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Evaluation of the Steam Explosion Pretreatment Upon the Anaerobic Digestion of Water Hyacinth Biomass: Influence on Liquid and Solid Fractions

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Abstract

Biochemical methane potential tests were performed to evaluate the effects of steam explosion on the liquid and solid substrates of thermal hydrolysis pretreatment applied to water hyacinth biomass. The operational conditions of the thermal hydrolysis applied the combination of two temperatures (170 and 210 ºC) and two cooking times (5 and 30 min). The higher solubilization factor was 22.9% for the sample pretreated at 210 ºC and 30 min followed by steam explosion effect (TH + SE). Steam explosion, temperature and time were, in order of importance, the more effective operational conditions for the biomass solubilization. The sample 210 ºC - 5 min TH + SE presented the higher methane production increase, in relation to the raw substrate, resulting in a increment factor of 2.43, for the solid sample. The higher methane production increase for the liquid sample was on a factor of 1.67, for sample 210 ºC - 30 min TH + SE. The higher biomethanization increase considering both biomass factors (solid + liquid) was obtained for the pretreatment 170 ºC - 30 min TH + SE. A combined model confirmed the hydrolysis limitation for the solid samples biodegradation; however, it was not clear for the prediction on the liquid samples. Micrographs evidenced the morphological changes of the solid substrate with the solubilization increase. Particle size reduction was the most effective effect of the pretreatment on the substrate morphology. Porosity increment was observed only in the surface of the sample 210 ºC - 30 min TH + SE.

Keywords: *Eichhornia crassipes*; lignocellulose biomass; methane production; thermal hydrolysis; flash effect.

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1. Introduction

Eichhornia crassipes (water hyacinth) is an aquatic plant recognized as a problematic weed on the world, due to its high growth rate and great adaptability [1]. Furthermore, it is an interesting biomass source as second generation for biofuel production, due to its renewable characteristic and as an abundant non-food biomass source [2].

Interest on biomethanation process for new biomasses has recently increased, focusing on pretreatment methods to improve methane production. Thermal hydrolysis pretreatment is the most studied method of pretreatment for sludge and lignocellulosic biomass, being successfully applied at industrial scale [3–5]. The main effect of this pretreatment technology is the significant increment on solids solubilization and biodegradability enhancement of the substrate, through the increase of the easily biodegradable biomass fraction [6].

The process presents a wide range of characteristics and configurations, mainly based on: operation temperature (low: < 110 ºC or high:> 110 ºC), pressure influence (steam explosion effect at the end of the process), and combination of thermal and other mechanisms (thermo-chemical or thermo-mechanical) [3,7]. The conventional thermal pretreatment is performed at high temperature, generally in the range of 160 ºC to 180 ºC, corresponding to a pressure of 6 to 10 bar (vapor liquid equilibrium), and a time operation ranging from 30 to 60 min [3,8,9].

As consequence of the use of high-pressure saturated steam as the energy source for the process, the reaction vessel operates at high pressure during the cooking time. In this process, after the operation time, the reactor is suddenly opened and the biomass shot to a flash reactor at atmospheric conditions. Thus, the biomass suffered an explosive decompression [4,10]. To ensure a high pressure variation, the recommended temperature inside the reactor is about 160 ºC to 240 ºC [7].

For lignocellulosic biomass, the steam explosion is predominantly related to the hemicellulose solubilization, whereas the lignin is transformed as consequence of the high operational temperature. Hence, the cellulose of the solid fraction becomes more accessible to enzymatic attack [7]. According to this, the biodegradability enhancement due to the pretreatment process reported for the thermal hydrolysis is related to both pretreatment temperature and time [11].

The main studies performed so far upon lignocellulosic biomass pretreated by thermal hydrolysis followed by steam explosion process are focused on the pretreatment for further biomass fermentation, as ethanol, sugars, acids and fiber production [7,12–14]. Furthermore, the influence of the operational pretreatment conditions for specific substrates has not yet been accessed [6], and relatively few works have focused on decoupling the effect of thermal hydrolysis and steam explosion on the anaerobic digestion performance of liquid and solid fractions.

Therefore, the aim of this research was to identify the effect of thermal hydrolysis (TH) combined with the steam explosion (TH + SE) process upon water hyacinth biomethanation, comparing the solubilization and its anaerobic digestion through biodegradability assay. In order to evaluate the influence of a thermal pretreatment on the biodegradability of the solid and liquid fractions, samples pretreated under different operational

conditions were fractionated. A combination of First Order and Modified Gompertz model was applied to fit the methane production of the assays, trying to identify kinetic parameters of the biomethanation process.

2. Materials and methods

2.1. Materials

Water hyacinth (*Eichhornia crassipes*) biomass was obtained from a garden center in the Netherlands. The plants were grinded down on a domestic crusher to a particle size of 0.5 to 2.0 cm and characterization (Table 1) performed according to the Standard Methods [15].

Table 1: Water hyacinth biomass characterization on terms of solids, organic matter and carbon to nitrogen ratio content

Parameter	Code	Units	Water hyacinth
Total solids	TS	%	4.8
Volatile solids	VS	%	3.8
Percentage of volatile solids	VS/TS	%	79
Chemical oxygen demand	tCOD	gO2 kg-1	50.5
Ratio tCOD/VS	tCOD/ VS	-	1.33
Ratio C/N	C/N	-	8.2

2.2. Pretreatment

Pretreatments were conducted on a lab-scale hydrolysis plant with a 5 L reaction tank and 50 L flash vessel. The pretreatment plant was connected to a steam boiler and controlled through time and temperature conditions upon a panel control. At the end of the cooking time an automatic valve opened suddenly and the decompression shoot the sample through outlet pipes to the flash tank, where it was collected.

Two pretreatment configurations were tested on this work: thermal hydrolysis without steam explosion (TH), and thermal hydrolysis followed by steam explosion (TH + SE). The first pretreatment (TH) was performed by opening the decompression valve slowly, allowing a smooth drop in the pressure inside the reactor. The second experiment (TH + SE) was performed by a suddenly opening of the decompression valve.

Prior to the experiments, the reaction tank was preheated with steam. Each pretreatment test was fed with 150 g of the crushed biomass. The operation conditions tested were: 170 ºC – 5 min; 170 ºC – 30 min; 210 ºC – 5 min; and 210 ºC – 30 min.

Pretreated lignocellulosic biomass of water hyacinth resulted in a heterogeneous substrate, with a liquid and solid biomass fraction. To analyze the pretreatment effect on the solubilized and particulate substrate, the pretreated medium was separated with a domestic sieve of approximately 2 mm mesh size during 10 min and characterized according procedures given in Standard Methods [15]. Scanning electron microscopy (SEM) was performed on a JSM 820, JOEL microscope on raw and pretreated solid fraction substrates to compare and analyze the superficial morphological characteristics changes after pretreatments.

