Thermal pre-treatment of lignocellulosic biomass as substrate for anaerobic digestion

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Abstract:

Purpose: Increasingly high energy demands have driven scientific research towards the production of alternative sources of energy. Lignocellulosic biomass represents a promising material for the production of renewable energy, such as biogas, mainly due to its wide availability and its relative low cost. Although the digestibility of lignocellulosic materials is usually low, suitably designed pre-treatment steps can significantly enhance the specific volumetric methane production. In this study, the thermal pre-treatment of lignocellulosic biomass and the simultaneous impact of the organic load rate (OLR) in anaerobic digestion (AD) were assessed for the first time.

Methods: Thermal pretreatment of wheat straw (WS) was carried out in an autoclave, (140°C, 2.75 bars pressure) operating at four different treatment retention times (0, 30, 60 and 90 minutes). The anaerobic biodegradability of the substrates was tested in batch scale under four different organic load rates (2, 4, 8 and 12 kg VS/m^3). The methane production data was modelled using a one phase exponential model and the kinetic coefficients hydrolysis constant (k) and maximum methane potential (Ymax) were calculated. The kinetic coefficients were used to estimate the output in a continuously fed reactor.

Results: For every OLR, the 60 minutes pre-treatment was superior to the other treatments in terms of the specific volumetric methane production. In the cases of OLR 4 and 8 kg VS/m^3, the pre-treatment resulted in a methane improvement up to 14%. At low OLR (2 Kg VS/m^3), methane improvement reached 95%, while in high ORL (12 kg VS/m^3) methane production was enhanced by 20%. Finally, for every OLR tested, the addition of the liquid fraction of the thermal treatment in the reactors was found to decrease the specific methane production compared to the runs that utilised solely the pre-treated solid WS residue.

Conclusions: Higher OLR could be favourable in anaerobic digestion systems operating with pre-treated lignocellulosic biomass as substrate.

Abbreviations

Wheat straw-WS, Anaerobic Digestion-AD, Organic Load Rate-OLR, Greenhouse Gases-GHG, High-Performance Liquid Chromatography-HPLC, United Kingdom-UK, Biochemical Methane Potential-BMP, Total Kjeldahl Nitrogen-TKN, Acid Soluble Lignin-ASL, Acid Detergent Lignin-ADL, Total Solids-TS, Volatile Solids-VS, Hydroxymethylfurfural-HMF.

Keywords

Anaerobic digestion, lignocellulosic biomass, Thermal pre-treatment, OLR, Wheat straw

1. Introduction

Driven by increasing life standards and an expanding human population, the energy demand worldwide is increasing rapidly. Waste production is following the same trend and the need for a sustainable way to manage them is imperative. The European Union is actively promoting the independence from traditional fuels, such as petroleum, and subsidizes the application of innovative, alternative and sustainable energy sources. In this direction, the UK has set a reduction target in greenhouse gas emissions (GHC) of 80% by 2050 [1]. Anaerobic Digestion (AD) transforms the energy that is present in a substrate in a non-utilizable form, such as cellulose, into renewable fuel, valuable nutrients and other macromolecules. The main product of AD is biogas, which can be used as a fuel in boilers and internal combustion engines. Even though AD is widely utilised as a valorisation method for low or even negative cost materials, many industrial scale reactors are still operating with energy crops as AD feed. [2]. The use of this type of substrate is offering substantial biomethane production but at the same time increases the cost of the whole process. Furthermore, the farming of energy crops is coming in direct competition with the land available for the cultivation of crops intended for human consumption, despite recent efforts to minimise this issue. An alternative candidate substrate, with high availability and low cost for AD systems, is lignocellulosic biomass, such as wheat straw (WS) [3]. Worldwide, straw is mostly used as feed and bedding material to ruminants while significant volumes are utilised as fuel for district heating. Finally, a small part of it is burned or left unused [3]. Unfortunately, the biodegradability of these materials is low due to the rigid structure of cellulose, hemicellulose and lignin [4]. Lignin is a non-anaerobically degradable and non-water soluble polymer and these two factors are limiting the biomethanation of lignocellulosic substrates. However, hemicellulose can be hydrolysed to simpler sugars that are assimilable by microorganisms during anaerobic digestion.

