DIFFERENT PHYSICOCHEMICAL PRETREATMENT METHODS ON LIGNOCELLULOSIC BIOMASS: THE EFEECT ON BIOCHEMICAL METHANE POTENTIAL

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ABSTRACT

Various pretreatment methods, such as thermal (24 h at 80ºC; 1 h at 120ºC), alkaline (2-20 gNaOH/100 gTS, 24 h at 80ºC) and acid (2-20 gH_2SO_4/100 gTS, 1 h at 120ºC; 2-20 g H_3PO_4/100 gTS, 1 h at 120ºC; 2-20 gHCl/100 gTS, 1h at 120ºC,) were applied on grass biomass and the effect of each pretreatment method on methane productivity and yield was evaluated. Biochemical Methane Potential (BMP) experiments were conducted for the whole pretreatment slurry (liquid and solid fraction obtained after pretreatment), as well as for both separated fractions and direct comparison of the methane yields was carried out. In addition, the effect of each pretreatment method on carbohydrate solubilization and lignocellulossic content fractionation (to cellulose, hemicellulose, lignin) was also evaluated. The possible release of compounds such as aliphatic acids (acetic and formic acid), furaldehydes (furfural and hydrolyl methyl furfural (HMF)) during the pretreatment, which could act as inhibitors, was also examined. The experimental results showed that the treatment with the acids leads to higher hemicellulose solubilization, while a lignin degradation is achieved when grass is treated with NaOH. The methane yield was enhanced with alkaline pretreatment and, the higher the NaOH concentration, the higher was the methane yield observed. Comparing the BMP of the whole pretreatment slurry with the sum of the BMPs of both fractions, no important differrences were found. Separation of both fractions was enhanced only by pretreatment with HCl 10 g/100 gTS (BMP of 458.5 compared to 371.7 LCH_4/kg VS, when the whole slurry was used).

Keywords: biochemical methane potential, grass lawn, biomass, pretreatment, lignocellulosic content

1. Introduction

The anaerobic digestion process has been applied to a wide range of lignocellulosic biomass types such as agricultural solid residues, leaves, grass and crop stalks and has received increased attention during the past few years [1]. Abundant and almost zero-cost feedstocks, such as agricultural residues and grasses do not contain easily fermentable free sugars, and their biotransformation to methane is not an easy task. This can be attributed to the complex structure of lignocellulose, where cellulose is embedded in an amorphous matrix of hemicellulose and lignin, as well as to the low efficiency with which lignocellulosic substrates are converted through biological processes, such as hydrolysis and fermentation [3]. In order to exploit lignocellulosic feedstocks to the highest possible degree, efficient fractionation, via pretreatment, has to be performed prior to bioconversion. By applying a proper pretreatment method, depolymerization of cellulose and hemicellulose and breaking of the lignin seal are observed. This way, the subsequent liberation and uptake of simple sugars (hexoses and pentoses) that can be readily converted by the microorganisms to methane is facilitated, leading to higher methane production yields.

In this work, various pretreatment methods, thermal or chemical (through alkali or acid addition) were applied on grass lawn and BMP experiments were conducted in the whole pretreatment slurry (liquid and solid fraction obtained after pretreatment), as well as at both separeted fractions.

2. Materials and methods

2.1. Pretreatment conditions

Grass was air dried, chopped, milled and sieved to a powder of 0.71mm. In the sequel, the biomass was air-dried at ambient temperature and used for the experiments. For all pretreatment methods used, the mass/volume ratio of solid (g TS) to liquid (mL) was 5:100. In table 1, the pretreatment conditions used in this study are presented. The biomass slurry that resulted from the pretreatment, was either used directly in BMP experiments or separated into solid and liquid fractions, using filters of 0.7μm for further analysis and characterization and then used for BMP experiments.