2.3. Biochemical Methane Potential Tests

Batch anaerobic digestion tests were carried out to assess the substrate biomethanation and methane production yield, based on standardized assays [16]. The inoculum originated from the mesophilic digested sludge of the municipal WWTP of Valladolid (27.4 gTS kg-1 and 15 gVS kg-1) was pre-incubated for 4 days at 35 ºC. The experiment was performed with blank bottles (only inoculum, to subtract the methane fraction generated by them), control bottles (feed with cellulose microcrystalline, to check the methane activity of the inoculum), raw substrate (water hyacinth biomass without pretreatment) and pretreated samples (solid and liquid fractions of pretreated biomass). The substrate-inoculum ratio used was 1:2 in terms of VS. The assays were performed in triplicate in reactor bottles of 160 mL total volume and a working volume of 30%. The experiments were conducted in a thermostatic room at 35 ºC and constant mixing in a shaker device. The biogas production was periodically quantified by pressure production and characterized by gas chromatography [17].

2.4. Parameters determination

The solubilization factor (SF) of the pretreated samples was calculated by the soluble organic matter (sCOD) released during the pretreatments in relation to the particulate organic matter fed (Eq. 1).

$$\%SF = \frac{(\mathrm{sCOD/tCOD})_{TH} - (sCOD/tCOD)_0}{\left((tCOD - sCOD)/tCOD\right)_0} \times 100 \quad (1)$$

where, the index 0 indicates sCOD and tCOD in the raw biomass and the TH indicates sCOD and tCOD after pretreatment.

Biomethanization factor of the substrates were estimated as the percentage of the experimental to the theoretical methane yield (Eq. 2).

$$\%BM = \frac{mLCH_4 / gVS_{fed}}{(350 mLCH_4 / gtCOD_{rem})(gtCOD / gVS)} \times 100 \quad (2)$$

The cumulative methane production from the experiments were fitted by a combination of the First Order model and the Modified Gompertz model (Eq. 3) [18].

$$B = B_{01}.[1-\exp(-k_H.t)] + B_{02}.\exp\left\{-\exp\left[\frac{R_m.e}{B_{02}}(\lambda - t)+1\right]\right\} \quad (3)$$

In this equation, B represents the cumulative methane production (mLCH4 gVS-1) and t the length of the assay (d). The estimated parameters were: the methane production potential from microorganisms resistant to inhibitory compounds, B01 (mLCH4 gVS-1), reported to systems were the hydrolysis reaction is the rate-limiting step of the global process; the methane potential from microorganisms able to acclimate in the pretreatment medium, B02 (mLCH4 gVS-1), reported to systems were inhibitory behavior is observed; the hydrolysis coefficient, kH (d-1); the maximum methane production rate, Rm (mLCH4 gVS-1 d-1); and the lag-phase, λ (d).

3. Results and discussion

3.1. Substrate characterization

Pretreated biomass was fractionated on liquid and solid phase and all substrate characterized in terms of solids content (total and volatile), COD (total and soluble) and pH (Table 2). The values of the solubilization factors (SF) are also presented in this table. The soluble fraction as a result of the pretreatments, named "liquid phase" in this work, refers to the portion of substrate which passed through the sieve (#1 mm). The sCOD parameter was performed by filtering, according to Standard Methods methodology, and thus used to calculate the solubilization factor.

Table 2: Pretreated substrates characterization in terms of solids, chemical oxygen demand, pH, and solubilization factors

	Temperature	Time	Phase	TS	VS	tCOD	sCOD	pH	SF
				g kg^{-1}	g kg^{-1}	g kg^{-1}	g kg^{-1}	-	%
TH	170 ºC	5 min	liquid	2.96	1.46	1.30	0.99	6.98	8.08
			solid	56.02	51.35	78.60	-	-	-
		30 min	liquid	2.07	1.15	1.41	0.44	6.99	11.82
			solid	66.64	61.98	88.42	-	-	-
	210 ºC	5 min	liquid	1.51	0.78	0.96	0.41	7.13	9.91
			solid	51.17	48.45	70.69	-	-	-
		30 min	liquid	1.19	0.70	0.89	0.44	6.47	16.09
			solid	63.68	59.43	85.33	-	-	-
TH + SE	170 ºC	5 min	liquid	3.39	2.68	2.49	1.06	6.43	11.64
			solid	59.18	54.33	64.35	-	-	-
		30 min	liquid	2.25	1.34	0.68	0.61	6.32	13.95
			solid	41.68	38.90	60.57	-	-	-
	210 ºC	5 min	liquid	2.76	1.69	2.21	0.96	7.78	17.05
			solid	58.49	54.19	78.35	-	-	-
		30 min	liquid	1.46	0.86	1.26	0.59	6.66	22.90
			solid	61.80	57.89	74.62	-	-	-

The mass balance of the pretreatment process, evaluated on relation to the total solid input on the hydrolysis plant and thus collected on the pretreated bulk (data not shown), for the condition without steam explosion evidenced the high mass loss in the experiments at low temperature and short cooking time. In the pretreatment at 170 ºC - 5 min (TH), only 66% of the biomass fed was recovered on the system, while for the pretreatment of 210 ºC - 30 min with flash (TH + SE) 87% of the solids were recovered. Steam explosion is related to become a mass loss for high severity process, where the flash intensity causes the material blown down from the vortex throw-out the exhaust port resulting in the evaporation of the products [19]. This author recovered 88% on a steam explosion test at 230 ºC - 45 min, which is a similar value to the one obtained in this work. This author considered this loss due to the flash effect. However, we observed on this work that the same aggressiveness without steam explosion resulted in a higher mass loss compared to the experiment with steam explosion. The reason is the equipment configuration and operation: for low variations in pressure (low temperatures) and short cooking time, there is a small water condensation and a high content of particles with low density. These particles of biomass remained attached to the reactor walls, and did not exit to the final vessel at the end of the pretreatment. In fact, the sudden decompression was the main effect for substrate recovery.

Regarding the characterization parameters, the TS concentration released to the liquid medium in the pretreatments ranged from 49% for the extreme pretreatment condition (210 ºC - 30 min TH + SE) to 23% for the mildest condition (170 ºC - 5 min TH), considering the dilution factor of each pretreatment. pH values in the liquid fraction presented a small variation, the pH value of 6.32 was the lowest, relative to the sample 170 ºC - 30 min TH + SE. This acidification is close to the inhibition limit for the biomethanation process, since the activity of methanogenic microorganisms decreases at pH values less than 6.5 [20].

The solubilization factor increased with the steam explosion effect for all the pretreatment conditions. The lower increment obtained was from 11.82% to 13.95% for conditions 170 ºC - 30 min TH and thus at TH + SE, respectively. In addition, the highest increment was from 9.91% to 17.05% for conditions 210 ºC - 5 min TH and thus at TH + SE, respectively. Consider the cooking time, higher increments were obtained for pretreatments without steam explosion. SF increased 46.3% from 5 min to 30 min at 170 ºC TH and 62.4% from 5 min to 30 min at 210 ºC TH. For pretreatments with steam explosion the increment was of 19.9% and 34.3% from 5 min to 30 min at 170 ºC and 210 ºC, respectively. For pretreatments without steam explosion, is evident the positive effect of cooking time parameter on the solubilization increment, however for pretreatments with steam explosion, the cooking time was less effective, approximately 50% lesser.

