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WASTE AND BIOMASS MANAGEMENT & VALORIZATION

Towards upscaling the valorization of wheat straw residues: alkaline pretreatment using sodium hydroxide, enzymatic hydrolysis and biogas production

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Abstract

Lignocellulosic biomass is considered as a recalcitrant substrate for anaerobic digestion due to its complex nature that limits its biological degradation. Therefore, suitable preprocessing for the improvement of the performance of conventional anaerobic digestion remains a challenge in the development of anaerobic digestion technology. The physical and chemical characteristics of wheat straw (WS), as a representative lignocellulosic biomass, have a significant impact on the anaerobic digestion process in terms of quantity and quality of the produced biogas. This study aimed at investigating the enzymatic saccharification and detoxification of straw prior to anaerobic digestion with the final objective of enhancing the performance of conventional anaerobic systems of recalcitrant fractions of agricultural waste. The experimental activity was performed in lab and pilot scale treating WS. Alkaline delignification of straw using sodium hydroxide (NaOH) was studied prior to enzymatic hydrolysis for the production of easily biodegradable sugars. After defining the optimum conditions for the pretreatment scheme, the anaerobic digestability of the effluents produced was measured. Finally, the final liquid effluents were fed to a pilot scale anaerobic digester of 0.5 m^3 volume, applying an increasing organic loading rate (OLR) regime (in terms of chemical oxygen demand (COD) from 0.2 to 15 kg COD/m^3/day). The optimum conditions for the delignification and enzymatic hydrolysis of WS were defined as 0.5 M NaOH at 50 °C for 3–5 h and 15 μL Cellic CTec2/g pretreated straw at 50 °C. It was proven that the resulting liquid effluents could be fed to an anaerobic digester in the ratio that they are produced with satisfactory COD removal efficiencies (over 70%) for OLRs up to 10 kg COD/m^3/day. This value is correspondent to a hydraulic retention time of around 7.5 days, much lower than the respective one for untreated straw (over 12 days).

Keywords Anaerobic digestion · Delignification · Enzyme loading · Particle size · Scale-up · Sugars yield

Introduction

Agricultural wastes, by-products and co-products are defined as animal or plant residues which are not (or may not be further processed into) feed or food, and generate economic and environmental issues in the primary processing sector as well as in farming (Guillard et al. 2018). This waste stream is also called agro-waste.

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Agro-waste represents a huge amount of biomass resources (around 50% of the fresh harvested crops representing a potential of 90 million tonnes of oil equivalent (Guillard et al. 2018) according to biomassfutures.eu) to be smartly converted into sustainable bioproducts (biomaterials, biomolecules, biofertilizers and bioenergy). In circular economy thinking, these conversion products can be considered as true resources for decoupling human well-being and economic growth from primary resources exploitation (Fischer-Kowalski et al. 2011). This prevents from exhausting land, from having detrimental impact on biodiversity and jeopardizing overall food security. There is a need to develop innovative holistic approach towards ecoefficient bioconversion pathways and 'smart' agro-waste management schemes which do not lead to penalising side-effects on soils, water and air quality (Gontard et al. 2018).

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Anaerobic digestion (AD) is a well-established biotechnology with numerous full-scale applications. Existing AD technology offers a way to bioconvert agro-waste into methane and fertilizer. Yet, inappropriate local agro-waste supply often leads to feeding AD plants with dedicated bioenergy crops. Furthermore, methane offers a low economic benefit and value, lignocellulosic-rich waste streams are not the usual substrate for AD systems and the efficiency rates are not that high (Gontard et al. 2018). Given the intrinsic complex structure of the plant's cell wall, substrates rich in lignocellulose are not effectively degraded in anaerobic digesters. In general, the driving force influencing the biomass hydrolysis is the surface area which is accessible to the microbes (Xu et al. 2019).

Nowadays, recent strategical schemes have set as goal to enhance the biogas production from lignocellulosic substrates. Lignocellulosic materials, such as straw, are primarily composed of lignin, hemicellulose and cellulose. Lignin is considered to be the major barrier to the biodegradation of lignocellulosic material. Therefore, removing lignin from the lignocellulose-rich raw materials is favorable to decreasing their recalcitrant nature and thus to increasing their biodegradability (Al-Battashi et al. 2019). In order to overcome this recalcitrant nature of lignocellulosic raw materials, a pretreatment stage is usually implemented. The principal objectives of pretreatment are as follows: to degrade hemicellulose and lignin, to decrease the cellulose's crystallinity and to increase the porous character of the lignocellulosic substrates, which possibly enhances the accessibility of enzymes and microbes during biogas production (Kumar et al. 2009; Feng et al. 2017). Therefore, selecting the most suitable pretreatment scheme is of vital importance as far as commercial biogas production is concerned.

Alkaline pretreatments with chemicals such as NaOH, potassium hydroxide, ammonia and calcium hydroxide have been proved to promote delignification reactions and thus have drawn much attention (Li et al. 2015a, b; Feng et al. 2017; Dey et al. 2020). Furthermore, the coupling of alkaline pretreatment and enzymatic hydrolysis has presented promising results so far but still needs further investigation. Feng et al. (2014) investigated the effect of several alkaline chemicals and temperatures on the pretreatment and subsequent enzymatic saccharification of WS. Lignin recovery around 50% was observed for most alkaline pretreatments and was in general increased as temperature was raised.

Zhao et al. (2008) investigated the enzymatic saccharification of spruce by alkaline pretreatment at low temperatures with or without urea. It was observed that the enzymatic hydrolysis rate and yield were greatly enhanced after the treatment process. The beneficial application of alkaline treatment on CH_4 production has been pointed out by several authors. Vasmara et al. (2017) studied the pretreatment of milled WS with dilutes NaOH prior to anaerobic digestion. This pretreatment gave much higher maximum cumulative methane production values (216 mL CH_4) and increased biogas yield by 23% compared with untreated samples (176 mL CH_4). Rice and WS presented a more than doubled CH_4 production (124% and 112% respectively) after NaOH pretreatment based on the results of Chandra et al. (2012a, b, c). In another work, that of Li et al. (2015b), the highest biogas yield of ammonia-pretreated WS was raised to 625 mL/g volatile solids (VS), in comparison with 400 mL/g VS of the untreated WS.

Moreover, in our previous work (Kontogianni et al. 2019), the effect of several alkaline pretreatment schemes on the enzymatic saccharification of straw was studied. It was observed that the most efficient pretreatments, as far as lignin removal was concerned, were NaOH 2% autoclave and alkaline treatment with hydrogen peroxide 10%. The respective delignification yields were 84.86% and 89.60% respectively. NaOH 2% at 50 °C for 96 h was also studied as a pretreatment alternative and ranked third with a delignification efficiency as high as 76.38%. Taking into consideration technical along with safety and economic issues, further investigation of this alternative may also be justified.

