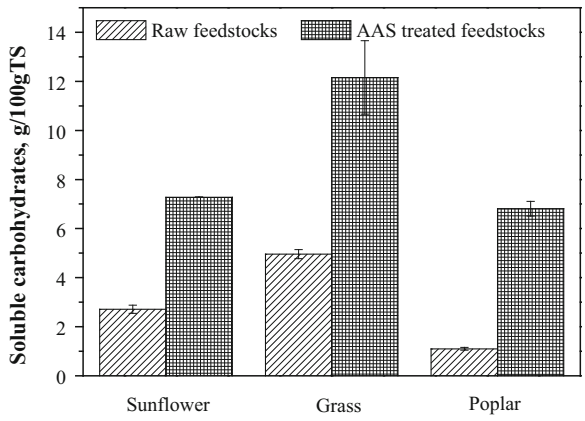

Fig. 1 Sugar content of raw and AAS pretreated feedstocks

Table 3 The main characteristics of the hydrolysates of AAS pretreated feedstocks

Characteristic (g/100g TS)	Sunflower straw	Grass	Poplar
Cellobiose	0.5 ± 0.	1.5 ± 0.1	0.4 ± 0.0
Glucose	0.4 ± 0.1	3.7 ± 0.1	0.3 ± 0.0
Xylose	0.8 ± 0.0	5.0 ± 0.1	0.1 ± 0.0
Arabinose	n.d.	n.d.	n.d.
Ethanol	n.d.	n.d.	n.d.
Formic acid	n.d.	0.8 ± 0.0	0.3 ± 0.0
Acetic acid	1.1 ± 0.1	0.2 ± 0.0	0.5 ± 0.1
Furfural	n.d.	n.d.	n.d.
HMF	n.d.	n.d.	n.d.

n.d. not detected

are also presented. These compounds may affect the downstream hydrolysis and fermentation steps [46] or could be toxic to methanogens during anaerobic digestion [47]. It is obvious that during AAS at room temperature, compounds such furfural or HMF, were not released. Only acetic and formic acids at low levels were produced, with the acetic acid concentration being higher for AAS pretreated sunflower straw. The fact that during AAS pretreatment at mild conditions the formation of toxic by-products is prevented is also confirmed by other studies [15]. In general, the formation of these compounds is possible, at extreme pretreatment conditions and especially under thermal and acidic pretreatment methods at high temperatures [47]. For these reasons and in combination with the lower energy requirements, the pretreatment methods at ambient temperatures and pressures are of interest [24].

Analysis of SEM Images

Selected SEM images that represent general observations in multiple images of raw and AAS treated sunflower straw, grass and poplar sawdust are illustrated in Figs. 2, 3 and 4. SEM revealed a change in morphology of all feedstocks after AAS treatment, but it is obvious that AAS affected differently each substrate. Thus, from Figs. 2 and 4 it may be seen that AAS treatment led to a different surface structure, while from Fig. 3 it is obvious that AAS caused smoothing of the surface. As shown in Fig. 2b, c, the raw sunflower straw is characterised a compact rigid structure and has few pores available for enzymatic hydrolysis. Figure 4a, where the untreated poplar sawdust is