The results evidence that the steam explosion is the major responsible of the increment in solubilization for this lignocellulose biomass, mainly for pretreatments at cooking time of 5 min, followed by the temperature condition and, finally, by the hydrolysis time, that has the lowest positive effect on the biomass solubilization. Nevertheless, for pretreatments without steam explosion, the hydrolysis time presented a higher increment effect on the biomass solubilization. Similar conclusions regarding the operation conditions were found on an experiment of sewage sludge pretreated at 170 ºC from 0 to 30 min [21].

Liquid phase samples of *Thypha angustifolia* thermal hydrolysis pretreated (Chapter 4.2) were more acidified than the samples of this experiment. This result corroborate with the low solubilization factor obtained on this

experiment, resulting in a less secondary degradation of monosacchrides to by-products [22]. Evidencing that *Eichhornia crassipes* has a more resistant structure for thermal hydrolysis pretreatment.

3.2. Methane production

Theoretical methane yield (NmLCH4 gVS-1) was determined from the performed substrate characterization [23] on raw, liquid and solid samples of *Eichhornia crassipes*, were as follows: 466.5 mLCH4 gVS-1 for raw, 429.9 mLCH4 gVS-1 for liquid phase, and 504.3 mLCH4 gVS-1 for solid phase. Increments on tCOD/VS ratio of solid samples increased the theoretical methane yield of this substrate, shown an increment on coefficient of specific organic matter conversion to COD of pretreated solid samples [24]. However, the tCOD/VS ratio reduction of liquid samples decreased its theoretical methane yield.

The methane production of raw and pretreated water hyacinth biomass were evaluated with BMP tests. The methane content in the biogas were always in the range 60 to 71% for all the substrates. Figure 1 shows the methane production profile of the samples pretreated at 170 ºC - 5 and 30 min at TH and TH + SE in the liquid and solid samples, including the untreated substrate (Raw) and the control (Cr).

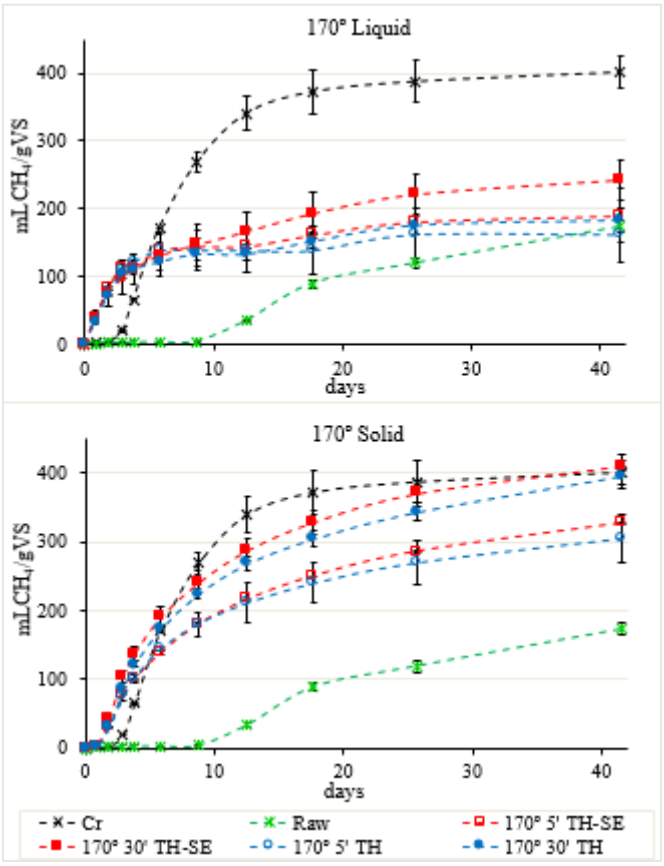

Figure 1: Cumulative methane production profile of control, raw, and liquid and solid fraction of water hyacinth biomass pretreated at 170 ºC for 5 and 30 min upon thermal hydrolysis + steam explosion (TH + SE) and only thermal hydrolysis (TH) process.

The total methane production of the raw substrate was 174 mLCH4 gVS-1, with approximately 9 days of lag-phase. The evaluation of the results of the solid fractions performed at pretreatments of 170 ºC evidence the enhancement in the methane yield with respect to the raw water hyacinth biomass for all pretreated substrates. The smaller improvement was 1.76 (170 ºC - 5 min TH), in relation to the raw substrate, and the best pretreatment condition improved 2.36 (170 ºC - 30 min TH + SE).

The methane production of the liquid fraction was lower than the one observed for the solid fraction. The lowest methane production factor was 0.93 (170 ºC - 5 min TH), in relation to the raw substrate, and the highest 1.40 for the higher cooking time and with steam explosion effect (170 ºC - 30 min TH + SE). This fact demonstrates the combined effect of time and steam explosion for large cooking time pretreatment for the liquid fraction substrate.

Different behavior was observed for the methane production of solid and liquid fractions. For solids, the cooking time was the most important operational condition to increase the methane production, whereas for the liquid phase, the combination of time and flash provided the higher increment in productivity for the liquid phase.

Figure 2 shows the methane production profile of the samples pretreated at 210 ºC for 5 and 30 min for TH and TH + SE in liquid and solid samples, including the untreated substrate (Raw) and the control (Cr). All pretreated substrates of the solid fraction presented an increment in methane production on relation to the raw biomass. The lowest factor of increment was 1.86 (210 ºC - 5 min TH), and the highest was 2.43 (210 ºC - 5 min TH + SE).

The main difference in the methane production among pretreatment conditions was for cooking time variation, for the solid samples 210 ºC from 5 to 30 min TH + SE. The cooking time decreased the methane production of the substrate 210 ºC – 30 min on 19%, as compared to the substrate 210 ºC – 5 min, as result of the steam explosion effect. The methane production of the substrates without flash (TH) did not presented a significant variation.

For the liquid fraction, it was also observed an increment on the methane production for all the substrates. The methane production factor for the samples pretreated at 5 and 30 min TH + SE were 1.30 and 1.67, respectively, and 1.02 and 1.09 for the samples submitted only to TH, on relation to the raw biomass productivity.

It is evident that the highest effect was obtained by the steam explosion in the samples at 30 min cooking time. Once again, it is clear the intrinsic combination of the time increase and the steam explosion effect upon the solubilization of the substrate.

For liquid fraction samples, also the main difference in methane production among pretreatment conditions was for cooking time variation, however as contrary of the trend found for solid sample. While for solid sample the methane production decreased 19% for sample 210 ºC – 5 min to 30 min, on liquid samples the methane production increased 22% form sample 210 ºC – 5 min to 30 min TH – SE.