To this end, the basic idea of this paper was to consider mostly unavoidable and continuously generated agro-wastes biomass, such as WS, as a true resource able to be fully converted into sustainable bioenergy and biofertilizers by the use of cascading processes. This paper aims to optimise the NaOH pretreatment process along with the enzymatic saccharification in order to maximise the release of fermentable sugars and glucose boosting the anaerobic biodegradability of the system. In order to optimise the pretreatment stages of straw before AD, the following parameters were studied: particle size, pretreatment kinetics, recycling of alkaline solution and enzyme loading. Finding the optimal hydrolysis conditions is important for increasing the yield of easily biodegradable compounds and the subsequent AD performance.

Materials and methods

Raw material

WS was harvested from Aspropyrgos province, Greece. For the comminution of straw, a FRITSCH Cutting mill Pulverisette 15 was used so as to achieve homogeneous and easy handlable raw material. By using proper sieves, the desired particle sizes (1 mm, 1–2 cm) were obtained. For coarse particle size of 3–5 cm, a grinder was used.

Treatment of WS

For the chemical pretreatment, 5-g WS was mixed with NaOH 0.5 M solution for 5 min in autoclavable bottles with a liquid to solid ratio (total solids, TS) of 10% w/w. NaOH

pretreatment was tested with two different operational setups; autoclaved at 121 °C for 1 h and under milder conditions (96 h, 50 °C).

The enzymatic saccharification of the pretreated solids was performed at 50 °C in 100-mL flasks after pH adjustment to 5 with sulfuric acid (H_2SO_4) 0.1 M. The reaction mixtures with 10% w/w dry pretreated solids and the cellulase, Cellic CTec2 provided by Novozymes were incubated at 50 °C and 300 rpm for 96 h in a rotary shaker incubator. The total cellulase activity was estimated based on the standard methods equal to 223 FPU/mL.

In order to maximise glucose release from straw, the following process parameters were studied: straw particle size, enzyme loading, NaOH pretreatment kinetics, recycling of alkaline solution and scale-up factor. By using proper sieves, the desired particle sizes (1–3 mm and 1–2 cm) were obtained and tested. For the examination of the effect of enzyme loading of the NaOH autoclaved straw on the solubilization efficiency, different enzyme loadings of CellicCTec2 were tested. Enzyme loadings of 5, 10, 15 and 20 μL/g pretreated straw were applied. The possibility of alkaline solution recycling was also investigated. Raw straw samples (10% TS) were pretreated with NaOH 0.5 M in an autoclave for 1 h. The resulting solid fractions were analysed whereas the liquid fractions were supplemented with fresh NaOH solution in order to achieve the proper solids ratio (10%). This process was repeated until the delignification efficiency was reduced more than desired (lower than 70%).

In all cases, the composition of raw and pretreated samples was analysed, as far as their liquid and solid fractions were concerned.

In order to examine the anaerobic digestibility of the WS (raw samples) and the hydrolysate produced under the optimal laboratory conditions, biomethane potential tests (BMP) were carried out. The tests were carried out in accordance with Angelidaki et al. (2009) in 0.5-L autoclavable containers. They were partly charged with inoculum and substrate (2:1 VS ratio). The seed sludge-inoculum was taken from a pilot-scale anaerobic digester that treated WS and had a VS concentration of 5% (50 g/L). In addition, two blanks with inoculum and no substrate were set as control experiments. Nutrient basic medium in accordance with Angelidaki et al. (2009) was added to a final volume of 0.25 L. The containers were securely closed and put in a shaking water bath at 150 rpm and 35 °C for AD. The biomethane production was determined every day based on the methodology of Esposito et al. (2012). Each BMP test was carried out in at least 2–3 dilutions and 4–5 replicates, so as to attain reliable results.

Upscaling

At first, an effort to upscale the whole pretreatment scheme from 5-g straw to a capacity of 200-g straw was studied under the optimum conditions that were determined at small scale. Then, as a next step, under the same conditions, further upscaling to a capacity of 3-kg straw was also considered. In the final effort of upscaling, WS was used as delivered, without any chopping, grinding or comminution.

A reactor of 4 L equipped with mechanical stirrer and double walls for water recirculation for temperature control was used in the first step of upscaling experimental trials. Alkaline pretreatment and enzymatic saccharification were performed under the optimum conditions that were determined in the lab scale experimental trials. In the end of the experiments, the liquid and solid fractions were analysed.

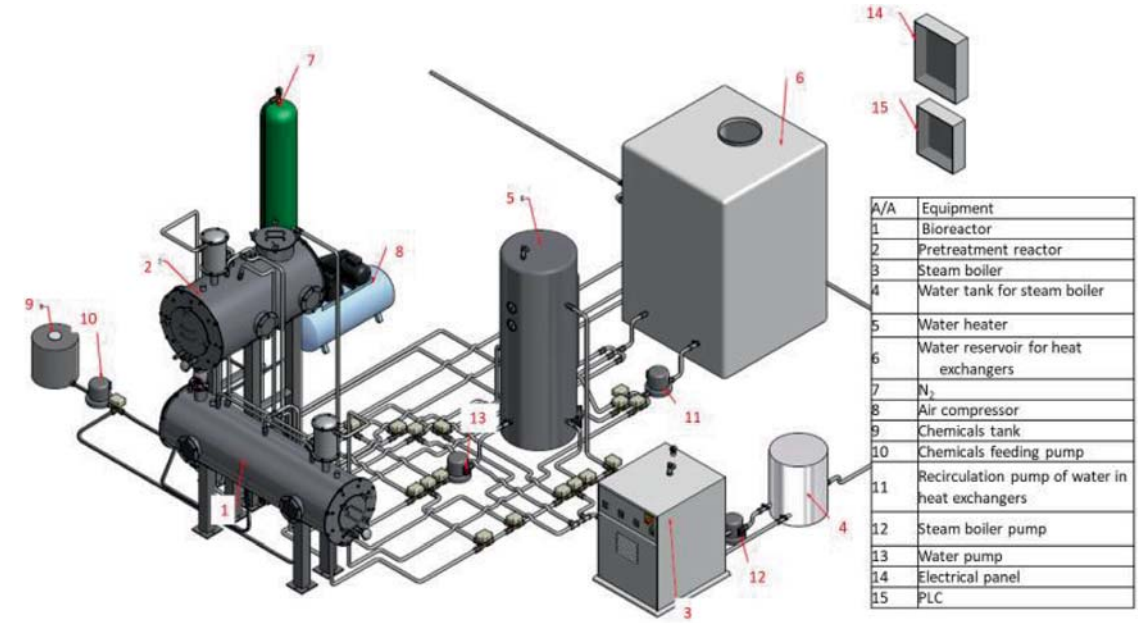
After this step, the pretreatment process unit was further upscaled by use of a bioconversion pilot scale facility (Fig. 1a) installed at the Unit of Environmental Science and Technology, National Technical University of Athens (NTUA), Greece. This system was utilised for the alkaline pretreatment and saccharification of WS. The pilot plant includes two stainless steel agitated horizontal rotating reactors having a capacity of 200 L each, which can operate independently. In the first reactor, the pretreatment took place while in the second the saccharification of straw. The temperature in the reactors is set through the circulation of steam or water in the double walls of the vessels. pH and temperature are controlled by a pH-meter and thermocouples. All pilot-scale experiments (pretreatment and enzymatic hydrolysis) were performed under the optimised conditions. In the end of experiments, the liquid and solid fractions were analysed.