In order to improve the digestion efficiency of lignocellulosic biomass, a pre-treatment stage that will target fibre breakdown is often necessary in order to improve the solubilisation and biosorption of hemicellulose. Different approaches have been examined by the scientific community in the past including biological, physical and chemical processes, targeting the breakdown of lignocellulosic building blocks. Examples of such methods represent the use of fungi or bacteria, the use of acids or bases and the use of grinders to decrease the particle size of the substrates. Thermal and high pressure techniques, such as steam explosion [5] and hydrothermal treatment [6], outweigh chemical pre-treatments mostly due to zero production of hazardous wastes. During these procedures, biomass structure opens up due to thermal expansion, and this is causing a reduction of the particle size and an increase in the pore volume. Moreover, the polysaccharides present in the lignocellulosic materials are hydrolysed to simple sugars leading to higher degradation rates from the microorganisms of AD. As a result, the efficiency of the whole process can be increased while the retention time required for optimum biogas production is decreased [7]. Wang et al [8] reported that steam explosion disrupts the lignin structure in lignocellulotic substrates. This disruption can enhance the anaerobic biodegradability of such substrates, while the cellulose content becomes more accessible for the microbes [8]. However, these studies were only focused on the harshness factor effect of the pre-treatment on the subsequent biomethane potential [9, 10]. To date, there is no published research dealing with the optimisation of anaerobic digestion of thermal pre-treated lignocellulosic substrates.

The main aim of the present study was to establish the effect of different retention times in a thermal pre-treatment and the correlation between this and the organic load rate of mesophilic anaerobic bioreactors. In the second stage of the experiment, both the whole slurry from the pre-treatment and only the solid part of this were evaluated in AD systems.

2. Materials and methods

Inoculum and substrate

The effluent of a full scale biogas plant, digesting crops and working at mesophilic temperatures (42±2°C), was used as inoculum. Wheat straw (WS) was utilised as feed substrate for the bioreactors, which was collected from fields in the wider area of Nottinghamshire, UK. After collection, WS was

manually chopped (2-3 cm) and stored in a freezer (-20±2°C) until use. The inoculum was incubated at 37±2°C and degassed for one week in order to minimise the endogenic methane production.

Pre-Treatment

WS was pre-treated through a thermo-mechanical process, by the application of high temperature (140 °C) and pressure (2.75 bars) at different retention times (0, 30, 60 and 90 minutes). An autoclave working at the above conditions was used as a reactor for the pre-treatments. WS samples were placed in a 500 mL bottles and deionized water was added in order to obtain a solid content of 35% (w/v). After heating treatment, a rapid pressure drop followed until atmospheric pressure was reached. Solids were separated from the liquid with the application of a filter pump and were placed at 40°C overnight.

Biomethane potential tests

The experiments took place in batch mode, in glass vials with total volume of 150 mL. This study was conducted in two stages. In the first stage, four different organic load rates (2, 4, 8 and 12 Kg VS/m^3) were examined. The working volume was set to 70 mL, and different amounts of inoculum and substrate were added, while deionized water was used in order to keep inoculum to substrate ratio (I/S) constant at a value of 2. This value has been commonly used for BMP tests [11]. Blanks (vials with only inoculum) were also used to measure the endogenous CH_4 production and the obtained values were subtracted from those acquired from the vials with the substrate. On the second stage of the experiment, the effect of the liquid fraction of the pre-treatment on the AD process was examined. Vials with treated substrate (60 min treatment) were prepared with and without the addition of the liquid phase generated from the pre-treatment. Before every experiment, the vials were flushed with nitrogen gas for five minutes, sealed with rubber stoppers and aluminium caps and incubated at 37±2°C for 35 days. Methane production was measured daily, with the application of a liquid displacement meter, while sodium hydroxide was used for scrubbing the CO_2. Furthermore, vials with microcrystalline cellulose (Avicel) were chosen as positive controls as proposed by Flores et al [12]. All experiments took place in triplicate. Results of methane production were expressed in standard conditions of temperature and pressure and per unit of volatile solid (g VS).

The biomethane production data of the solid fraction of the pre-treated substrate was modelled using a one phase exponential model [13], which gave a good fit for all the curves tested. According to the model, the biomethane yield followed Equation 1.

$$Y = Ymax\ (1 - e^{-k(t-t_{lag})}) \qquad \text{Equation 1}$$

where Y= biomethane yield at time (t), Y_{max} = ultimate biomethane yield, k = hydrolysis constant and t_{lag} = lag phase.

The kinetic parameters were used to estimate the biomethane potential in a continuous reactor, using Equation 2;

$$Y_{RT} = Y_{max}\ (1 - \frac{1}{1+k \cdot RT}) \qquad \text{Equation 2}$$

where RT = retention time in the continuous reactor.