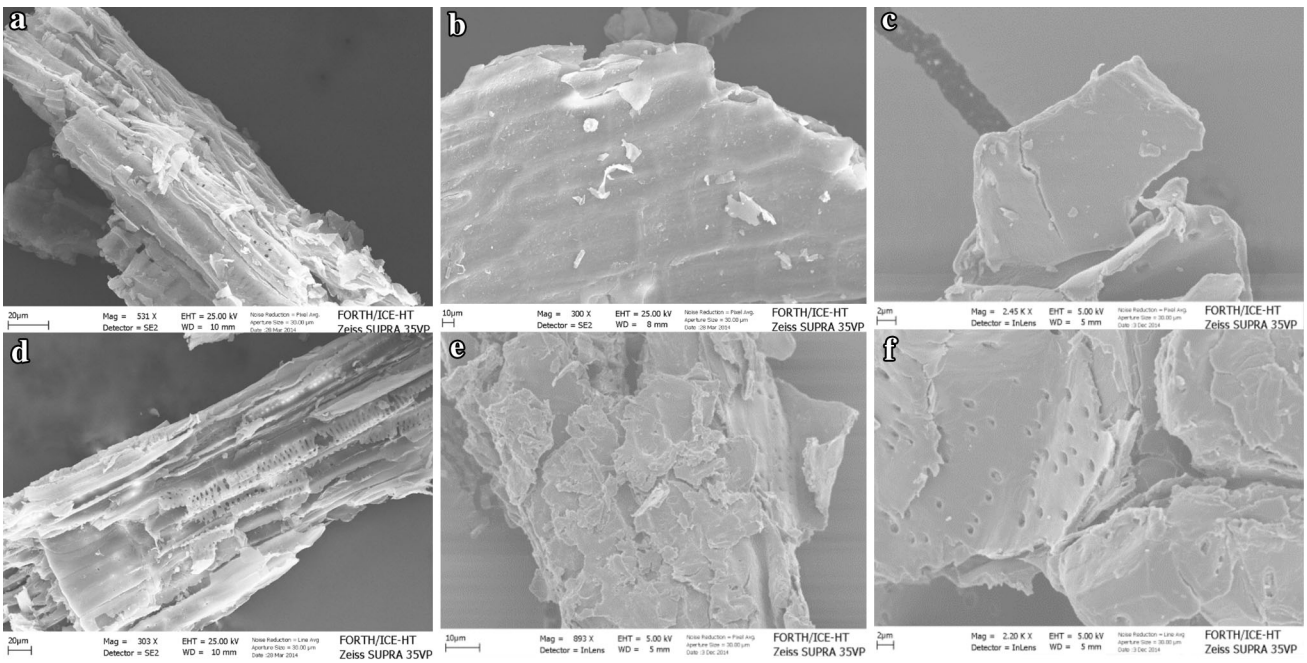

Fig. 2 SEM images from raw (**a–c**) and AAS pretreated sunflower straw (**d–f**)