The AD process was upscaled by use of a pilot scale apparatus (Fig. 1b), operational at the NTUA facilities that consists of a cylindrical, stainless-steel anaerobic reactor with a working volume of 0.5 m^3. The pilot plant is controlled by a PLC (programmable logical computer) and is fully automated.

The digester's loading and operational parameters such as pH and temperature can be controlled. Biogas flow rate and composition can be continuously monitored. A biogas utilization system for the heating of the digester is also included in the pilot plant. The excess biogas is burnt in suitable combustion device.

The anaerobic digester's start-up started by acclimatising the environment using low loading of the feeding (0.2 kg COD/m^3/day). The reactor was fed with the treated effluent following an increasing OLR regime. The OLR applied on the system was increased when statistically constant effluent was observed for at least 3 days. The state of the reactor was defined as statistically constant when the daily COD removal efficiency was within 10% variation, for at least 3 consecutive days, as is the common practice in dynamic loading of anaerobic digestion (De Francisci et al. 2015; Wu et al. 2019). The loading rates applied were 0.2, 0.4, 0.7,1, 1.5, 2, 2.5, 3, 5, 7, 10, 12 and 15 kg COD/m^3/day. In order to prevent a system's failure as a result of pH drop below 6.5, the pH of the pilot plant was carefully monitored and controlled every day.

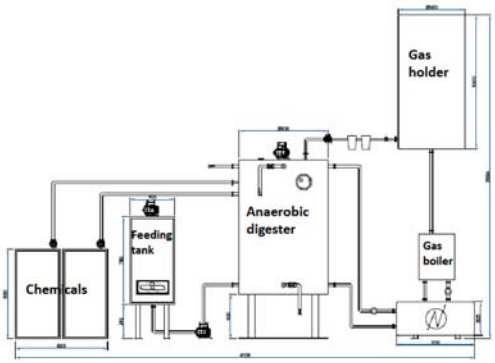
The anaerobic system's performance was closely monitored daily.



A/A	Equipment
1	Bioreactor
2	Pretreatment reactor
3	Steam boiler
4	Water tank for steam boiler
5	Water heater
6	Water reservoir for heat exchangers
7	N_2
8	Air compressor
9	Chemicals tank
10	Chemicals feeding pump
11	Recirculation pump of water in heat exchangers
12	Steam boiler pump
13	Water pump
14	Electrical panel
15	PLC

(a)

(b)



(c)

Fig. 1 The pilot plant (**a**), the bioconversion unit (**b**) and the anaerobic digestion unit (**c**)

Inoculum

The seed sludge utilised in the continuously stirred tank (CSTR) anaerobic reactor was obtained from the Psyttalia Wastewater Treatment Plant, Attica, Greece. More specifically, the seed sludge came from the anaerobic, mesophilic, high-rate digestion plant treating the thickened sludge mixture of primary sludge and surplus activated sludge. The reactor was also supplemented with some micronutrients and macronutrients according to Angelidaki et al. (2009).

Chemical analyses

Lignin (acid soluble lignin, ASL and acid insoluble lignin, AIL), hemicellulose, cellulose, extractives, moisture and ash in raw and pretreated samples were analysed based on the National Renewable Energy Laboratory's process (Sluiter et al. 2012). The chemicals were of analytical grade and were utilised without any purification. In the liquid phase, volatile fatty acids, VFA, total organic carbon, TOC, and phenolic compounds were also measured according to standard methods (APHA, AWWA 2005). Furthermore, the glucose content was assessed by a commercial kit which applies the Glucose Oxidase–Peroxidase method (Biosis S.A., Greece). All the analytical measurements were performed in triplicate.

Regarding the inoculum, the following parameters were measured based on the standard methods for the examination of water and wastewater (APHA, AWWA 2005): COD, TOC, total suspended solids (TSS) and volatile suspended solid (VSS).

Results and discussion

The composition of the milled straw was the following: 16.4% lignin (1.0% acid-soluble lignin and 15.4% Klason lignin), 45.1% hemicellulose, 33.8% cellulose and 4.7% ash, consistent with literature (Barakat et al. 2015; Solé-Bundó et al. 2017), whereas for the inoculum of the pilot scale anaerobic digester, the mean TSS, VSS, COD and TOC concentrations were measured equal to 12.25, 10.21, 0.31 and 0.158 g/L, respectively.

Particle size

In order to study the effect of particle size of straw on its pretreatment, the following tables derived (Tables 1 and 2), presenting its chemical composition after pretreatment. The degradation compounds determined in the liquid phase after alkaline pretreatment were phenolic compounds and VFA originating from the break down lignin.

The AIL degradation was very efficient, ranging from 74.43 to 84.86%. Similarly, Sambusiti et al. (2013) observed that the highest lignin reduction (53%) for WS was observed at 100 °C with 0.4 M NaOH, which is a lower lignin reduction compared with our study. Liu et al. (2015) reported a lignin degradation of more than 50% (54.6%) during WS pretreatment with 50% potassium hydroxide (Bolado-Rodríguez et al. 2016). In the study of Smit and Huijgen (2017), it was also verified that alkaline pretreatment (121 °C, 60 min, 2.5% w/w) solubilises most of the lignin (up to 77.5%) of WS as well as a part of the hemicellulose fraction. Solé-Bundó et al. (2017) also reported a minimal lignin reduction of almost 10% but more pronounced hemicellulose degradation (25%) after calcium oxide (CaO) pretreatment of WS (10 g CaO/100 g TS at 72 °C for 24 h). The concentration of soluble substances (including inter alia glucose, xylose, ramnose and acetate) after pretreatment was also similar (8.4% and 9.5% in our study). Similarly, Bolado-Rodríguez et al. (2016) detected a concentration of 5.80-g degradation compounds (phenolics and organic acids) per 100 g of WS after the basic pretreatment.