Excel solver was used to fit the model to the biomethane production data.

The model did not provide a good fit for the liquid fraction of the substrate and therefore, for the second phase of the experiment, the methane potential values were those obtained at the end of the trial (day 35) as opposed to the ultimate methane potential Y_{max}.

Analytical techniques

Total solids were determined by drying the samples at 105°C overnight. Subsequently, every sample was ignited at 550°C for two hours. The VS content was calculated as the difference between the TS content and the produced ash (after the 550°C drying) divided by the wet sample, in accordance with the standard methods for examination of water and wastewater [14]. The nitrogen content of WS was

measured with a total Kjeldahl Nitrogen (TKN) analyser. Acid soluble lignin (ASL) and the concentrations of sugars in untreated and the solid fraction of the treated WS were measured after a two-step acid hydrolysis process according to the National Renewable Energy Laboratory (NREL) protocol [15]. According to the same protocol, the samples were analysed for furfurals and hydroxymethylfurfural (HMF). Glucose, xylose and arabinose were measured by high-performance liquid chromatography (HPLC) in an Agilent 1260 series system, coupled with an Aminex HPX-87H column (Biorad) and a DAD and RI detector in series. The temperature of the column was set at 65 °C and a 0.6 mL/min flow rate with 5 mM H_2SO_4 as mobile phase was used. The sample volume was 20 μL. Sugars were detected in an RI detector (Agilent) and were quantified based on calibration curves of commercial sugars used as external standards. Acid Detergent Lignin (ADL) was measured based on the analytical method of Van Soest and Wine [16].

Statistical analysis

The statistical analyses were conducted with a paired student's t-test and statistically significance was assigned when $P<0.05$.

3. **Results**

Physicochemical characteristics

The chemical composition of WS before and after the pre-treatment is presented in Table 1. Cellulose content, expressed as glucose, in the untreated WS samples accounted for 33% of the whole biomass. The percentage for hemicellulose, expressed as xylose plus arabinose, was 22%. Finally, the ADL content represented 14% of the whole biomass. These percentages in the untreated WS are similar to the measured values for WS from previous studies. Ferreira et al [17] stated that the percentage of cellulose in raw wheat straw range between 30 and 40%, while the fractions for hemicellulose and lignin, range between 20-30% and 10-20% respectively. After the pre-treatment, glucose and xylose content of the WS seemed to have slightly increased. The highest values for both sugars were present in the WS samples pre-treated for 90 min. The protein content in the untreated WS was 5.06 % and found to follow a slightly increasing trend after thermal pre-treatment. For every different retention time of pre-treatment, the protein content ranged between 5.35 and 5.45 %. The pre-treatment also increased the moisture content in WS samples. Due to this, the TS content decreased, from 50% in the untreated samples to approximately 18% in the pre-treated WS (Table 1), indicating an absorption of water by the fibres. ADL values exhibited an increasing trend after each pre-treatment, from 14% in the untreated WS, to higher than 16% after the 90 min treatment. Finally, trace quantities of furfurals and hydroxymethylfurfurals (HMF) were produced during the pre-treatment (data not presented).

Specific volumetric CH_4 production

Different combinations of the pretreatment time and the OLR of the AD system were evaluated. The methane production data was modelled and the ultimate methane yield was calculated for all conditions tested (Figure 1). The calculated kinetic coefficients are presented in Table 1. Thermal pre-treatment, in every retention time, except for 90 min, improved the ultimate biogas potential when OLR 2 kg VS/m^3 was applied. For the same OLR, when WS was subjected to 90 min pre-treatment, no statistical difference was observed. Under this OLR, a maximum methane yield of 365 mL CH_4/g VS was obtained. Interestingly, the pre-treatment did not affect the ultimate methane potential, which varied between 241 and 279 mL CH_4/g VS, when an OLR of 4 kg VS/m^3 was applied. At an OLR of 8 kg VS/m^3, only the 60 min pre-treatment time affected the ultimate biomethane potential, which increased by ~9%, compared to the untreated substrate. Finally, in the highest OLR (12 kg VS/m^3), CH_4 production increased along with the increase in the pre-treatment time, except for the 30 min retention time. In that case, the highest production was achieved for the 60 and 90 min treatments, yielding 350 and 383 mL CH_4/g VS, respectively (Fig.1d).