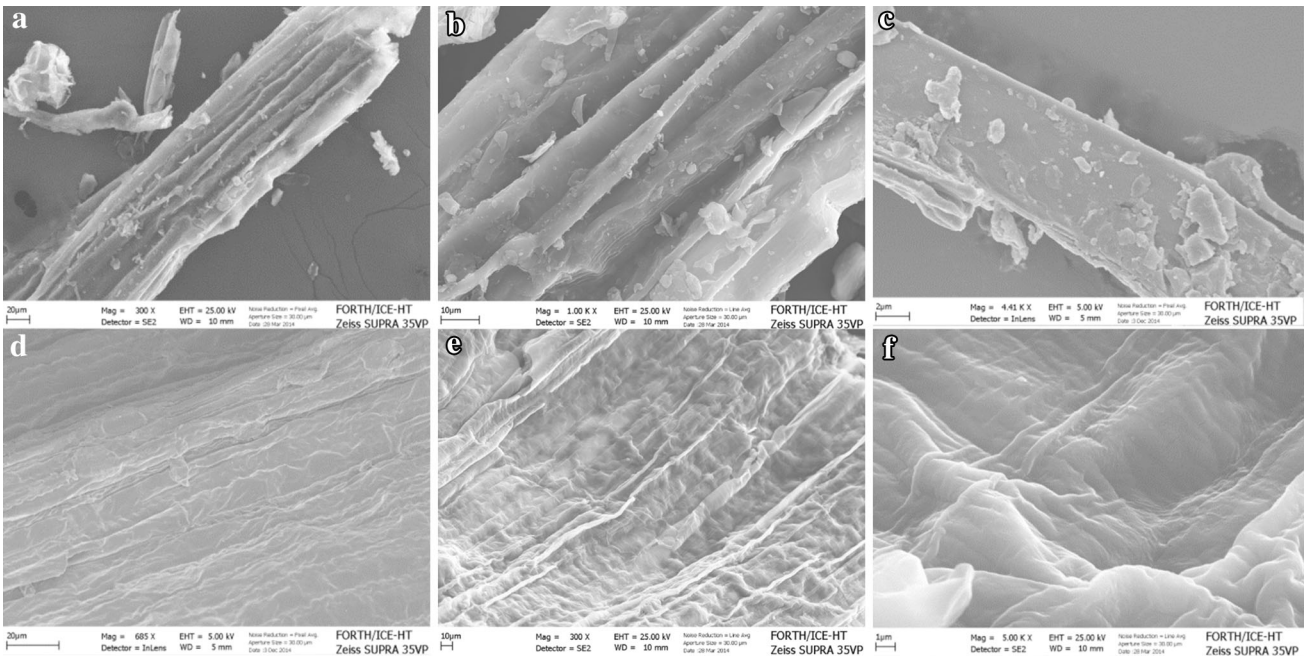

Fig. 3 SEM images from raw (**a–c**) and AAS pretreated grass (**d–f**)

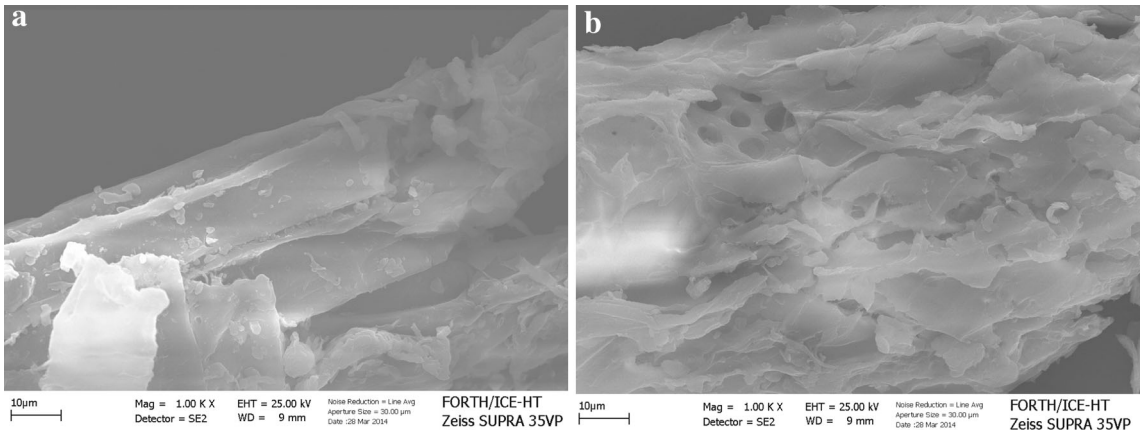

Fig. 4 SEM images from raw (**a**) and AAS pretreated poplar sawdust (**b**)

depicted, shows rigid, ordered fibrils, and connected structure. In the AAS treated samples (Figs. 2d–f, 4b), the fibers are somewhat separated and exposed. A large amount of mass seems to have been removed from the initial connected structure. Pinholes and gaps are also visible in the treated sunflower straw and poplar sawdust, leading to the speculation that the surface area and the porosity, have also increased. On the other hand, Fig. 3a–c show that grass was partly covered by debris, which vanished after AAS treatment (Fig. 3d–f), resulting in a smoother surface. Similar smoothing after AAS treatment, was observed through SEM and AFM images of digested and raw manure fibers, which were AAS treated at the same conditions [48]. These observations are also in agreement with Donohoe et al. [49] who applied ammonia pretreatment on switchgrass. The different morphology of grass compared with the other AAS treated samples could possibly be attributed to the cellulose loss from the solid matrix, which was also observed, accompanied by the loss of hemicellulose and lignin, which occurred at all AAS treated biomasses.