From the experimental data, it was obvious that the particle size in the range of 1 to 20 mm did not affect significantly the performance of the pretreatment scheme, since both chemical pretreatment and enzymatic hydrolysis presented similar efficiencies within the statistical error of the experimental trials. Thus, all the experimental trials beyond this point were conducted using coarse straw particles (10–20 mm).

Enzyme loading

The composition of the liquid as well as the solid fraction after sequential chemical pretreatment with NaOH in an autoclave and enzymatic hydrolysis of straw with CellicCtec2 is presented in Table 3.

From the results presented in Table 3, it was calculated that glucose yield ranged from 12 to 51% when cellulolytic formulation is added. A higher glucose yield (68%) was reported by Smit and Huijgen (2017) after applying alkaline pretreatment and enzymatic hydrolysis (2.5 filter paper units (FPU) Accelerase Trio/g substrate, 50 °C for 72 h at 140 rpm) on WS. It is also obvious that for enzyme loadings up to 15 μL/g pretreated straw, there is an almost linear correlation between enzyme loadings and performance indicators of straw hydrolysis such as cellulose degradation, soluble organic carbon and glucose released in the liquid phase. On the other hand, further increase of enzyme dosage brought up similar cellulose degradation efficiency. Nevertheless, a noticeable decrease in concentration of soluble compounds was also observed, as presented in Fig. 2, although the hydrolysis of the solid phase in terms of TS and cellulose is almost the same, within the limits of the experimental error. This fact may be attributed to the inhibition on the solubilization of the oligomers to monomers or to possible side-reactions consuming soluble organic carbon. Thus, 15 μL/g pretreated straw was selected as the optimum cellulase dosage.

Pretreatment kinetics

The enzymatic saccharification of the solid residue after both alkaline pretreatment schemes (autoclaving 121 °C, 1 h or 50 °C, 96 h) with the cellulolytic formulation CellicCtec2 yielded similar cellulose degradation efficiencies, 82.4% and 75.2% respectively. These elevated cellulose degradation efficiencies were accompanied by high saccharification yield

Table 1 Effect of particle size on the liquid and solid fractions of straw after alkaline pretreatment with NaOH (121 °C and 1 h)

Mean particle size (mm)	Liquid fraction	TOC (mg/g straw)	Glucose (mg/g straw)	VFA (mg/g straw)	Phenolics (mg/g straw)
1		159.65 ± 9.86	0.62 ± 0.06	98.30 ± 14.99	5.60 ± 0.14
10–20		137.20 ± 6.10	0.90 ± 0.01	67.20 ± 2.80	4.10 ± 0.20
Mean particle size (mm)	Solid fraction	%TS hydrolysis	%cellulose degradation	%AIL degradation	%ASL degradation
1		36.47 ± 2.78	33.52 ± 3.44	84.86 ± 0.45	49.86 ± 5.72
10–20		30.89 ± 0.89	19.68 ± 5.27	74.43 ± 3.55	99.49 ± 0.06

Table 2 The influence of particle size on enzymatic saccharification of alkaline-pretreated straw; composition of the liquid and solid fractions

Mean particle size (mm)	Liquid fraction	TOC (mg/g pretreated straw)	Glucose (mg/g pretreated straw)	VFA (mg/g pretreated straw)	Phenolics (mg/g pretreated straw)
1		327.18 ± 12.31	422.46 ± 21.02	71.04 ± 11.15	0.83 ± 0.05
10–20		305.04 ± 15.24	489.24 ± 17.31	56.82 ± 9.35	2.05 ± 0.02
Mean particle size (mm)	Solid fraction	%TS hydrolysis	%cellulose degradation	%AIL degradation	%ASL degradation
1		80.72 ± 3.18	75.20 ± 7.58	9.94 ± 0.63	80.67 ± 8.65
10–20		70.74 ± 2.21	70.59 ± 5.41	40.14 ± 3.78	62.3 ± 0.07

ranging from 207 to 225 mg glucose/g straw. This similar performance may be attributed to the fact that for alkaline media, pH is reduced as temperature rises (for example the pH of NaOH is reduced from 12.7 to 11.9 as temperature rose from 20 to 50 °C). Apart from pH, the impact of chemicals and temperature on the substrate may be attributed to the ionic concentration along with the respective impact on lignocellulose (Feng et al. 2014).

Since both performances were similar, but the cost infrastructure and energy needs of the two pretreatment approaches differ in magnitude, the kinetics of NaOH pretreatment of straw under milder thermal conditions was studied.

The timeline of the concentrations of the main components of liquid and solid fractions was studied. Figure 3 presents the evolution of delignification efficiency with time during NaOH pretreatment at 50 °C, since it has already been revealed that lignin degradation is the most crucial parameter for the successful implementation of the following stages. The timeline of organic carbon concentration in the liquid phase follows the same pattern.

From this experimental set, it was proved that after 6 h of pretreatment (up to 96 h), the delignification efficiency remained nearly constant.

Recycling

For the recycling experiments, regarding the solid fraction content, in respect to the number of cycles, the lignin, cellulose and hemicellulose degradations range as presented in Table 4.

In the liquid phase of the final cycle, the following parameters were measured:

- TOC 194.00 ± 11.3 mg/g straw
- Glucose 3.09 ± 0.12 mg/g straw
- VFA 106.78 ± 5.36 mg/g straw
- Total phenolic compounds 5.40 ± 1.05 mg/g straw.

It is evident from the concentrations presented above that the recirculation of the alkaline solution did not influence significantly the liquid phase, since these concentrations are only slightly higher than the respective of the first cycle. This may be attributed to the fact that fresh solution of NaOH was also added for each subsequent cycle. The fresh solution addition was almost 50% of the total volume, due to the increased the water holding capacity of WS.