In the second stage of the study, a comparison between the digestion of the whole slurry from the pre-treatment and its solid fraction in the performance of AD was conducted. For every examined OLR, lower methane production was obtained from the whole slurry than from the solid substrate. When the

highest dilution of the inoculum was applied (2 kg VS/m^3), the whole slurry produced the same amount of methane as the untreated sample. In these two cases, methane production was 40% lower than the production from the solid treated substrate (Fig. 2a). For the OLR 4 kg VS/m^3, the CH_4 production of the whole slurry was 40% lower than the production from the treated solid (Fig. 2b). Finally, at higher OLRs (8 and 12 kg VS/m^3), the decrease on the CH_4 production from the samples of the solid substrate to the samples of the whole slurry was statistically insignificant (Fig.2c-d).

4. Discussion

The thermal pre-treatment affected the composition of WS as it can be seen in Table 2. Due to the increased temperatures, it is likely that the pores of the substrate were opened and increased the water absorption. After the treatment, WS found to have a TS content of 18% while for the samples that didn't pass through this process the TS content measured represents the 50% of the substrate. In the past, similar results have been reported from other researchers [7]. Boonterm and his team [18] reported that the fibre surface wettability increases along with the increase in the harshness factor of the thermal treatment. According to the sugars analyses, concentrations of both glucose and xylose increased in WS solid fraction (Table2), indicating that part of the sugars were released from the lignocellulosic structure and passed into the liquid phase. It is possible that these sugars remained in the solid WS, as no washing step of the pre-treated WS was carried out, a fact which led to an increase of their concentration in treated WS solids. In accordance with these results, studies in the past reported an increase in the concentration of glucose in the solid product of the treatment [7, 19] Different studies on lignocellulosic material have shown a reduction of hemicellulose in the solid fraction after thermal treatment [20]. Bauer et al [21] on the other hand, reported an increase in the hemicellulose content after the treatment of 160°C followed by a decrease in this content when harsher pre-treatments (in terms of temperature) were used. These results reveal that the temperature plays a vital role in the depolymerisation of hemicellulose. Finally, the acid detergent lignin (ADL) seems to increase after the thermal pre-treatment. Similar increase in the ADL content of lignocellulosic materials, after thermal pre-treatment has already been reported in previous studies [7]. In these studies, the amount of recovered ADL after pre-treatment is stated as pseudolignin. Vivekanand et al [22] attributed this increase to the formation of cross-linked aromatic compounds due to the pre-treatment. The same team proposed that the amounts of pseudolignin which are formed during the thermal treatment can be anaerobically degradable.

The ultimate target of the pre-treatment on the WS was to increase the anaerobic biodegradability of this lignocellulosic material. This is the first time that the combined effect of the retention time of thermal pretreatment and the OLR of AD system was investigated. The results of the ultimate volumetric methane production in this study indicate that, the thermal pre-treatment had a positive effect in the ultimate methane yield, especially at the lowest and highest OLR, as depicted in Fig 1. The results showed that if an OLR of 2 kg/ m^3 is to be applied, a retention time of 60 min would be more efficient, while if an OLR of 12 kg/ m^3 was to be applied, a pre-treatment of 60 or 90 min would lead to increased biomethane production from WS.

During the second stage of the experiment, AD systems with lower organic loads seemed to be more affected by the addition of the liquid fraction of the pre-treatment. It is believed that the amount of active microorganisms is playing vital role for the digestion of the thermal treated samples. A synergetic effect of the different type of microorganisms and the presence of the nutrients from the inoculum, might also affect the digestion efficiency. Further investigation is required in order to identify the exact mechanisms that are present during the whole process and the ways that these treatments can enhance the anaerobic digestion of lignocellulotic substrates.

The highest ultimate biomethane yield from WS obtained in this work (350 l/kg VS; OLR of 2 and 12 kg/ m^3 and pre-treatment conditions 60 and 90 min) was within the range usually reported for maize (375 l/kgVS) [23]. This result indicates that using a heat pre-treatment may be an important tool to aid replacing energy crops with WS.

5. Conclusions

Thermal pre-treatment was successfully applied on WS prior to anaerobic digestion. This specific pre-treatment seems to have different effect on the process under different OLR. The effect of the pre-treatment was higher when the highest and the lowest tested OLR were applied. The optimum condition tested was at OLR of 2 and pre-treatment time of 60 min and OLR of 12 kg/ m^3 and pre-treatment time of 60 and 90 min, which led to a biomethane potential in the range of those reported for energy crops such as maize. In that way, higher volumetric methane yields can be achieved, maintaining the biogas output of existing Plants.