Table 1: The pretreatment schemes which were tested in grass lawn in this study

Pretreatment	Chemical	Temperature,ºC	Chemicals loading (g/100 gTS)	Time, h
Thermal (blank experiments)	-	80	-	24
		120		1
Acid	H_2SO_4	120	2,10,20	1
	H_3PO_4	120	2,10,20	1
	HCl	120	2,10,20	1
Alkaline	NaOH	80	2,10,20	24

2.2. BMP experiments

BMP experiments were carried out in duplicate at 35ºC according to Owen and Chynoweth [2]. Anaerobic sludge was used as inoculum. For the experiments with the solid fractions or the whole slurry obtained after pretreatment, serum bottles of 160 mL were seeded with 20 mL mixed anaerobic culture and appropriate amounts of samples were added, in order to acquire the desirable VS content of 2g VS / L. For the experiments with the liquid fractions, 2 gCOD/L of the hydrolysates, were added in the vials, which were also seeded with 20 mL mixed anaerobic sludge. The microbial culture was supplemented with nutrient solutions and trace metals. The methodology followed has been described in Antonopoulou *et al*. [3].

2.3. Analytical methods

Raw samples were air-dried and then used for a two-step, water and ethanol extraction prior to compositional analysis. Carbohydrate and lignin contents were determined according to the National Renewable Energy Laboratory (NREL)'s standard laboratory analytical procedure (LAP) [4]. Detection and quantification of sugar monomers were performed with HPLC-RI with an Aminex HPX-87H column (Biorad). For the characterization of the pretreated samples, a separation of liquid and solid fractions was made, through filtering with 0.7μm filters. The solid fractions were washed with water, air-dried and characterized as described above, but without perfrorming the extraction process, prior to the characterization. The liquid fractions were used for soluble carbohydrate content determination, according to Joseffson [5] as well as for the identification of furaldehydes (5-hydroxymethylfurfural (HMF) and furfural) and aliphatic acids (formic and acetic acid), which were probably released during pretreatment. The measurements of total solids (TS) and volatile solids (VS) were carried out according to Standard Methods [6]. Raw and extractive-free samples were also used to determine Total Kjeldahl Nitrogen (TKN) according to Standard Methods [6]. The 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) equipped with a TCD (thermal conductivity detector) and helium used as carrier gas.

3. Results and discussion

3.1. Feedstock composition before and after pretreatment

The composition of grass used in this study is: TS (%) =92.2 ± 0.1, VS (g/100 gTS)= 83.4 ± 0.1, cellulose (g/100 gTS)= 20.4 ± 0.1, hemicellulose (g/100 gTS)= 24.0 ± 2.0, lignin (g/100 gTS)= 12.3. ± 1.2, extractives (g/100 gTS)= 25.6 ± 3.1 and proteins (g/100 gTS)= 10.5 ± 0.5. Figure 1 summarizes the effect of pretreatment on the fractionation of biomass in terms of lignin, cellulose and hemicellulose.

The values are expressed per kg of initial grass TS, meaning that the material recovery due to the loss of weight during pretreatment has been taken into account. The loss of biomass increased with pretreatment severity. For example, the percentage material recovery ($gTS_{pretreated\ biomass}$/g $TS_{initial\ biomass}$) after thermal treatment was 72.4%, while after treatment with 10 gH_2SO_4/100 gTS it was 58.4% and after treatment with 20 gH_2SO_4/100 gTS and 20 gHCl/100 gTS it was only 49.8 and 40.6%, respectively. From figure 1, it is obvious that acid pretreatment resulted in hemicellulose fraction reduction due to hemicellulose solubilization. This has also been reported in other studies [7]. The hemicellulose content decreased with acid strength and concentration. The lignin fraction was affected only by alkaline pretreatment, which is in agreement with the literature [7].

Hemicellulose solibilization which occured during acid pretreatment is also confirmed by soluble sugars concentration (measured as glucose equivalent), which is presented in table 2. It is obvious that the soluble sugar content increased after all pretreatments, and its highest value was observed in the liquid fractions obtained from acid pretreatments. This is attributed to higher solubilization of solid material (hemicellulose), resulting in higher xylose release.
Several toxic compounds are usually formed during pretreatment, as a result of lignin and carbohydrates' degradation, which may affect the downstream hydrolysis and fermentation steps. Working at extreme pH favors the formation of these compounds, with thermal and acid pretreatments entailing a greater risk of formation of furaldehydes and aliphatic acids. In table 2, the concentrations of formic and acetic acid, as well as those of furfural and HMF, released during thermal (1h at 120ºC) and acid pretreatments (with all inorganic acids), are presented.