Table 3 The influence of enzyme dosage on the enzymatic saccharification of alkaline-pretreated (121 °C and 1 h) straw; composition of the liquid and solid fractions

Enzyme loading (μL/g pretreated straw)	Liquid fraction	TOC (mg/g pretreated straw)	Glucose (mg/g pretreated straw)	VFA (mg/g pretreated straw)	Phenolics (mg/g pretreated straw)
0		17.7 ± 1.3	0.3 ± 0.03	11.4 ± 2.1	0.6 ± 0.02
5		108.0 ± 15.9	51.5 ± 28.6	44.3 ± 4.0	1.1 ± 0.2
10		160.6 ± 9.0	97.9 ± 6.1	55.1 ± 3.5	1.2 ± 0.01
15		293.3 ± 7.2	213.5 ± 9.6	62.5 ± 3.9	1.6 ± 0.01
20		191.3 ± 32.8	157.6 ± 14.5	59.6 ± 2.6	1.8 ± 0.03
Enzyme loading (μL/g pretreated straw)	Solid fraction	%TS hydrolysis	%cellulose degradation	%AIL degradation	%ASL degradation
0		13.22 ± 4.21	1.31 ± 2.32	6.92 ± 2.85	16.44 ± 8.02
5		43.18 ± 6.03	4.69 ± 0.11	31.35 ± 13.19	26.97 ± 2.95
10		53.38 ± 0.12	34.80 ± 7.50	37.59 ± 5.76	40.93 ± 1.24
15		70.74 ± 1.37	70.59 ± 6.15	40.14 ± 2.31	62.33 ± 1.52
20		68.16 ± 1.38	65.28 ± 1.64	34.08 ± 4.32	67.81 ± 2.07

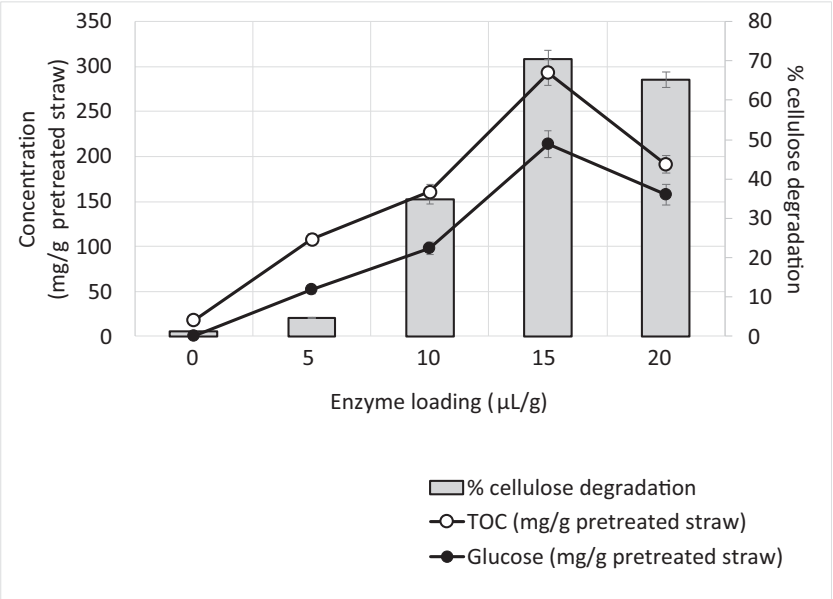


Fig. 2 Effect of enzyme dosage on the straw solubilization and cellulose degradation during enzymatic hydrolysis

Regarding the solid phase, for all the pretreatment cycles, the acid soluble lignin degradation was kept at very high levels (over 99%) while the cellulose degradation remained low (< 9%) in all cases. On the other hand, the recirculation of NaOH had a more pronounced effect on the delignification efficiency. The latter was reduced from 86.36 to 79.51% from the first to the second cycle. Then it remained almost constant for the following two cycles and at the fifth cycle it dropped further to 65.96%.

Thus, it is evident that the solution could be recirculated at least 4 times before being 'saturated' although after each experiment, it needed to be supplemented with some fresh solution. Nevertheless, despite the addition of fresh NaOH, the consumption of alkaline solution is reduced nearly 40% since the alkaline solution needs dropped from 10 to 6.25 kg/kg straw.

Conclusively, alkaline pretreatment with 0.5 M NaOH at 50 °C for 3–5 h followed by enzymatic saccharification with 15 μL Cellic CTec2/g pretreated straw at 50 °C resulted in the highest solubilization of WS (88% TS hydrolysis). Recycling up to four cycles of alkaline solution does not affect the delignification efficiency.

Anaerobic digestability of pretreated straw

Figure 4 shows the biomethane production of untreated straw along with the final effluents of the pretreatment (hydrolysate and alkaline solution). Furthermore, the hydrolysates of the 4 cycles were mixed with the 'saturated' alkaline solution (alkaline solution/ hydrolysate = 1/6) and also fed to the BMP tests.

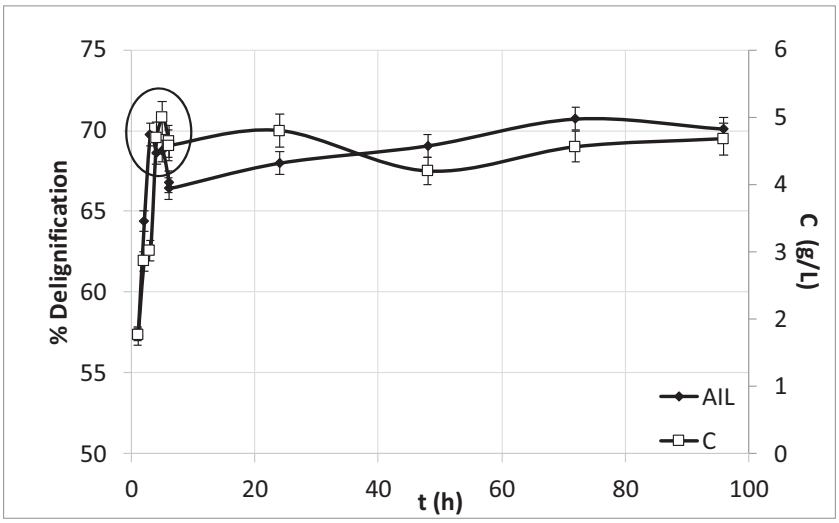


Fig. 3 Timeline of TOC (g/L) and delignification efficiency (%) during NaOH 0.5 M pretreatment at 50 °C

Table 4 Solid fractions' degradations after 1 to 5 cycles of pretreatment with NaOH at 50 °C for 6 h of WS

Cycle no.	%TS hydrolysis	%cellulose degradation	%AIL degradation	%ASL degradation	%hemicellulose degradation
1	36.61 ± 0.12	2.74 ± 0.26	86.36 ± 2.16	99.54 ± 2.84	51.71 ± 2.59
2	30.16 ± 0.21	4.12 ± 1.47	79.51 ± 3.58	99.48 ± 2.93	26.34 ± 2.31
3	26.80 ± 0.32	7.34 ± 1.61	72.21 ± 5.23	99.41 ± 3.65	15.67 ± 3.26
4	30.08 ± 0.78	8.50 ± 2.44	76.47 ± 2.45	99.41 ± 5.62	44.02 ± 6.19
5	14.03 ± 0.13	5.05 ± 2.63	65.96 ± 1.36	99.38 ± 3.18	23.65 ± 4.36