The process of AD seemed to be affected by the addition of the liquid fraction of the pre-treatment when lower OLR were applied. According to these results, anaerobic bioreactors are more likely to handle higher OLR when fed with thermally pre-treated lignocellulosic biomass.

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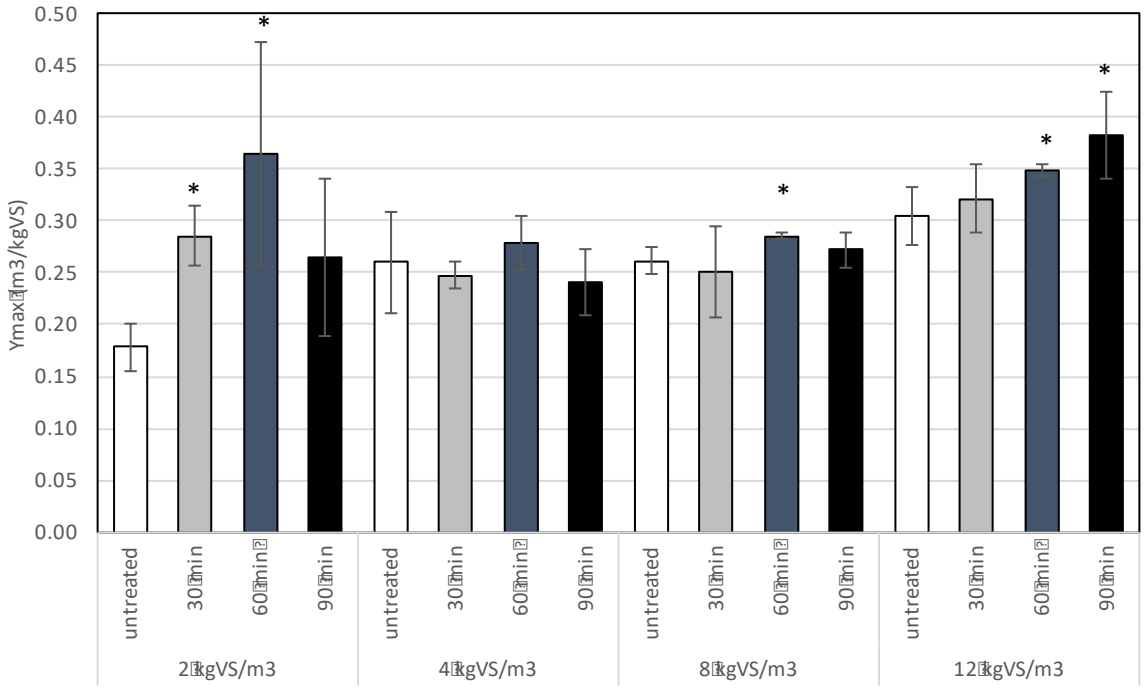
Fig. 1: Specific ultimate volumetric CH_4 yield (Y_{max}) in batch vial experiments with OLR of (a) 2, (b) 4, (c) 8 and (d)12, using WS subjected to different intensity of pre-treatment



* Asterisks indicate statistical difference between the untreated and the treated samples in the same OLR.