Biochemical Methane Potential (BMP) Experiments

Batch experiments were performed in order to determine the methane potential of raw and AAS pretreated sunflower straw, grass and poplar sawdust. Different organic loadings (10, 20 and 60 g TS per L of inoculum) of raw and AAS

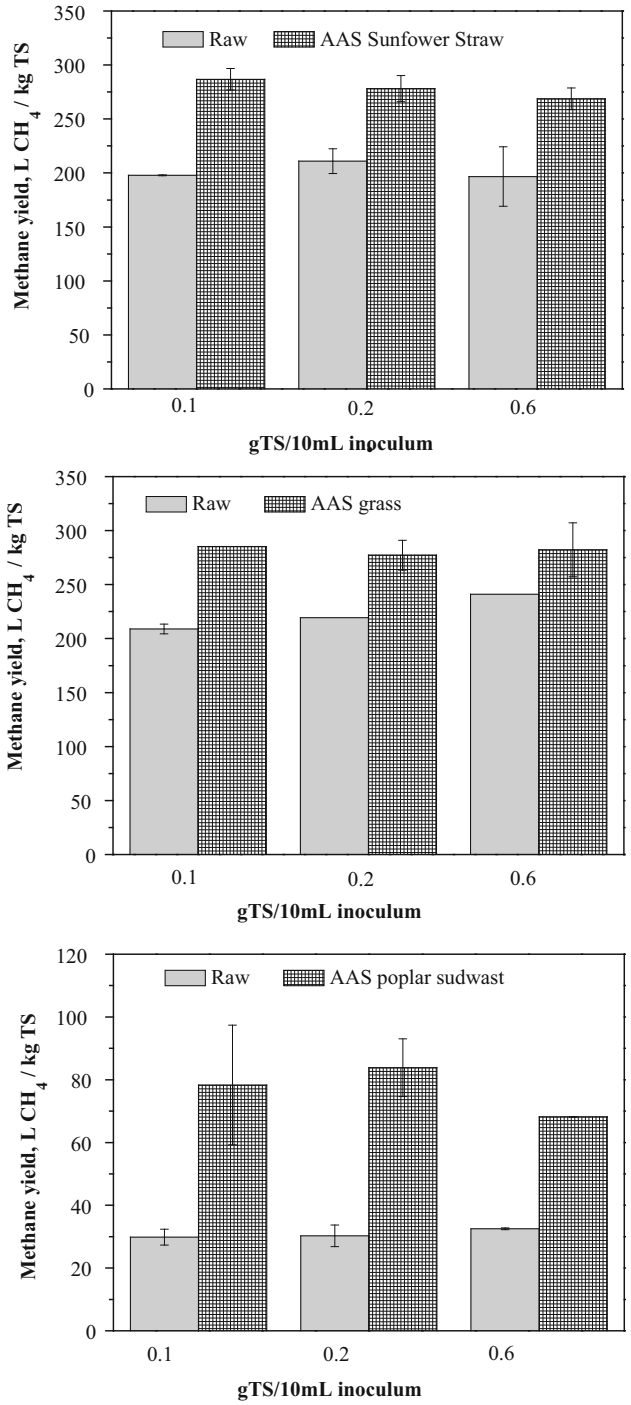

Fig. 5 Final methane yields, of raw and AAS treated, sunflower straw, grass and poplar sawdust at different organic loadings (0.1, 0.2 and 0.6 gTS/10 mL inoculum)

pretreated feedstocks were tested, in order to determine possible inhibition due to components that might be formed during the pretreatment. The final methane yields of raw and AAS-pretreated sunflower feedstocks, after 60 days of batch anaerobic digestion, at different organic loadings, are shown in Fig. 5.

It is obvious that the methane yield for raw poplar sawdust was significantly lower than the respective of grass and sunflower straw. This could be attributed to its higher lignin content. Based on Monlau et al. [50], who developed a model to predict the BMP of lignocellulosic feedstocks as a function of their compositional and structural features, the most important parameter, which is negatively correlated to the BMP, is the lignin content, followed by the soluble sugars content (positively correlated), the proteins content (positively correlated), the crystalline cellulose content (negatively correlated) and the amorphous holocelluloses (amorphous cellulose and hemicellulose) content (positively correlated).