As shown in Fig. 4, AD was greatly accelerated when alkaline pretreatment and enzymatic hydrolysis were initially applied. For the untreated straw, BMP was equal to 183 ± 6 mL/g straw, while for the hydrolysate, its potential was over 270 ± 8 mL/g straw, implying serious improvement in anaerobic digestability of WS. From Fig. 4, it is also obvious that the kinetics of AD process was also affected by the applied pretreatment scheme. Ninety percent of the maximum BMP was achieved after 12.7 days for untreated straw, while the respective value was 7 days for the hydrolysate. As depicted in Fig. 4, the BMP of the alkaline solution was as low as 35 ± 8 mL methane (CH_4)/g straw. The mixture of hydrolysate with alkaline solution presented the highest BMP equal to 325 ± 9 mL/g straw, reaching 90% of this value after 7 days. This fact may lead to significant efficiency improvements especially for the case of continuous systems. Thus, the synergistic effect of the mixing of the two streams is obvious, implying that inhibition factors such as phenolic compounds were alleviated due to the dilution factor. Therefore, it was decided that the anaerobic pilot plant would be fed by the mixture of hydrolysate with alkaline solution.

Sambusiti et al. (2013) also concluded that the combination of chemical and thermal (at 100 °C) pretreatments presented a beneficial synergetic effect, that led to elevated methane yields, much higher than the yields observed when no pretreatment (BMP 189 L_{CH4}/g straw) or just chemical pretreatment was applied. The most efficient pretreatment conditions for WS reported were 10% NaOH at 100 °C, improving the methane yield up to 67% (BMP 315 L_{CH4}/g straw).

Feng et al. (2017) studied the in situ injection of KOH (0.8–10% w/w) as a pretreatment step for the briquetting process of WS and concluded that the highest CH_4 production from WS briquettes was as high as 353 mL/g VS after injection of KOH solution of 6.27% w/w, almost 15% higher than the raw WS. They also observed that untreated WS exhibited considerably slow kinetics that could not be attributed to nutrient deficit, whereas the reaction kinetics were undoubtedly enhanced after the pretreatment step; there was a k constant increase from 0.046 to 0.123 day^{-1}.

In the same context, Vasmara et al. (2017) concluded that the NaOH pretreatment of milled WS was very effective, with a 23% increase in the maximal biomethane production and the 85% increase in the daily rate of CH_4 accumulation in comparison with no pretreatment. Similarly, an enzymatic pretreatment with a mixture of cellulolytic enzymes showed 14% CH_4 production enhancement.

Scale-up

During upscaling, both delignification and saccharification efficiencies remained in the same range, implying that scale-up factor of 100 could be applied in the process successfully.

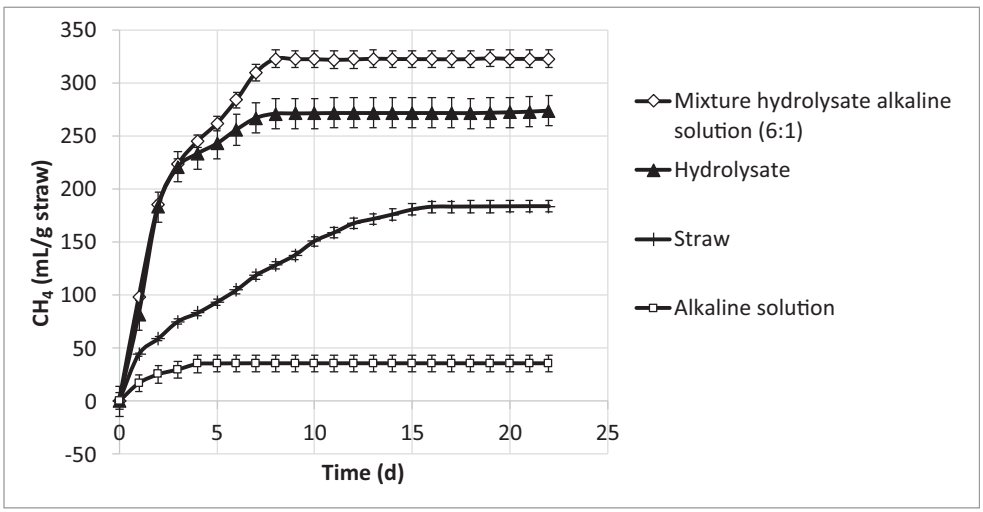


Fig. 4 Cumulative biomethane production of WS, hydrolysate, alkaline solution and mixture of hydrolysate with alkaline solution

Further upscaling to a capacity of 3-kg straw was also successful.

Figure 5 presents the effect of upscaling on the performance parameters of the NaOH pretreatment at 50 °C for 5 h. It is evident that the delignification efficiency and phenols release were slightly increased, while the VFA concentration was much higher. Consequently, scale-up of NaOH pretreatment could be considered more than successful. To this point, the difficulties of handling untreated straw should be pointed out. The apparent density of untreated straw is very low and thus its feeding in the treatment systems was difficult. By alkaline pretreatment application, this macroscopic behaviour of the raw material was completely changed and a slurry easily stirred and managed derived.

As a next step, for all cases, the pretreated straw was enzymatically hydrolysed. The scale-up of the enzymatic hydrolysis had a negative effect on the overall performance with decrease on glucose release up to 25% since at the capacity of 5-g straw per batch, 244 ± 5 mg glucose/g straw were produced, while at 200-g straw per batch, this yield dropped to 160 ± 7 mg glucose/g straw and finally at the larger scale of 3000-g straw per batch, the glucose yield was further reduced to 145 ± 9 mg/g straw.

The average composition of mixture of hydrolysate with the 'saturated' alkaline solution that derived from the pilot plant runs is presented in Table 5. This effluent was used as a feedstock for the anaerobic pilot plant.