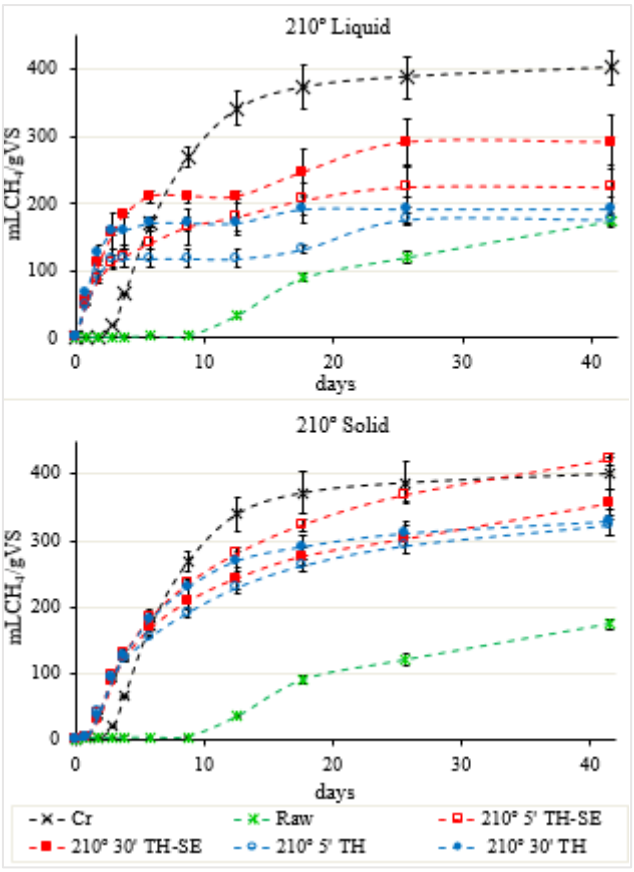

Figure 2: Cumulative methane production profile of control, raw, and liquid and solid fraction of water hyacinth biomass pretreated at 210 ºC for 4 and 30 min upon thermal hydrolysis + steam explosion (TH + SE) and only thermal hydrolysis (TH) process.

Performing the relationship evaluation of the methane production and the solubilization factor (Figure 3). The first evidence is that the solubilization increased with the pretreatment aggressiveness and was higher for the pretreatments with steam explosion (TH + SE). The methane production also increased in the samples with steam explosion, exhibiting a relationship between solubilization increment with the steam explosion and the consequent enhancement in methane production. However, the trend was not the same for the operational conditions, and especially for the solid samples, because the methane production decreased for the highest temperature.

Figure 3 also shows a great variation between the samples of liquid and solid fractions. The best condition for the liquid substrate biomethanization was for 210 ºC - 30 min TH + SE, while 210 ºC - 5 min TH + SE was the best for the solid fraction. The variation in the methane production between these fractions was higher than 100 mLCH4 gVS-1.

For the pretreatments 170 ºC - 30 min TH and 210 ºC - 5 min TH + SE, it is evident the increment in the methane yield of the solid fraction with respect to the liquid, even in spite of the low solubilization increase in these substrates. A possible explanation may be the increment in the surface area and exposure of accessible carbohydrates in the particle structure, allowing the microorganisms attack. Whereas, for higher aggressiveness

pretreatments (210 ºC at 5 min TH and 210 ºC at 30 min TH + SE) the solubilization increase, with the consequent solid biomethanation decrease as well as the methane production of the liquid fraction. The methane production increment of the liquid phase pretreated at aggressiveness conditions found for this biomass, *Eichhornia crassipes*, evidenced a positive effect, contrary at thus found for Typha angustifolia, where all pretreatment at 210 ºC decreased the methane production of the substrate. This behavior shown the structural resistance of *Eichhornia crassipes* biomass to degradation, reducing the secondary degradation of monomers to by-products, and it lost as volatilization, and inhibition or recalcitrance compounds production.

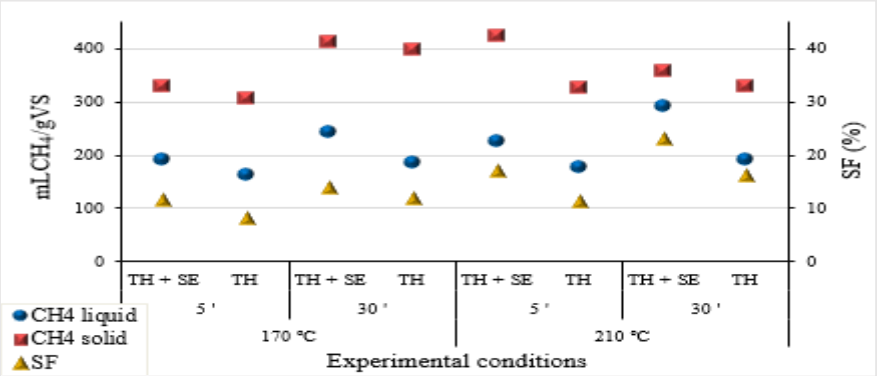

Figure 3: Solubilization factor and total methane production of liquid and solid fractions of the pretreated substrates submitted to thermal hydrolysis (TH) and to thermal hydrolysis + steam explosion (TH + SE) effect.

The biomethanization factor is an important parameter to evaluate the methane yield with respect to the maximum theoretical methane production of the substrate. The solubilization factor and the substrate biomethanization are plotted in Figure 4, comparing the biomass solubilization of the pretreatments and the potential of the microorganisms to degrade the substrate fed upon the methane production. The biomethanization of the control sample (microcrystalline cellulose) was 96.8%, evidencing the high methanogenic activity of the inoculum, for raw substrate the biomethanization was 37.3%.

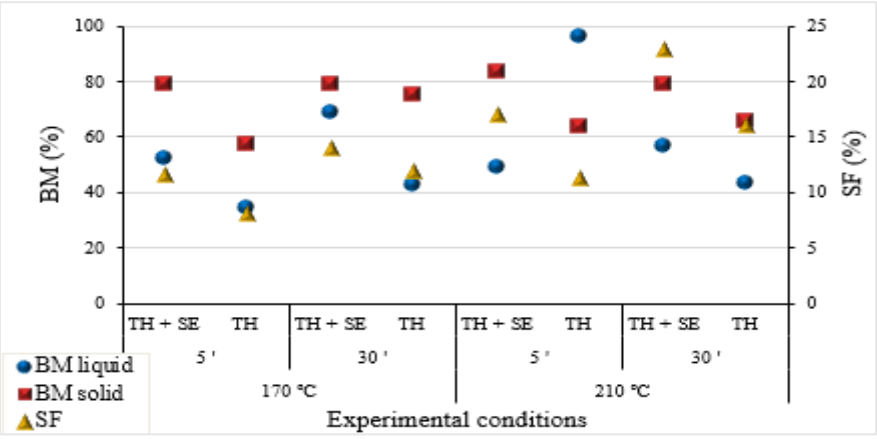

Figure 4: Solubilization and biomethanization factor of liquid and solid fraction substrates on biomethanation process after thermal hydrolysis pretreatment (TH) and thermal hydrolysis followed by steam explosion pretreatment (TH + SE).

All the substrates showed an increase in biomethanization, except for the liquid fraction pretreated at 170 ºC - 5 min TH, which presented a biomethanization ratio of 0.90 on relation to the raw substrate. The highest BM increment (2.2) was obtained for the solid fraction of pretreatment 210 ºC - 5 min TH + SE.

The biomethanization of the substrate fractions presented the same trend for both pretreatments at 170 ºC - 5 min (TH and TH + SE), being the ratio of 2.1 and 1.6 for TH + SE and TH, respectively, on relation to the raw substrate. All the solid substrates presented a higher biomethanization compared to the liquid fraction. The sample 170 ºC - 30 min TH + SE shown a higher biomethanization increment for both substrate fractions. On the other samples, the solid fraction was higher than the liquid one.