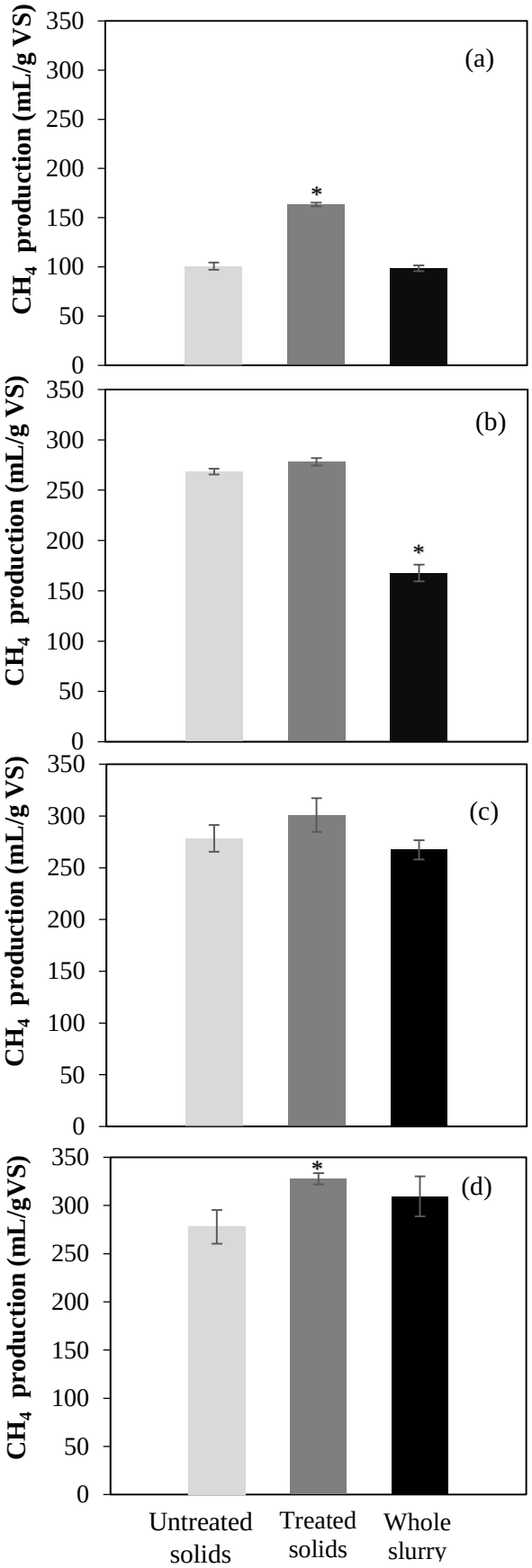


Fig. 2: Specific volumetric CH_4 production in batch vial experiments with OLR of (a) 2, (b) 4, (c) 8 and (d)12 after 35 days of incubation, using untreated solids, pre- treated solids (60 min treatment) and the whole slurry of the pre-treatment.

* Asterisks are indicating statistical difference between the untreated and the treated samples.

Table 1: Kinetic coefficients calculated the biomethane production data model output.

OLR	Conditions	K (d-1)	Ymax (l/gVS)	tlag	SSR
2 kgVS/m3	**untreated**	0.047722569	0.178067188	0.373239461	0.001372444
	30 min	0.045082047	0.285633272	1.09569512	0.00370164
	60 min	0.03852062	0.3647508	0.726158038	0.00477793
	90 min	0.051584045	0.264655191	1.036334374	0.00297911
4 kgVS/m3	**untreated**	0.059543578	0.25988905	0.787180862	0.003298857
	30 min	0.063010284	0.247830499	0.224799105	0.001269991
	60 min	0.066099707	0.278996039	0.755692259	0.002502513
	90 min	0.062549693	0.240927305	0.673145238	0.001995025
8 kgVS/m3	**untreated**	0.071580933	0.261770637	1.067434941	0.001518872
	30 min	0.061393432	0.250686782	0.658761002	0.036598081
	60 min	0.063436265	0.285615113	1.292430342	0.002005682
	90 min	0.06903502	0.271992643	0.866761466	0.001880627
12 kgVS/m3	**untreated**	0.040680321	0.304579635	0.689595468	0.000907789
	30 min	0.047451522	0.321564177	0.54764396	0.000726041
	60 min	0.045080037	0.349384072	0.509393875	0.000821888
	90 min	0.042537967	0.383099902	0.839067711	0.000861114

Table 2: Physicochemical characteristic of the untreated and the treated WS (solid fraction). Data are expressed as percentage of dry matter.

	Untreated	30 min treatment	60 min treatment	90 min treatment
Total Solids % (g TS/g wet sample)	50.00±4.11	17.87±0.61	18.32±0.40	18.82±0.12
Volatile solids % (g VS/g wet sample)	47.79±4.16	17.19±0.73	17.61±0.28	17.99±0.03
Crude protein (g/100g)	5.06±0.13	5.35±0.40	5.45±0.28	5.35±0.13
Xylose (g/100g)	20.70±0.22	22.06±1.62	22.57±1.99	23.91±2.73
Arabinose (g/100g)	1.90±0.01	1.91±0.06	2.14±0.29	1.94±0.29
Glucose (g/100g)	33.68±0.86	34.14±0.13	36.20±1.31	37.19±1.85

ASL (g/100g)	1.04±0.35	0.88±0.25	0.92±0.27	0.89±0.24
ADL (g/100g)	14.20±0.46	15.64±1.03	15.00±1.27	16.64±0.34