Table 6 presents the strategy applied and the results obtained during the anaerobic reactor operation after the statistically constant effluent has been achieved. Figure 6 depicts the OLR step-wise increase in relation to %COD removal efficiencies in the anaerobic digester during the whole operational period. At first, 0.2 kg COD/m^3/day was fed to the digester. Seven days passed before the digester completely stabilised its performance, due to high fluctuations in daily biomethane production. At the lowest OLR of 0.2 kg COD/m^3/day, the %COD removal efficiencies reached up to more than 98%. A steady state of 4 days followed, and then the digester was then fed with 0.4 kg COD/m^3/day. At this point, 9 days, a longer period, where necessary for the biodegradation, after which the COD removal efficiency was maintained stable around 90% for 3 consecutive days. Nevertheless, the longer time period necessary for stabilization may be attributed to the digester's response to the change of OLR. Then, the OLR was further raised to 0.7 kg COD/m^3/day, corresponding to an HRT (hydraulic retention time) of 101.27 days. A continuous increase in OLR was applied up until the %COD removal efficiency dropped to 44.8% at 15 kg COD/m^3/day. Each time OLR was raised, a noticeable reduction in the COD removal efficiency was detected; yet, the anaerobic system could recover soon and get adapted to the next operational conditions. It can be observed that at an OLR of 10 kg COD/m^3/day, the COD removal efficiency was almost 70% and this was set as the highest limit for satisfying performance under the conditions of this study. This value of OLR

Table 5 Composition of the AD influent that derived from mixing the hydrolysate with the 'saturated' alkaline solution

Component	Value
TSS (mg/L)	19,929 ± 1562
VSS (mg/L)	18,527 ± 1261
COD (mg/L)	75,302 ± 8541
TOC (mg/L)	28,238 ± 3203
VFA (mg/L)	5544 ± 232
Phenols (mg/L)	118 ± 23
Glucose (mg/L)	32,095 ± 512
pH	7.5 ± 0.6

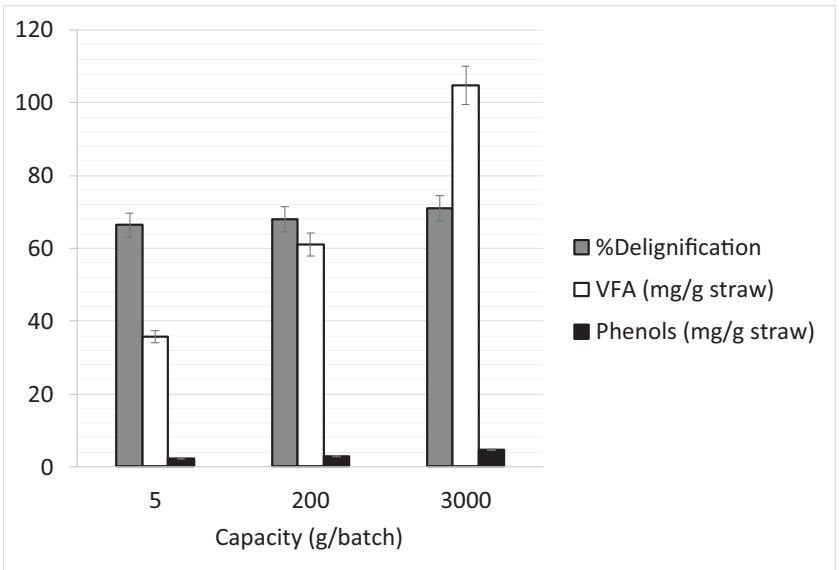


Fig. 5 Effect of increasing capacity of alkaline pretreatment scheme on key performance indicators of alkaline pretreatment

Table 6 Operational parameters and results of the pilot anaerobic digester

Day	OLR (kg/m^3/day)	Feed flow rate (L/day)	HRT (day)	COD removal efficiency (%)	Biogas production (L/day)
1–7	0.2	1.01	396.04	98.3 ± 0.13	31.46 ± 0.04
8–16	0.4	2.29	174.67	90.3 ± 0.29	60.62 ± 3.38
17–23	0.7	3.95	101.27	81.2 ± 0.20	89.64 ± 2.45
24–29	1	5.74	69.69	72.5 ± 1.37	119.08 ± 5.98
30–41	1.5	8.51	47.00	70.1 ± 0.26	181.63 ± 4.41
42–51	2	11.99	33.36	73.4 ± 0.08	258.68 ± 0.30
52–60	2.5	13.79	29.01	71.6 ± 0.74	303.19 ± 19.40
61–71	3	17.26	23.17	72.9 ± 0.51	376.14 ± 8.35
72–87	5	24.30	16.46	72.6 ± 0.40	608.10 ± 31.90
88–102	7	38.00	10.53	69.6 ± 0.31	835.45 ± 44.71
103–117	10	53.49	7.48	70.1 ± 2.01	1060.44 ± 49.58
118–128	12	64.53	6.20	66.2 ± 0.17	800.47 ± 28.48
129–139	15	76.54	5.23	45.3 ± 0.17	646.50 ± 24.84

corresponds to an HRT of 7.5 days, very similar to the values obtained from the BMP tests.

Figures 7 and 8 depict the biogas and CH_4 production rates for the increasing OLRs. Figure 7 shows the total biogas production during the pilot anaerobic digester operation. After acclimatization which lasted almost 7 days, the initial OLR of 0.2 kg COD/m^3/day was progressively increased to 15 kg COD/m^3/day up until biogas production dropped to 645 L/day in the last step. A moderate drop of CH_4 production was experienced for 0.7 and 1 kg COD/m^3/day. This was principally attributed to the stress of the digester caused by the OLR increments that seriously affected the microorganisms' methanogenic activity. Hence, counter measures are necessary to address discontinuity of the anaerobic process. From the starting stage, changes in CH_4 content were noticed from a minimal of 0.1566 L CH_4/gCOD (day 1) to a maximum of 0.2363 L CH_4/gCOD added at OLR 2 kg COD/m^3/day. A similar trend was recorded for 3, 5 and 7 kg COD/m^3/day until CH_4 production was kept almost steady and around a mean value of 0.1734 L CH_4/gCOD added.

On the other hand, the biogas and the CH_4 production rates were reduced remarkably as the COD loading rate in the last phases (12 and 15 kg COD/m^3/day) increased. The trend in Fig. 7 demonstrates that a high COD loading rate leads to an elevated concentration of organic compounds readily available for bioconversion to biogas leading to CH_4 production.