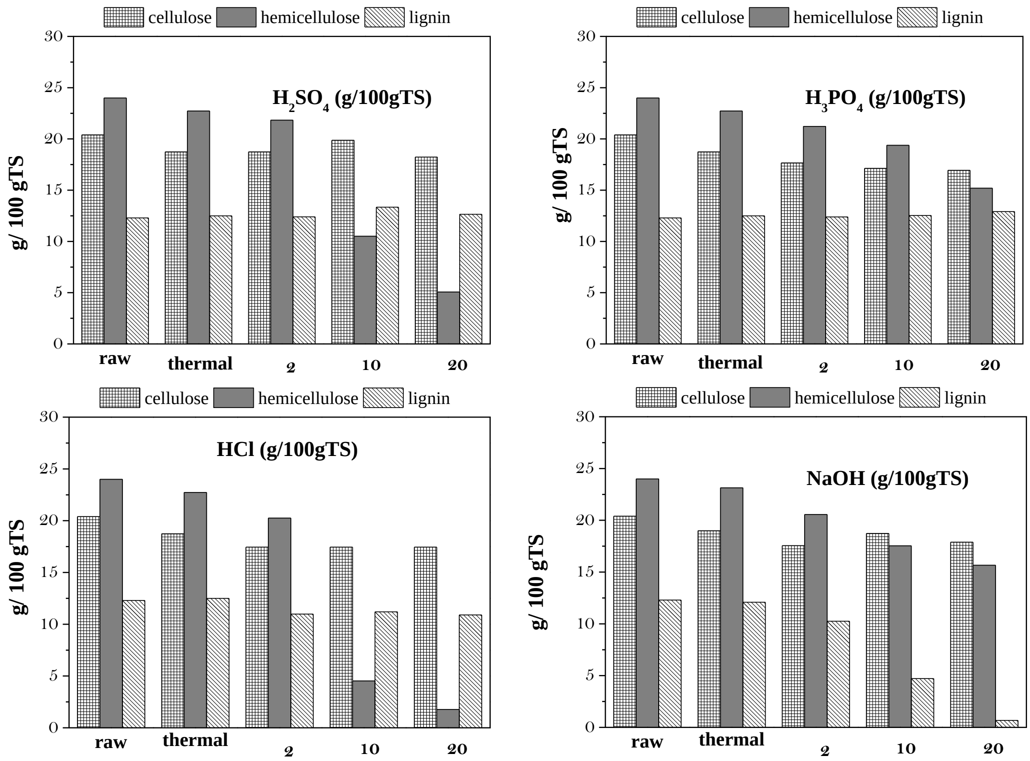

Figure 1: Effect of pretreatment on grass composition

Table 2: Sugar content of raw and pretreated samples

Pretreatment		Sugars (g/100gTS)	Furaldehydes (g/100gTS)	Aliphatic acids (g/100gTS)
Raw		4.96 ± 0.18	-	1.04 ± 0.02
Thermal	80ºC	5.21 ± 0.18	n.m	
	120ºC	6.36 ± 0.10	-	1.08± 0.04
H_2SO_4	2 g/100gTS	6.71 ± 0.34	0.05± 0.00	1.17± 0.02
	10 g/100gTS	14.77. ± 0.11	0.33± 0.00	0.96± 0.04
	20 g/100gTS	15.12 ± 0.14	0.41± 0.09	1.07± 0.09
H_3PO_4	2 g/100gTS	6.24 ± 0.03	-	0.52± 0.03
	10 g/100gTS	7.69 ± 0.74	0.07± 0.00	0.77± 0.02
	20 g/100gTS	11.17 ± 1.78	0.41± 0.02	0.79 ± 0.04
HCl	2 g/100gTS	8.08 ± 0.12	0.08± 0.00	0.57± 0.04
	10 g/100gTS	18.21± 0.69	0.73± 0.02	1.81± 0.01
	20 g/100gTS	19.03 ± 0.58	1.11± 0.01	2.78± 0.08
NaOH	2 g/100gTS	5.34 ± 0.78	n.m	n.m
	10 g/100gTS	6.80 ± 0.20	n.m	n.m
	20 g/100gTS	8.58 ± 0.30	n.m	n.m

n.m= not measured

It is obvious that treatment with H_2SO_4 and HCl resulted in higher furaldehydes' concentrations. The higher the acid concentration, the higher the concentrations of furfural and HMF observed.