It should be noted that the methane potential of poplar sawdust, obtained in this study, was noticeably lower than the respective obtained using the same feedstock but different inoculum (anaerobic sludge collected from the anaerobic digester of a municipal treatment plant) [51]. The fact that the different inoculum source led to a different methane potential, could be attributed to the different population of hydrolytic bacteria which might be contained in different inocula. This has also been reported in other studies [52, 53].

Figure 5 shows that the increase of TS loading did not affect the final methane yield of either raw or AAS pretreated feedstocks. In addition, AAS pretreatment had a positive effect on methane yield from all three biomass types tested, with the most impressive one being the increase observed when AAS was applied on poplar sawdust. The methane production from raw poplar increased from 30.9 ± 1.4 to 76.8 ± 7.9 mL CH_4/g TS, after pretreatment, taking into account the values obtained from all loadings. The increase of the final methane yield of the AAS-poplar sawdust compared to the raw substrate was as high as 148.7 %. A *t* test of the BMP of poplar, before and after AAS pretreatment, showed that for experiments with organic loading of 20 and 60 g TS per L of inoculum, the average methane yields after pretreatment, were significantly higher than the yields before pretreatment ($p = 0.017$ and $p = 0.0000378$, respectively, i.e. in both cases $p < 0.05$), (5 %). However, no significant difference was found in the methane yields of poplar, with the lower organic loading (10 g TS per L of inoculum), ($p = 0.07 > 0.05$).

For sunflower straw and grass, the enhancement of the methane yield was lower. Thus, for sunflower straw, the increase was 37. 7 % (from 201.8 ± 7.9 to 277. 9 ± 9.0 mL CH_4/g TS, taking into account the values obtained from all loadings), while for grass the increase was 26.2 % (from 223.1 ± 16.4 to 281.6 ± 4.1 mL CH_4/g TS, taking into account the values obtained from all loadings). For sunflower straws, the *t* test analysis of the BMP before and after AAS pretreatment, showed that for experiments with organic loading of 10 and 20 g TS per L of inoculum, the average

methane yields after pretreatment were significantly higher than the yields before pretreatment ($p < 0.05$), (5 %), while no significant difference was found in the methane yields of sunflower straws with 60 g TS per L of inoculum, ($p > 0.05$). However, for the BMPs with grass for all organic loadings, the difference between the methane yields before and after AAS were not significant ($p = 0.17, 0.27$ and 0.22 which are >0.05 for 10, 20 and 60 g TS per L of inoculum).

The experimental results obtained are in accordance with those presented by Jurado et al. [31], who found that AAS was highly efficient when applied on biomasses with low methane potential, such as willow. In that study, the increase of the ultimate methane yield of willow, due to AAS pretreatment was 94 %, which is much higher when compared with the respective of feedstocks with lower lignin content, such as miscanthous (25 % increase) and wheat straws (37 % increase). However, the BMP of poplar, obtained in the present study is quite low, even after AAS pretreatment. Comparing the higher heating value of poplar (19.38 MJ/kg TS) [54] with the energy that could be obtained when poplar is converted to methane (almost 3 MJ/kg TS, assuming that the energy yield from methane is 0.0364 MJ/L), it is obvious that through the anaerobic digestion process, the maximum energy recovery for this type of biomass is not achieved.

Conclusions

In the present study, aqueous ammonia soaking was investigated as a moderate pretreatment method for enhancing the methane potential of sunflower straw, grass and poplar sawdust. Among the three biomasses tested, grass and sunflower straw were the most promising in terms of methane production, due to their low lignin content. AAS treatment led to an increase of the ultimate methane yields of all biomasses, with the increase in the case of poplar being as high as 148.7 %. The enhancement of the methane yield was 37.7 and 26.2 % for sunflower straw and grass, respectively. In addition, the increase of TS loading did not affect the final methane yield of either raw or AAS pretreated biomasses. Regarding the effect of AAS on the chemical composition of three different lignocellulosic biomasses, a loss of hemicellulose and a partial removal of cellulose was observed, for all biomasses. Higher cellulose and hemicellulose degradation took place for grass biomass, which exhibited the higher loss of mass and a different morphology than the other treated samples was shown in SEM images.

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