Sample 210 ºC - 30 min TH + SE provided the highest solubilization for the liquid and solid substrates, however the biomethanization of the liquid decreased on relation to the pretreatment 170 ºC - 30 min TH + SE for the solid in relation 210 ºC - 5 min TH + SE. As a conclusion, biomethanization of the liquid was negatively affected by the temperature increase, probably due to the production of inhibitors and recalcitrant compounds. In addition, the solid biomethanization was affected by the steam explosion combined to the cooking time, which resulted in the intensification of the flash effect.

3.3. Kinetic parameters

Kinetic parameters give substantial information of the anaerobic digestion system, like details of the microorganism activity and the metabolism upon the substrate available on the system. In this way, a recent study proposed a new mathematic model [18], as a result of the combination of two known models, the First Order and the Modified Gompertz. Based on this combination, the authors referred to the possibility to estimate the methane production according with the limiting step of the process. The limiting steps considered on this equation were: the microorganisms resistance to inhibitory compounds (parameter B01), being the enzymatic hydrolysis the limitation; and the methane production related to microorganisms able to acclimate in the pretreated medium (parameter B02). Therefore, the methane production profile obtained by BMP tests was fitted by this model combination and the results are presented in Table 3.

The solid substrates provided a higher methane production for the parameter B01. As expected, the enzymatic hydrolysis on the biomethanation process is the limiting step for the particle degradation. All pretreatments conditions for hydrolysis coefficient, maximum methane production rate and lag-phase parameters presented similar values, with low variation.

Evidencing that the pretreatments conditions evaluated on this study had low effects on the kinetics parameters of the solid fraction of the *Eichhornia crassipes* biomass. Comparing the pretreated samples to the raw biomass, it is observed a lag-phase reduction of approximately 7 days for all the solid fraction substrates and an increment of approximately 24 mLCH4 gVS-1 d-1 on the maximum methane production rate. These results evidence that the lag time is related to the enzymatic hydrolysis reaction of the particulate substrates and the pretreatment conditions tested in this work were able to reduce expressively this critical step of the anaerobic digestion.

Table 3: Kinetic parameters calculated by fitting the methane production of the raw and pretreated substrates using the Combined First Order + Modified Gompertz model

			B_{01} mLCH4 gVS^{-1}	k_H d^{-1}	B_{02} mLCH4 gVS^{-1}	R_m $mLCH_4$ gVS^{-1} d^{-1}	λ d	R^2 -
Raw			0	4.984	176	8.4	8.4	0.989
Liquid	TH + SE	210º 5'	162	0.102	69	51.4	0.1	0.998
		210º 30'	163	0.040	165	64.0	0.1	0.987
		170º 5'	97	0.048	108	50.0	0.2	0.994
		170º 30'	187	0.057	74	38.2	0.1	0.999
	TH	210º 5'	235	0.001	104	78.6	0.2	0.967
		210º 30'	44	0.079	150	91.8	0.2	0.995
		170º 5'	65	0.020	126	45.7	0.1	0.991
		170º 30'	108	0.040	98	44.0	0.2	0.995
Solid	TH + SE	210º 5'	361	0.052	105	31.1	1.6	0.999
		210º 30'	279	0.044	119	38.1	1.6	0.999
		170º 5'	286	0.055	71	23.3	1.6	0.999
		170º 30'	340	0.063	97	32.7	1.6	0.999
	TH	210º 5'	269	0.068	72	32.3	1.5	0.999
		210º 30'	203	0.079	135	32.5	1.5	0.998
		170º 5'	238	0.052	94	27.9	1.5	0.999
		170º 30'	325	0.049	115	31.7	1.7	0.999

The liquid fraction presented variations on the kinetic parameters upon the conditions tested. The light aggressiveness pretreatment (170 ºC - 5 min) on TH and TH + SE evidenced that microorganisms were affected by inhibitory compounds due to the high B02 methane production. The intermediate pretreatments (170 and 210 ºC at 30 and 5 min, respectively) shown the hydrolysis as the limiting step due to the high B01 methane production. Half of the methane production of the sample 210 ºC - 30 min also belongs to the First Order model, demonstrating also the hydrolysis limitation. At last, the sample 170 ºC - 5 min TH + SE and TH was also limited by microorganism's inhibition. The limiting effects obtained according to the combined model presented by Bolado-Rodríguez and his colleagues (2016) did not provided clear information for the liquid samples,

probably due to the high content of soluble organic matter and due to the aggressiveness of the pretreatments, that are largely related to inhibitory effects [3,25,26]. However, an expressive increase on the maximum methane production rate was obtained in relation to the raw material and for the solid fractions. Also was calculated a lag-phase reduction of approximately 8 days in relation to the raw substrate.

The hydrolysis coefficient did not show a clear trend for the pretreated substrates with high B01 values (First Order model), being not a good indicator of kinetic effects of the pretreatments [27].

Additional research work regarding the characterization of the compounds released on the liquid phase is necessary to explain the behavior of the methane production and the limiting effects predicted by the combined model evaluated by this work.

3.4. Morphology effects

SEM analysis was performed to evaluate the surface structure of the solid substrate, comparing the raw and the pretreatments effects due time, temperature and steam explosion conditions. Figure 5 shows the effects of pretreatments and the structural changes of the samples pretreated at 170 ºC, for 5 and 30 min, also as TH + SE and TH, compared to the raw biomass and Figure 6 also shows the effects of the pretreatments at 210 ºC.

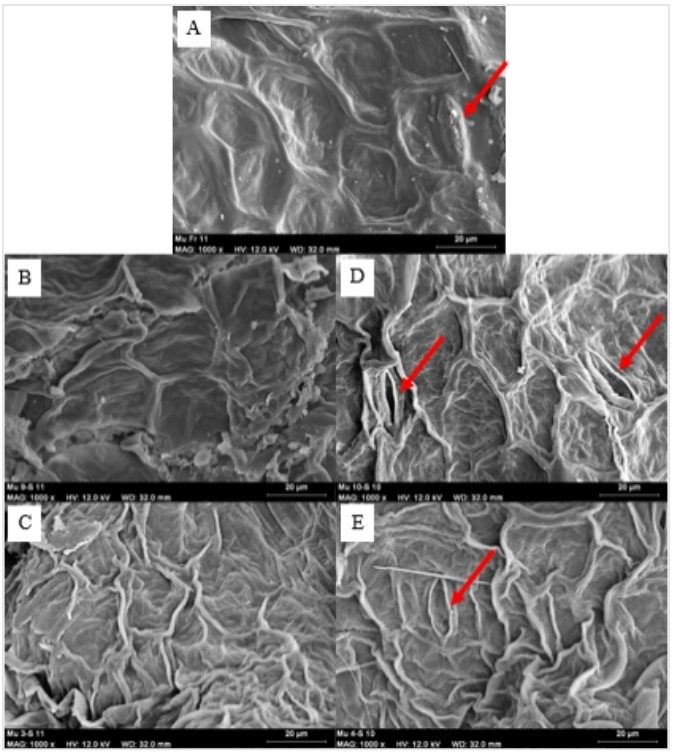

Figure 5: SEM micrographs of raw water hyacinth biomass (A) with detail of a closed stoma (arrow); on 170 ºC - 5 min TH (B); 170 ºC - 5 min TH + SE (C); 170 ºC - 30 min TH (D) with detail of an opened stoma (arrow); and 170 ºC - 30 min TH + SE (E) with detail of a completely open stoma (arrow). Scale bar of 20 µm.