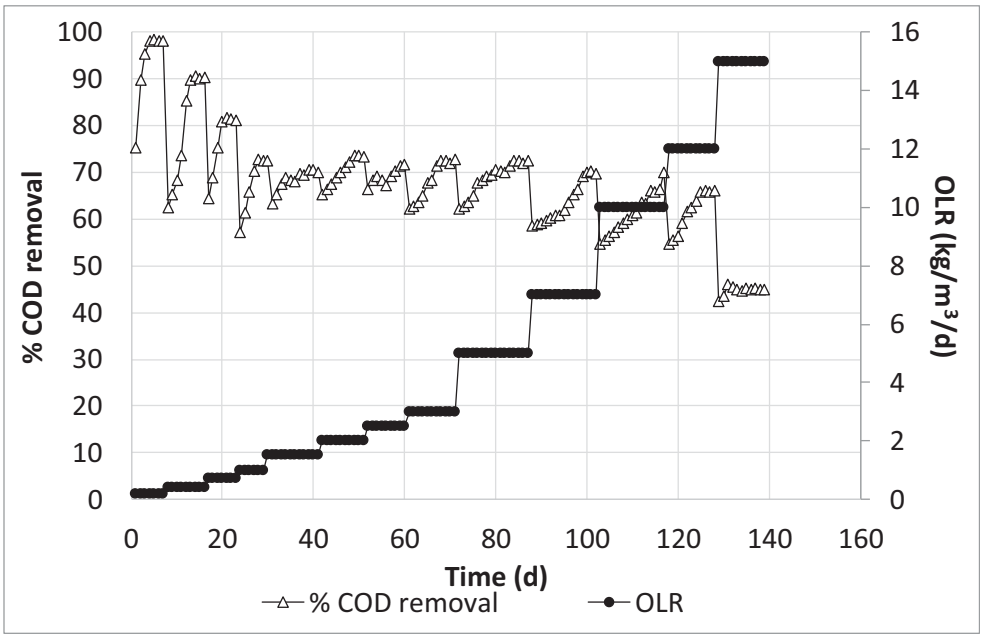


Fig. 6 Influence of OLR on the %COD removal efficiency

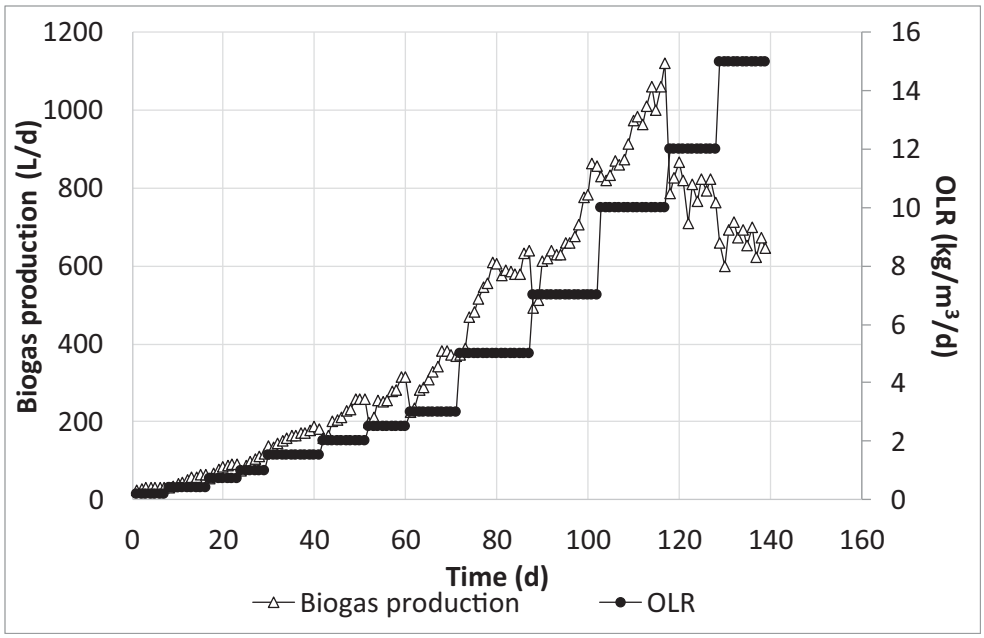


Fig. 7 Influence of OLR on biogas production

Nevertheless, the drop in the overall efficiency at 15 kg COD/m^3/day OLR may be attributed to the fact that VFA accumulated inside the bioreactor (over 635 mg/L).

Conclusively, it was made evident that the resulting liquid effluents from the pretreatment (chemical and enzymatic) of WS could be fed to an anaerobic digester in the ratio that they are produced with satisfactory COD removal efficiencies (over 70%) for OLRs up to 10 kg COD/m^3/day, corresponding to an HRT of around 7.5 days, much lower than the respective one for untreated straw (over 12 days).

Conclusions

Conclusively the optimal parameters for saccharification of WS are as follows: particle size up to 2 cm, alkaline pretreatment with NaOH 0.5 M at 50 °C for 6 h followed by enzymatic hydrolysis at enzyme loading of 15 μL CellicCTec2/g pretreated straw. The alkaline solution could be recycled up to 4 times without compromising the delignification efficiency. Upscaling process capacity from 5 g to 3 kg of straw presented similar yields, implying that the whole process could be incorporated in full scale systems. It was also made evident that

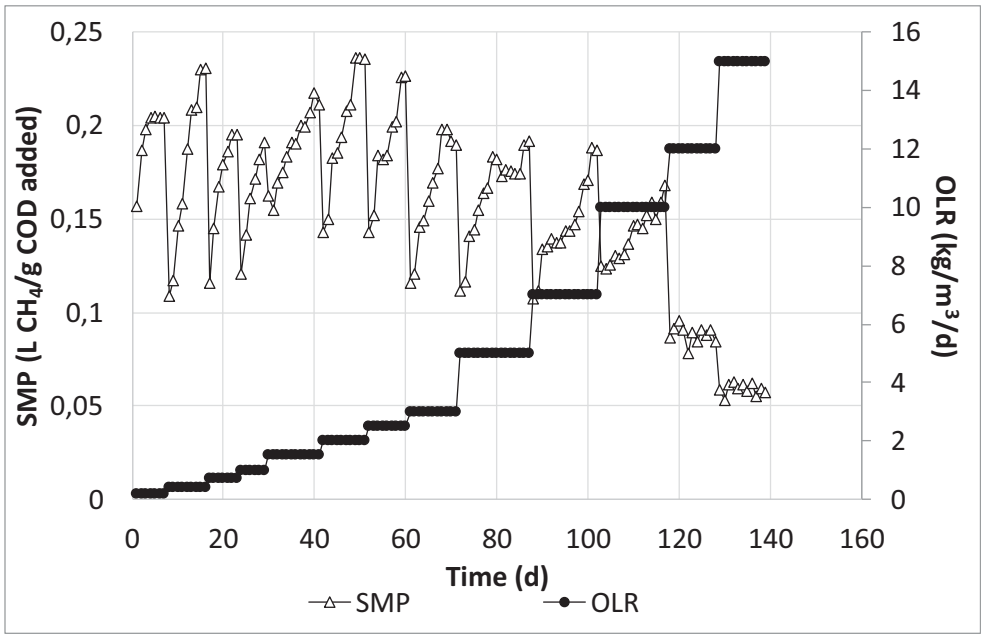


Fig. 8 Influence of OLR on SMP (Specific Methane Production)

the resulting liquid effluents from the pretreatment (chemical and enzymatic) of WS could be fed to an anaerobic digester in the ratio that they are produced with satisfactory COD removal efficiencies (over 70%) for OLRs up to 10 kg COD/m^3/day; corresponding to a hydraulic retention time of around 7.5 days, much lower than the respective one for untreated straw (over 12 days).

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