3.2. BMP experiments

In figure 2a, the BMP of grass lawn is presented when the whole slurry obtained after pretreatment was used. In figures 2b and 2c, the BMP of the liquid and the solid fractions, are presented. The values are expressed per kg of initial grass VS, meaning that the material recovery due to the loss of weight during pretreatment, has been taken into account. It is obvious that all alkaline pretreatment methods enhanced methane production from grass lawn and the higher the alkaline concentration, the higher was the methane production. Thus, the methane yield for 20 gNaOH/100 gTS was 414.81 ± 26.54 for the whole slurry and 430.46 ± 15.64 mL CH_4/g VS for the sum of both fractions, respectively, corresponding to a 29 and 35 % increase in methane yield compared to the untreated sample. In the alkali-pretreated sample, the lignin content was reduced, and this is associated with higher methane yields [7]. When only the liquid fractions were used, H_2SO_4 and H_3PO_4 resulted in lower methane yields, compared to using HCl, meaning that the compounds resealed during pretreatment had no toxic affect on methanogens. For the case of BMPs with the solid fractions, it was observed that the higher the concentration of each acid used, the lower was the BMP obtained.

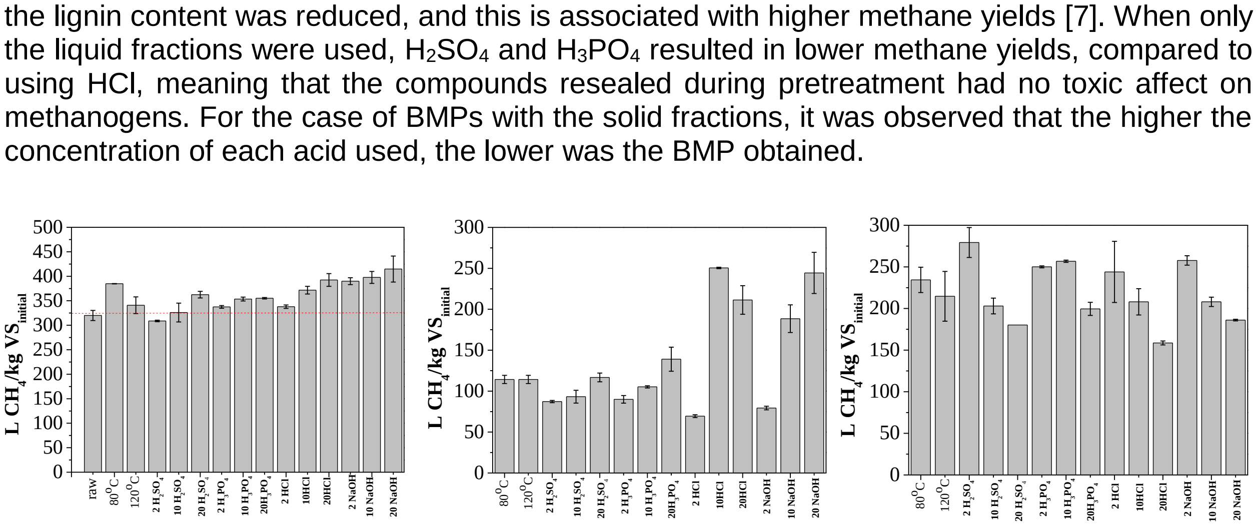

Figure 2: Effect of pretreatment on BMP of grass lawn, when the whole pretreatment slurry (a), as well as both separeted fractions (liquid (b) and solid (c)) obtained after pretreatment, were used

4. Conclusions

The experimental results obtained showed that the treatment with acids led to higher hemicellulose solubilization, while lignin removal from the solid matrix was achieved, when grass was treated with NaOH. Higher acids concentrations led to higher solubilization of hemicellulose. The BMP of grass lawn was enhanced with alkaline pretreatment and the higher the NaOH concentration, the higher was the methane yield observed.

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