The raw water hyacinth substrate (Figure 5A) evidenced the cell wall forming the epidermal cell. The epidermal cell appears to be wilt, as a probable consequence of the sample drying (proceedings for the analysis performance); however, the structure is intact, with smooth and homogeneous surface, without indication of degradability. In addition, it is possible to identify the structure of a closed stoma (arrow).

The samples at 5 min cooking time for TH and TH + SE (Figure 5B-C) presented a significant change on the biomass surface, with the increase on the roughness of the plant tissue, especially for the TH + SE pretreatment. The same trends are observed for the operation time of 30 min (Figure 5D-E). The turgidity loss of the cells epidermal is proportional to the pretreatment aggressiveness. The roughness is greater for the pretreatments with steam explosion, putting in evidence the anticlinal cell wall due to the high relief of this structure and the formation of concavities in the central surface of the cell. On these images, it is possible to identify stomas structures that present a degraded morphology due to the pretreatment effect.

The condition 170 ºC - 5 min presented the great morphology change between the pretreatments TH + SE and TH. The effect of the sample TH + SE was similar for 30 min, corroborating with the biodegradability increment and evidenced that the main effect of the pretreatment was the particle size reduction. The water hyacinth substrate pretreated at 210 ºC (Figure 6) compared to the raw biomass (Figure 6A) also evidenced the degradation increase of the substrate morphology upon the pretreatment process. In this case, the conditions without steam explosion (Figure 6B-D) demonstrated the increment on the biomass degradability with similar effects of those found for the pretreatments at 170 ºC - 5 and 30 min TH + SE. Also it is possible to observe an expressive increment on the roughness of the plant tissue, evidencing the anticlinal wall, due to the high relief of this structure and the concavities formation in the central surface of the cell. The stomata identified on this pretreated image also demonstrates the degradation effect of the process, visible due to the presence of breaks on this fiber structure. On the other hand, the steam explosion evidenced the additional anticlinal wall degradation, being this effect more aggressive for the cooking time of 30 min (Figure 6E) and not evidenced on pretreatments at 170 ºC. The fibers that crop up on the pretreatment without steam explosion were pulled out of the matrix (delignification), being released to the bulk and exposed the matrix. Micrographs of delignification was also observed on switchgrass for pretreatments of cellulose solvent-based lignocellulose fractionation and soaking in aqueous ammonia [28]. On this structure, it was not possible to identify stomas, probably due to the high degradability of the whole structure. The increase on the roughness surface was likewise observed on a solid state fermentation and alkali pretreatment of bagasse for cellulose production [29] and as a consequence of the intensity increase of the pretreatment [30]. In this study, the authors evaluated the alkali pretreatment on narrow-leaf cattail for ethanol production. The porosity increment was an additional effect observed on the sample 210 ºC -30 min TH + SE (Figure 6F) as a result of the pretreatment effect. The results obtained demonstrated the effect of the temperature of reaction and the flash (steam explosion) on the solid substrate, however the low temperature presented similar morphology degradation, evidencing that the time had a low influence on the porosity increment. These pores are largely reported to increase the enzyme-accessible surface area which increases the enzymatic hydrolysis step of the anaerobic digestion [31]. The porosity increment on the surface biomass did not provided clear evidence regarding the biodegradability increment on this substrate, demonstrating that the particle size reduction by the thermal hydrolysis pretreatment presented higher effect on the biomethanization process of water hyacinth biomass than the porosity increment.

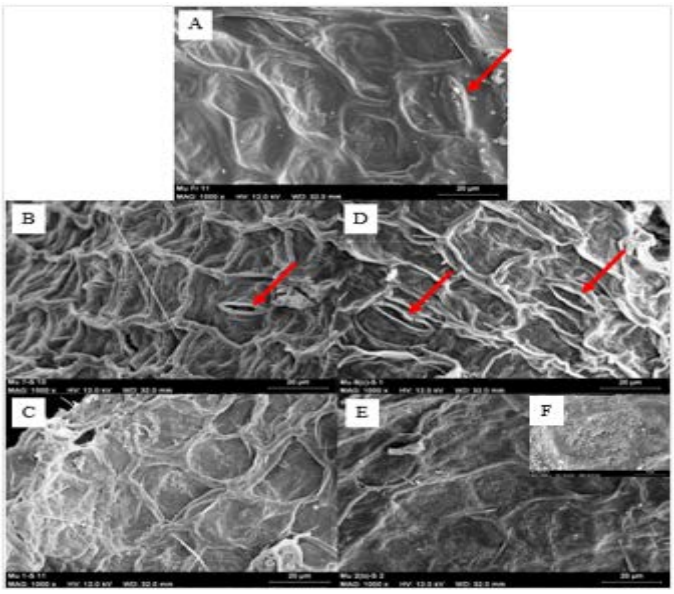

Figure 6: SEM micrographs of raw water hyacinth biomass (A); 210 ºC - 5 min TH (B) with detail of an opened stoma (arrow); 210 ºC - 5 min TH + SE (C); 210 ºC - 30 min TH (D) with detail of a opened stoma (arrow); and 210 ºC - 30 min TH + SE (E) with detail of a zoom image at scale bar of 5 µm (F). Scale bar of 20 µm.

Furthermore, the presence of lignin drops was observed at high density on the sample 210 ºC - 30 min TH + SE and on lower density on the sample of 210 ºC - 5 min TH + SE (Figure 7). Evidencing that the lignin recondensation was also influenced by the temperature and flash effect on the pretreatment. Study comparing different temperature regimes upon microwave assisted chemical pretreatment concluded that temperature values up to 200 ºC increased substantially the deposition of lignin droplets on the biomass surface [32], [33].

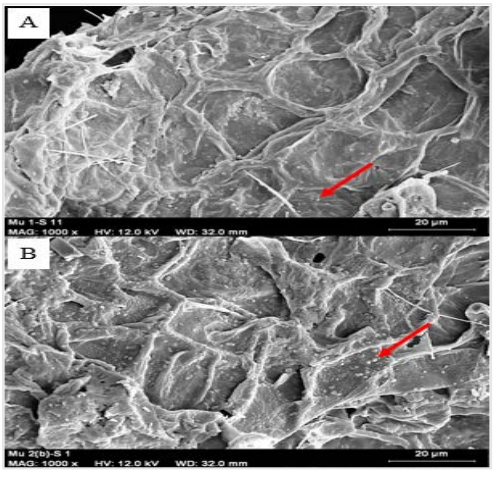

Figure 7: SEM micrographs of the lignin drops formed on the solid surface through the soluble lignin recondensation at high temperature on TH + SE pretreatment. 210 ºC - 5 min (A); and 210 ºC - 30 min (B). Scale bar of 20 µm.

The lignin droplets, also named as a new Klason lignin, are related to be produced on stream exploded biomass and acid treated. This substance is formed by carbohydrates that are modified by acid catalysis or reforming reactions, forming unsaturated carbon, such that the products are polyphenolic in structure [34]. This compound consume carbohydrates, that are an important substrate released on the pretreatment and could hamper the microorganism activity [34]. Vivekanand and his colleagues (2013) observed no inhibitory effect of the anaerobic consortia to substrate with this compound and observed an apparently consumption of these structures as substrates.

All the physic changes on the substrate morphology are in agreement with the variations of the solubilization factor by the pretreatments. The solubilization and these structural effects on the biomass tissue corroborate with the evidence that the temperature and the steam explosion are important factors that drive the behavior of the pretreatment process. Time had a small effect on the pretreatment, especially for low temperature of operation. In this way, the higher effect was found for the operation at 210 ºC - 5 min TH + SE. The biomethanization also followed this trend, however with an increment on the solid and liquid fraction biodegradability of the substrates that presented a low degradation due the mildest temperature. These results evidenced that the solubilization is not directly associated to the biomethanization. Operational conditions at weak aggressiveness are in general terms more biodegradable compared to the high aggressiveness effect, as is the case of the sample 210 ºC - 30 min TH + SE, which presented the higher solubilization, however with no proportional increment on the biomethanization. The biodegradability decrease on the liquid samples are probably related to the formation of inhibitory compounds due to the pretreatment aggressiveness, as evidenced by the combined mathematical model through the low production of the B01 (methane production potential from microorganisms resistant of inhibitory compounds) parameter (from 187 to 163 mLCH4 gVS-1, at 170 ºC – 30 min to 210 ºC - 30 min TH + SE, and from 235 to 44 mLCH4 gVS-1, at 210 ºC – 5 min to 210 ºC - 30 min TH, respectively).

The biomethanization stabiliti of the solid fraction at 210 ºC TH + SE, as compared to the substrate at 170 ºC TH + SE, evidenced that the aggressiveness temperature condition of 170 ºC TH + SE is enough to reach the maximum biomethanization of the solid fraction substrate. The release of the easily biodegradable compounds at this condition resulted in the remaining of slow biodegradable organic matter on the particulate substrate. Also low biomethanization increment was observed when comparing the treatments at 170 ºC TH + SE and 210 ºC TH + SE.

Study demonstrated that the increase in cellulose accessibility is more important than the enhancement of the delignification of the biomass [28]. It could be the consequence to the high biodegradability of a low solubilized biomass. The pretreatments at 170 ºC avoided the access to the easily biodegradable substrates, fact that are more effective on the biomethanation process than the increase in the solubility, such as the lignification and the degradation of sugars in recalcitrant compounds due to a secondary degradation [22], [36]–[38].

4. CONCLUSIONS

Thermal hydrolysis pretreatment provided increment on the solubilization factor which was in agreement with the aggressiveness increase. Steam explosion, temperature and time, in order of significance, provided the

higher increments on the biomass solubilization. The methane production and biomethanization of the solid fraction was higher than the liquid for all experiments. Sample pretreated at 170 ºC - 30 min followed by steam explosion presented the higher biomthanization increment considering both fractions. The combined model proposed by Bolado-Rodríguez and his colleagues (2016) evidenced the enzymatic hydrolysis reaction as the limiting step of the particulate substrate of the anaerobic digestion. The biodegradability limitation of the liquid fraction evidenced an inhibitory effect. The increase on the surface area of the substrate was the most effective effect on the biomethanization obtained by the pretreatments, according to the substrate morphology.

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References

[1] P. E. Ndimele, C. A. Kumolu-Johnson, and M. A. Anetekhai, “The Invasive Aquatic Macrophyte, Water Hyacinth {Eichhornia crassipes (Mart.) Solm-Laubach: Pontedericeae}: Problems and Prospects,” Res. J. Environ. Sci., vol. 5, no. 6, pp. 509–520, 2011.

[2] R. Chandra, H. Takeuchi, and T. Hasegawa, “Methane production from lignocellulosic agricultural crop wastes : A review in context to second generation of biofuel production,” Renew. Sustain. Energy Rev., vol. 16, no. 3, pp. 1462–1476, 2012.

[3] J. Ariunbaatar, A. Panico, G. Esposito, F. Pirozzi, and P. N. L. Lens, “Pretreatment methods to enhance anaerobic digestion of organic solid waste,” Appl. Energy, vol. 123, pp. 143–156, 2014.

[4] H. Carrere, G. Antonopoulou, R. Affes, F. Passos, A. Battimelli, G. Lyberatos, and I. Ferrer, “Review of feedstock pretreatment strategies for improved anaerobic digestion: From lab-scale research to full-scale application,” Bioresour. Technol., vol. 199, pp. 386–397, 2015.

[5] F. Fdz-Polanco, R. Velazquez, S. I. Perez-Elvira, C. Casas, D. del Barrio, F. J. Cantero, M. Fdz-Polanco, P. Rodriguez, L. Panizo, J. Serrat, and P. Rouge, “Continuous thermal hydrolysis and energy integration in sludge anaerobic digestion plants.,” Water Sci. Technol., vol. 57, no. 8, pp. 1221–6, Jan. 2008.

[6] M. Carlsson, A. Lagerkvist, and F. Morgan-Sagastume, “The effects of substrate pre-treatment on anaerobic digestion systems: a review,” Waste Manag., vol. 32, no. 9, pp. 1634–50, Sep. 2012.

[7] V. B. Agbor, N. Cicek, R. Sparling, A. Berlin, and D. B. Levin, “Biomass pretreatment: fundamentals toward application.,” Biotechnol. Adv., vol. 29, no. 6, pp. 675–685, 2011.

[8] H. Carrère, C. Dumas, a Battimelli, D. J. Batstone, J. P. Delgenès, J. P. Steyer, and I. Ferrer,

"Pretreatment methods to improve sludge anaerobic degradability: a review.," J. Hazard. Mater., vol. 183, no. 1–3, pp. 1–15, Nov. 2010.

[9] H. Shahriari, M. Warith, M. Hamoda, and K. J. Kennedy, "Anaerobic digestion of organic fraction of municipal solid waste combining two pretreatment modalities, high temperature microwave and hydrogen peroxide.," Waste Manag., vol. 32, no. 1, pp. 41–52, Jan. 2012.

[10] P. Kumar, D. M. Barrett, M. J. Delwiche, and P. Stroeve, "Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production," Ind. Eng. Chem. Res., vol. 48, no. 8, pp. 3713–3729, Apr. 2009.

[11] Z. Yu, B. Zhang, F. Yu, G. Xu, and A. Song, "A real explosion: The requirement of steam explosion pretreatment," Bioresour. Technol., vol. 121, pp. 335–341, 2012.

[12] X. Hou, F. Sun, D. Yan, H. Xu, Z. Dong, Q. Li, and Y. Yang, "Preparation of lightweight polypropylene composites reinforced by cotton stalk fibers from combined steam flash-explosion and alkaline treatment," J. Clean. Prod., vol. 83, pp. 454–462, 2014.

[13] Y. Sun and J. Cheng, "Hydrolysis of lignocellulosic materials for ethanol production: a review.," Bioresour. Technol., vol. 83, no. 1, pp. 1–11, May 2002.

[14] M. Zoulikha, M. Thierry, Z. J. Qiuyu, A. Nouviaire, and R. Sid-ahmed, "Combined steam-explosion toward vacuum and dilute-acid spraying of wheat straw. Impact of severity factor on enzymatic hydrolysis," Renew. Energy, vol. 78, pp. 516–526, 2015.

[15] APHA, Standard Methods for the Examination of Water and Wastewater, 21st ed. Washington: American Public Health Association, 2005.

[16] I. Angelidaki, M. Alves, D. Bolzonella, L. Borzacconi, J. L. Campos, a. J. Guwy, S. Kalyuzhnyi, P. Jenicek, and J. B. van Lier, "Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays," Water Sci. Technol., vol. 59, no. 5, p. 927, Mar. 2009.

[17] L. C. Ferreira, P. J. Nilsen, F. Fdz-polanco, and S. I. Pérez-elvira, "Biomethane potential of wheat straw : Influence of particle size , water impregnation and thermal hydrolysis," Chem. Eng. J., vol. 242, pp. 254–259, 2014.

[18] S. Bolado-Rodríguez, C. Toquero, J. Martín-Juárez, R. Travaini, and P. A. García-Encina, "Effect of thermal, acid, alkaline and alkaline-peroxide pretreatments on the biochemical methane potential and kinetics of the anaerobic digestion of wheat straw and sugarcane bagasse," Bioresour. Technol., vol. 201, pp. 182–190, 2016.

[19] A. Elliston, D. R. Wilson, N. Wellner, S. R. A. Collins, I. N. Roberts, and K. W. Waldron, "Effect of steam explosion on waste copier paper alone and in a mixed lignocellulosic substrate on saccharification and fermentation," Bioresour. Technol., vol. 187, pp. 136–143, 2015.

[20] R. Leitao, A. Vanhaandel, G. Zeeman, and G. Lettinga, "The effects of operational and environmental variations on anaerobic wastewater treatment systems: A review," Bioresour. Technol., vol. 97, no. 9, pp. 1105–1118, 2006.

[21] A. Donoso-Bravo, S. Pérez-Elvira, E. Aymerich, and F. Fdz-Polanco, "Assessment of the influence of thermal pre-treatment time on the macromolecular composition and anaerobic biodegradability of sewage sludge," Bioresour. Technol., vol. 102, no. 2, pp. 660–666, 2011.

[22] C. K. Nitsos, K. A. Matis, and K. S. Triantafyllidis, "Optimization of Hydrothermal Pretreatment of Lignocellulosic Biomass in the Bioethanol Production Process," ChemSusChem, vol. 6, no. 1, pp. 110–122, Jan. 2013.

[23] L. C. G. Ferreira, "Evaluación De La Biodegradabilidad Anaerobia De Residuos Orgánicos Pre-Tratados Térmicamente," Universidad de Valladolid, 2013.

[24] S. F. de Aquino, S. de Q. Silva, and C. A. de L. Chernicharo, "Considerações práticas sobre o teste de demanda química de oxigênio (DQO) aplicado a análise de efluentes anaeróbios," Eng. Sanitária e Ambient., vol. 11, no. 4, pp. 295–304, 2006.

[25] F. Monlau, C. Sambusiti, A. Barakat, M. Quéméneur, E. Trably, J.-P. Steyer, and H. Carrère, "Do furanic and phenolic compounds of lignocellulosic and algae biomass hydrolyzate inhibit anaerobic mixed cultures? A comprehensive review.," Biotechnol. Adv., vol. 32, no. 5, pp. 934–951, 2014.

[26] Y. Zheng, J. Zhao, F. Xu, and Y. Li, "Pretreatment of lignocellulosic biomass for enhanced biogas production," Prog. Energy Combust. Sci., pp. 1–19, Feb. 2014.

[27] A. Donoso-Bravo, S. I. Pérez-Elvira, and F. Fdz-Polanco, "Application of simplified models for anaerobic biodegradability tests. Evaluation of pre-treatment processes," Chem. Eng. J., vol. 160, no. 2, pp. 607–614, Jun. 2010.

[28] J. a. Rollin, Z. Zhu, N. Sathitsuksanoh, and Y. H. P. Zhang, "Increasing cellulose accessibility is more important than removing lignin: A comparison of cellulose solvent-based lignocellulose fractionation and soaking in aqueous ammonia," Biotechnol. Bioeng., vol. 108, no. 1, pp. 22–30, 2011.

[29] S. Anuradha Jabasingh and C. Valli Nachiyar, "Utilization of pretreated bagasse for the sustainable bioproduction of cellulase by Aspergillus nidulans MTCC344 using response surface methodology," Ind. Crops Prod., vol. 34, no. 3, pp. 1564–1571, 2011.

[30] A. Ruangmee and C. Sangwichien, "Statistical optimization for alkali pretreatment conditions of narrow-leaf cattail by response surface methodology," Songklanakarin J. Sci. Technol., vol. 35, no. 4, pp. 443–450, 2013.

[31] N. Mosier, R. Hendrickson, N. Ho, M. Sedlak, and M. R. Ladisch, "Optimization of pH controlled liquid hot water pretreatment of corn stover," Bioresour. Technol., vol. 96, no. 18 SPEC. ISS., pp. 1986–1993, 2005.

[32] Z. Zhu, D. J. Macquarrie, R. Simister, L. D. Gomez, and S. J. M. Mason, "Microwave assisted chemical pretreatment of Miscanthus under different temperature regimes," Sustain Chem Process, vol. 3, no. 15, pp. 1–13, 2015.

[33] M. J. Selig, S. Viamajala, S. R. Decker, M. P. Tucker, M. E. Himmel, and T. B. Vinzant, "Deposition of Lignin Droplets Produced During Dilute Acid Pretreatment of Maize Stems Retards Enzymatic Hydrolysis of Cellulose," Biotechnol. Prog., vol. 23, no. 6, pp. 1333–1339, 2007.

[34] P. Sannigrahi, D. H. Kim, S. Jung, and A. Ragauskas, "Pseudo-lignin and pretreatment chemistry," Energy Environ. Sci., vol. 4, no. 4, pp. 1306–1310, 2011.

[35] V. Vivekanand, E. F. Olsen, V. G. H. Eijsink, and S. J. Horn, "Effect of different steam explosion conditions on methane potential and enzymatic saccharification of birch.," Bioresour. Technol., vol. 127, pp. 343–9, 2013.

[36] B. Danon, G. Marcotullio, and W. De Jong, "Mechanistic and kinetic aspects of pentose dehydration towards furfural in aqueous media employing homogeneous catalysis," Green Chem., vol. 16, pp. 39–54, 2014.

[37] G. Lissens, A. B. Thomsen, L. De Baere, W. Verstraete, and B. K. Ahring, "Thermal wet oxidation improves anaerobic biodegradability of raw and digested biowaste," Environ. Sci. Technol., vol. 38, no. 12, pp. 3418–3424, 2004.

[38] R. L. Souza, H. Yu, F. Rataboul, and N. Essayem, "5-Hydroxymethylfurfural (5-HMF) Production from Hexoses: Limits of Heterogeneous Catalysis in Hydrothermal Conditions and Potential of Concentrated Aqueous Organic Acids as Reactive Solvent System," Challenges, vol. 3, no. 2, pp. 212–232, 2012.